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Research report

Aging process alters hippocampal and cortical secretase activities of Wistar rats

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HIGHLIGHTS

- Aged brains have an imbalance between amyloidogenic and non-amyloidogenic pathways.
- Lower cortical TACE activity was linked to aging-induced aversive memory impairment.
- Treadmill exercise was unable to alter hippocampal and cortical secretase activities.

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ABSTRACT

A growing body of evidence has demonstrated amyloid plaques in aged brain; however, little attention has been given to amyloid precursor protein (APP) processing machinery during the healthy aging process. The amyloidogenic and non-amyloidogenic pathways, represented respectively by β- and α-secretases (BACE and TACE), are responsible for APP cleavage. Our working hypothesis is that the normal aging process could imbalance amyloidogenic and non-amyloidogenic pathways specifically BACE and TACE activities. Besides, although it has been showed that exercise can modulate secretase activities in Alzheimer Disease models the relationship between exercise effects and APP processing during healthy aging process is rarely studied. Our aim was to investigate the aging process and the exercise effects on cortical and hippocampal BACE and TACE activities and aversive memory performance. Young adult and aged Wistar rats were subjected to an exercise protocol (20 min/day for 2 weeks) and to inhibitory avoidance task. Biochemical parameters were evaluated 1 h and 18 h after the last exercise session in order to verify transitory and delayed exercise effects. Aged rats exhibited impaired aversive memory and diminished cortical TACE activity. Moreover, an imbalance between TACE and BACE activities in favor of BACE activity was observed in aged brain. Moderate treadmill exercise was unable to alter secretase activities in any brain areas or time points evaluated. Our results suggest that aging-related aversive memory decline is partly linked to decreased cortical TACE activity. Additionally, an imbalance between secretase activities can be related to the higher vulnerability to neurodegenerative diseases induced by aging.

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1. Introduction

Aging process and age-related diseases are usually accompanied by cognitive decline and structural alterations in brain areas such as cortex and hippocampus [1–3]. Substantial data have shown that β-amyloid (Aβ) brain deposition, a 39 to 43 amino acid peptides derived from amyloid precursor protein (APP) is implicated in both Alzheimer disease (AD) and normal aging process [4–6]. Although

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amyloid plaques have been found in aged human brain [7,8] little attention has been given to APP processing machinery during the healthy aging process.

APP can be proteolytically cleaved by amyloidogenic or non-amyloidogenic pathways. The amyloidogenic pathway comprises the sequential APP cleavages by β-secretase and γ-secretase leading to Aβ production [9,10]; β-secretase activity is represented by BACE (β-site APP-cleaving enzyme-1) [11]. There are paradoxical findings about the relationship between Aβ and cognitive performance since Aβ has demonstrated a dual neuromodulatory effect, which can improve or impair memory performance [12–15]. Moreover, recent in vivo imaging studies have failed to find any correlations between cognitive performance and amyloid deposition [16]. Taken together, we can suggest that in fact APP processing machinery rather than the Aβ levels is related to cognitive performance during the aging process.

The non-amyloidogenic APP processing pathway is represented by α–secretases. The members of a Disintegrin and Metalloprotease (ADAM) family, such as tumor necrosis factor (TNF)-α converting enzyme (TACE or ADAM17) and ADAM10 have α–secretase activity and generate α-APPs that play a role in synaptic plasticity, neuroprotection and synaptogenesis [17,18]. Although sAPPα levels are reduced in cerebrospinal fluid (CSF) of 23-month-old rats [19], to the best of our knowledge, there are no studies evaluating the non-amyloidogenic pathway role such as TACE activity during normal brain aging process. However, increased TACE activity and consequently higher sAPPα levels may be related to neurotrophic effects as synaptic formation and cognitive improvement [17,20].

A significant body of clinical and experimental evidences has indicated that regular physical exercise improves cognitive functions during normal aging and age-associated disorders [21–24]. The effects of forced and voluntary exercise protocols on Aβ-associated neuropathology and APP pathways processing have been investigated [25–29]. Recently, Zhao et al. [30] demonstrated that forced treadmill exercise ameliorates the spatial memory impairment accompanied by alterations in hippocampal plaques in APP/PS1 mice. In accordance, this transgenic model subjected to a voluntary exercise had improved memory, lower Aβ aggregation and BACE activity in hippocampus and cortex [31]. Despite these findings demonstrating that exercise can modulate secretase activities in AD models little is known regarding the relationship between APP processing and exercise effects during the healthy aging process.

Previously, we demonstrated that the treadmill exercise protocol used here (20 min/day during 2 weeks) was able to reduce in vitro ischemic damage in hippocampal slices of Wistar rats [32]. Besides, this forced exercise protocol transiently improved the inhibitory avoidance aversive memory performance in aged and young rats [33,34]. Recently, it has been described the impact of this neuroprotective protocol on inflammatory and epigenetic marks in hippocampus and frontal cortex of rats [33–37].

Our working hypothesis is that the normal aging process could imbalance amyloidogenic and non-amyloidogenic pathways, specifically BACE and TACE activities. Moreover, we expect that our exercise protocol would be able to alter aging-induced impairments. Taken together, the aim of this study was to evaluate the effects of aging process and a daily running exercise protocol on hippocampal and cortical BACE and TACE activities in Wistar rats.

2. Methods

2.1. Animals

Male Wistar rats of different ages, 3-month-old (n = 38), 21-month-old (n = 27) and 26-month-old (n = 24) were used. The animals were provided by the Centro de Reprodução Animal de Laboratório (CREAL) and maintained under standard conditions (12-h light/dark, 22 ± 2 °C) with food and water *ad libitum*. All animal procedures and experimental conditions were approved by the Local Ethics Committee (CEUA – Comissão de Ética no Uso de Animais – UFRGS; nr.23464). Their ages were chosen based on previous studies by Takahashi et al. [10] describing increased Aβ1-42 levels in brains of 26-month-old healthy rats. Additionally, work performed by our group demonstrated that this exercise protocol improves the cognitive performance of young adult and 20–21-month-old rats [33,34].

2.2. Treadmill exercise protocol

The animals were divided in sedentary (SED) or exercised groups (EXE). The aged groups, 21 and 26-month-old animals, had 12–14 rats per group; since we had control animals for each set of experiments and because the youngest animals (3-month-old) were taken as a control group an increased number per group was examined (n = 18–20). Exercise training consisted of running sessions on a motorized rodent treadmill (AVS Projetos, São Paulo, Brazil) using a moderate daily treadmill protocol (20 min running session each day for 2 weeks). All animals ran at 60% of their maximal oxygen uptake (VO_{2max}), which was measured indirectly prior to training [34,36]. Animals in the SED group were daily placed on the treadmill for 5 min without any stimulus to run. Gentle tapping on their back encouraged rats that initially refused to run. Neither electric shock nor physical prodding was used in this study, and the treadmill exercise was performed between 2:00 and 5:00 PM

2.3. Inhibitory avoidance test

Single-trial step-down inhibitory avoidance conditioning was used as an established model of fear-motivated memory where the animals learned to associate a location in the training apparatus (a grid floor) with an aversive stimulus (foot shock). In the training session, the rats received a 0.6 mA foot shock for 3.0 s. The test session was performed 24 h after a single training session to evaluate long-term memory; no foot shock was delivered, and the step-down latency (maximum 180 s) was used as memory retention [38]. The behavioral test was conducted 30 min after the last exercise training and 30 min prior to euthanasia. Previously, we described a transitory treadmill exercise effects on aversive memory [33,34]. After behavioral test rats were randomly subdivided into 1 and 18 h groups in order to verify transitory and delayed exercise effects on biochemical outcomes (Fig. 1).

2.4. Sample preparation

Rats were decapitated 1 and 18 h after the last exercise training session, consequently 30 min or 17.5 h respectively, after behavioral measurement (Fig. 1). It is important to cite that 1 h after exercise was performed in the afternoon while 18 h was taken early in the morning. Each exercised group had its sedentary control group. The hippocampi and prefrontal cortices were quickly dissected, immediately snap-frozen in liquid nitrogen and stored at −80 °C.

2.5. β-secretase (BACE) activity

BACE activity was evaluated using a commercially available kit (Abcam, catalog number ab65357) according to the manufacturer's instructions. The hippocampus and prefrontal cortices (n = 5–9) were homogenized, incubated on ice for 10 min and centrifuged 10.000 × *g* for 5 min at 4 °C; thereafter, the supernatant was collected. In a black 96-wells plate, 30 μL of the sample (total protein

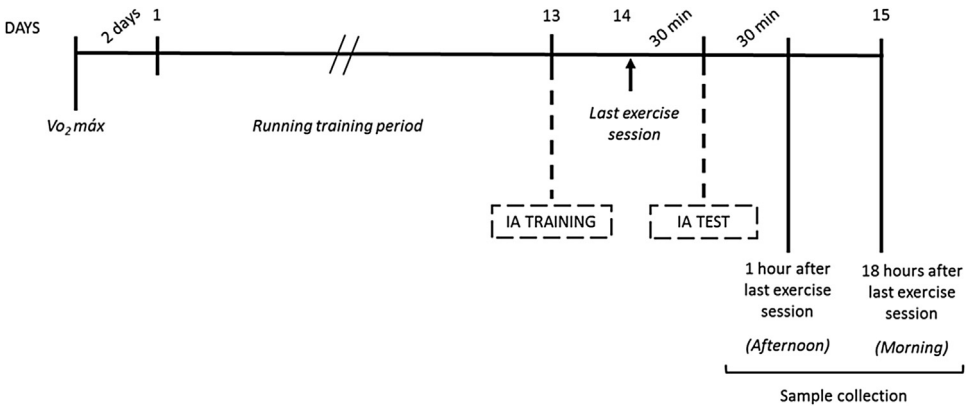


Fig. 1. Schematic diagram showing experiment design used in this study. The horizontal line represents the timeline of exercise protocol (14 days), inhibitory avoidance (IA), training and test, and sample collection. Exercised groups were subjected to a moderate daily treadmill protocol (20 min running session each day for 14 days). All animals were submitted to IA training trial and 24 h later IA test (30 min after the last exercise session). Samples collection was performed 1 h (afternoon) or 18 h (morning) after the last exercise session.

~200 μg) was added to each well followed by 70 μL of 2 × reaction buffer and 2 μL of the BACE substrate. The plate was incubated in the dark at 37 °C for 1 h. Fluorescence was read at excitation and emission wavelengths of 345 and 520 nm, respectively. BACE activity was expressed as relative fluorescence units (RFU) per mg of protein.

2.6. α-secretase (TACE) activity

TACE Activity Fluorimetric Assay Kit was used according to the manufacturer's instructions (Anaspec Inc. catalog number 72085). The hippocampus and prefrontal cortex (n = 5–9) were homogenized in assay buffer containing 0.1% Triton-X 100, incubated for 15 min at 4 °C and centrifuged at 2.000 x *g* at 4 °C, after which the supernatant was collected. In a 96-wells plate, 50 μL of the sample (total protein ~400 μg) was added into each well followed by 50 μL of the TACE substrate solution, after the reagents were mixed completely by an orbital shaker for 30 s. The plate was incubated in the dark for 1 h, after which 50 μL of stop solution was added, and the fluorescence was read at excitation (490 nm) and emission (520 nm) wavelengths. TACE activity was expressed as relative fluorescence units (RFU) per mg of protein.

2.7. Western blot analysis

Western blot was performed to evaluate the hippocampal APP content. The hippocampus (n = 4 animals per group) was homogenized with RIPA buffer (1:10) with phosphatase and protease inhibitors cocktails (1:100, Sigma Aldrich, Missouri, USA). Proteins were separated by 10% polyacrylamide gel electrophoresis (SDS-PAGE, 1.5 mm, 120 V) and transferred to PVDF membranes (Millipore). The membrane was blocked for 1 h with 4% nonfat dry milk in TBS-T and then blotted with primary antibodies anti-APP (Millipore, 1:5000) and GAPDH (Cell Signaling, 1:5000) overnight at 4 °C and a secondary horseradish peroxidase anti-rabbit antibody (Cell Signaling, 1:1000) for 2 h a room temperature. The proteins were quantified by measuring the band intensity (area × OD) using the Image J software (http://rsb.info.nih.gov/ij) and normalizing to GAPDH.

2.8. Protein quantification

The total protein was measured by the Coomassie blue method using bovine serum albumin as standard [39].

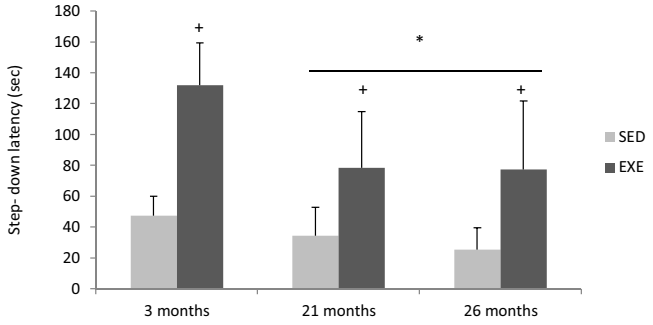


Fig. 2. Effects of aging and treadmill exercise on step-down latency in the inhibitory avoidance test. Three month-old SED (n = 20) and EXE (n = 18); 21 month-old SED (n = 13) and EXE (n = 14); 26 month-old SED and EXE (n = 12). The columns represent the mean ± SD; two-way ANOVA followed by Tukey's test. *Values significantly different from 3-month-old groups ($p < 0.05$). +Values significantly different from its respective sedentary control group ($p < 0.05$).

2.9. Statistical analysis

Behavioral data and secretase activities results were expressed as the mean ± SD and were analyzed using two-way analysis of variance (ANOVA) with age and exercise as factors followed by Tukey post hoc test. Taken that APP content was non- parametric data it was employed Kruskal–Wallis. In all tests the accepted significance level was $p < 0.05$.

3. Results

In the inhibitory avoidance test two-way ANOVA showed an effect of both factors, age ($F_{(2,88)} = 22.172$; $p < 0.001$) and exercise ($F_{(1,88)} = 122.751$; $p < 0.001$). Aged rats exhibited impaired aversive memory performance compared to young adult rats; however, daily moderate exercise increased the step-down latency in all groups ($p < 0.05$; Fig. 2).

Two-way ANOVA showed a significant age effect on hippocampal TACE activity in 1 h groups ($F_{(2,40)} = 24.080$; $p < 0.001$; Fig. 3A) as well as 18 h groups ($F_{(2,36)} = 46.280$; $p < 0.001$; Fig. 3B) since 26-month-old rats showed decreased TACE activity compared to other ages in both tested time points (Tukey post hoc test; $p < 0.001$). Neither exercise nor aging process induced changes on hippocampal BACE activity (Fig. 4A and B). It is important to note that a similar profile of tested time points, 1 and 18 h groups was observed in hippocampal secretase activities.

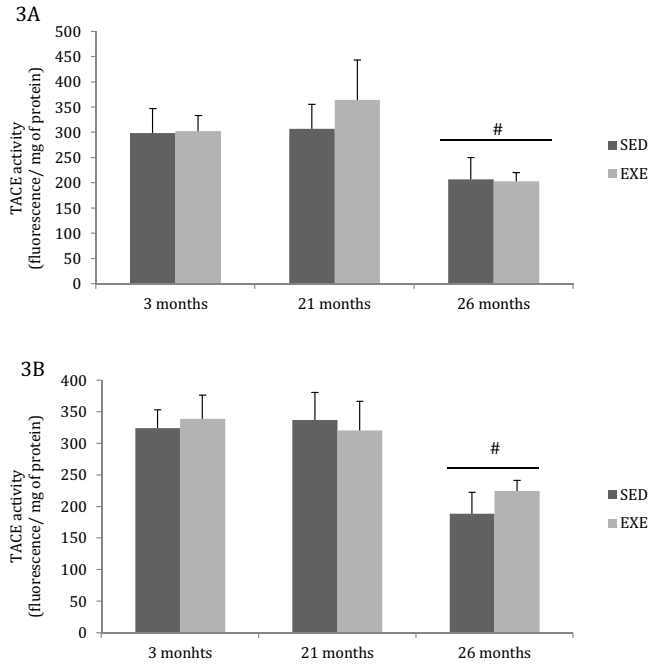


Fig. 3. Effects of aging and treadmill exercise on the hippocampal TACE activity in both tested time points. (Panel A) 1 h groups, 3 month-old SED (n = 6) and EXE (n = 9); 21 month-old SED and EXE (n = 7); 26 month-old SED and EXE (n = 6); (Panel B) 18 h groups, 3 month-old SED and EXE (n = 7); 21 month-old SED (n = 6) and EXE (n = 7); 26 month-old SED and EXE (n = 5). The columns represent the mean ± SD; two-way ANOVA followed by Tukey's test. #Values significantly different from 3- and 21-month-old groups ($p < 0.05$).

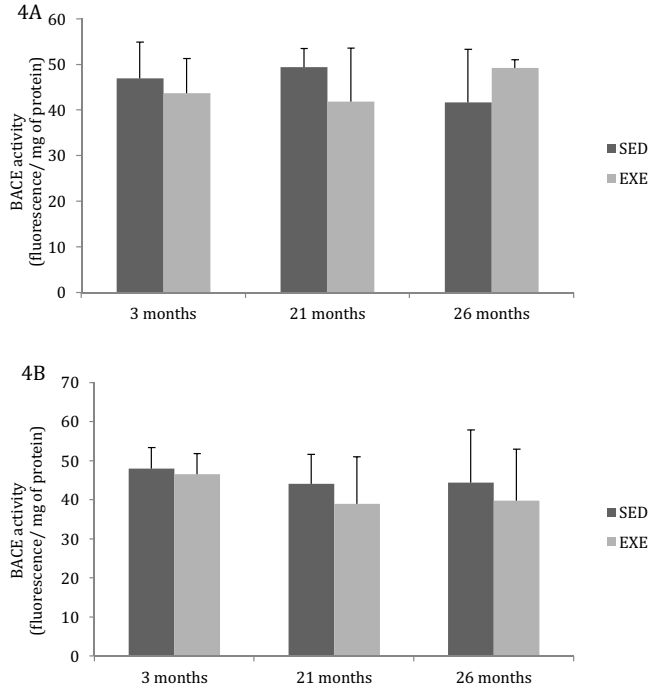


Fig. 4. Effects of aging and treadmill exercise on the hippocampal BACE activity in both tested time points. (Panel A) 1 h groups; 3 month-old SED (n = 9) and EXE (n = 6); 21 month-old SED (n = 6) and EXE (n = 7); 26 month-old SED and EXE (n = 5). (Panel B) 18 h groups; 3 month-old SED (n = 6) and EXE (n = 9); 21 month-old SED (n = 5) and EXE (n = 6); 26 month-old SED and EXE (n = 5). The columns represent the mean ± SD; two-way ANOVA followed by Tukey's test.

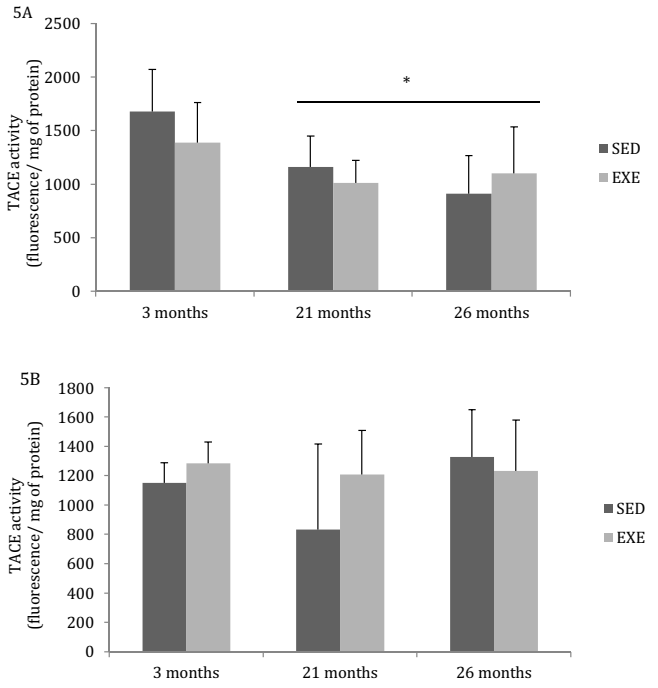


Fig. 5. Effects of aging and treadmill exercise on cortical TACE activity in both tested time points. (Panel A) 1 h groups; 3 month-old SED (n = 7) and EXE (n = 8); 21 month-old SED (n = 7) and EXE (n = 6); 26 month-old SED and EXE (n = 7). (Panel B) 18 h groups; 3 month-old SED (n = 7) and EXE (n = 9); 21 month-old SED and EXE (n = 7); 26 month-old SED and EXE (n = 7). The columns represent the mean ± SD; two-way ANOVA followed by Tukey's test. *Values significantly different from 3-month-old groups ($p < 0.05$).

In contrast, a significant aging process effect was observed on TACE activity in the prefrontal cortex in 1 h groups ($F_{(2,41)} = 10.075$; $p < 0.001$; Fig. 5A), given that there was a decreased TACE activity in both aged groups, 21 and 26-month-old rats compared to young adult ones (Tukey post hoc test; $p = 0.004$ and $p = 0.001$, respectively). However, this profile was not observed in prefrontal cortex collected in the early morning (18 h groups, Fig. 5B) demonstrating a clear temporal profile.

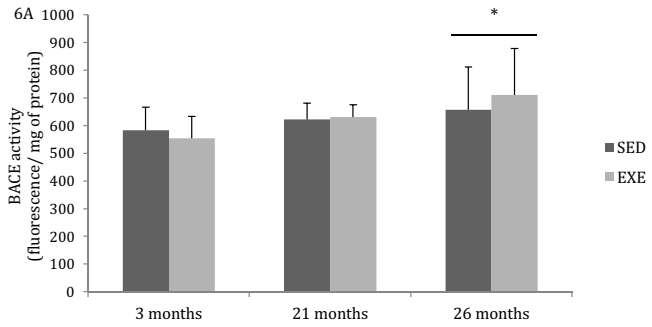
Besides, cortical BACE activity was significantly higher in 26 month-old rats in afternoon (1 h groups) than in other tested ages ($F_{(2,42)} = 4.580$; $p = 0.017$; Fig. 6A) corroborating the results observed with cortical TACE activity there were no changes in BACE activity in the early morning (18 h groups, Fig. 6B).

Our exercise protocol was unable to impact amyloidogenic and non-amyloidogenic pathways, specifically cortical and hippocampal BACE and TACE activities neither transitorily nor delayed, respectively 1 or 18 h after the last exercise session.

Additionally, the ratio between TACE and BACE activities was evaluated showing a significant age effect in the afternoon groups (1 h) in hippocampi ($F_{(2,43)} = 4.845$; $p = 0.013$; Fig. 7A). Hippocampal TACE/BACE ratio was decreased in 26-month-old rats compared to other ages (Tukey post hoc test; $p = 0.012$), however this ratio remained unchanged in 18 h groups (Fig. 7B).

In accordance with the results described above a significant age effect was observed in cortical TACE/BACE ratio ($F_{(2,40)} = 10.494$; $p < 0.001$; Fig. 8A), since it was showed a decreased TACE/BACE ratio in both aged groups (Tukey post hoc test; $p = 0.001$); interestingly this profile was not observed in samples collected in early morning (18 h groups, Fig. 8B).

In addition, APP content (KW = 2.33; $p = 0.8019$) remained unchanged in all groups (data not shown).



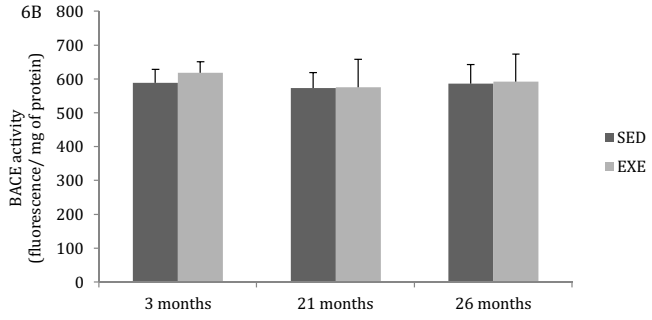
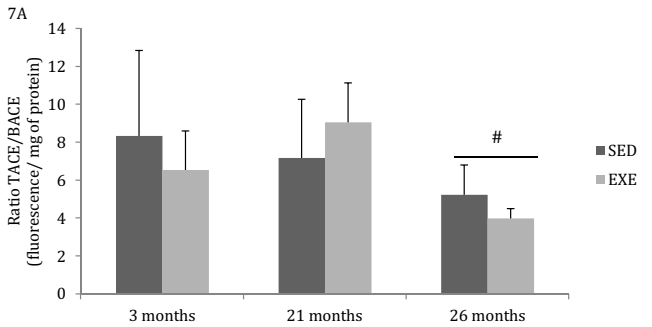


Fig. 6. Effects of aging and treadmill exercise on cortical BACE activity in both tested time points. (Panel A) 1 h groups; 3 month-old SED (n = 9) and EXE (n = 8); 21 month-old SED and EXE (n = 7); 26 month-old SED and EXE (n = 6). (Panel B) 18 h groups; 3 month-old SED (n = 6) and EXE (n = 8); 21 month-old SED (n = 6) and EXE (n = 5); 26 month-old SED and EXE (n = 6). The columns represent the mean ± SD; two-way ANOVA followed by Tukey's test. *Values significantly different from 3-month-old groups ($p < 0.05$).



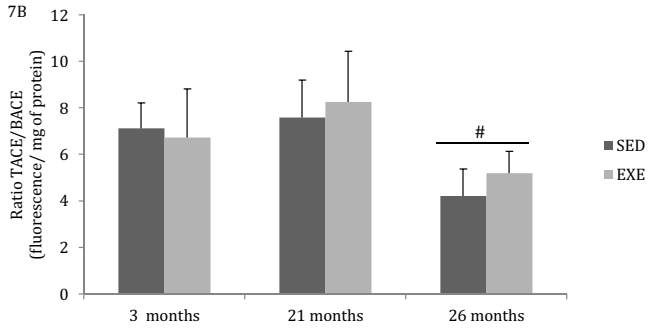


Fig. 7. Effects of aging and treadmill exercise on hippocampal TACE/BACE ratio in both tested time points. (Panel A) 1 h groups; 3 month-old SED and EXE (n = 9); 21 month-old SED and EXE (n = 7); 26 month-old SED and EXE (n = 6). (Panel B) 18 h groups; 3 month-old SED (n = 7) and EXE (n = 9); 21 month-old SED and EXE (n = 5); 26 month-old SED and EXE (n = 6). The columns represent the mean ± SD; two-way ANOVA followed by Tukey's test. #Values significantly different from 3- and 21-month-old groups ($p < 0.05$);.

4. Discussion

Our work adds evidence for the role of non-amyloidogenic and amyloidogenic pathways, specifically of secretases in the aging process. In addition, to our knowledge, this is the first study evaluating TACE activity in healthy aged brains. In this context, we observed a diminished α-secretase activity, specifically TACE (ADAM17). Consistent with our results, lower soluble APPα (sAPPα) levels, a TACE-derived product, have been found in cerebrospinal fluid (CSF) of 23-month-old rats [19]. Moreover, individuals with Alzheimer's disease present low sAPPα levels in the CSF suggesting reduced TACE-dependent α-secretase activity [40,41]. Taken that sAPPα appears to have beneficial functions including neuroprotection, neurotrophism and neurogenesis [19,42], it is possible to suppose that reduced TACE activity observed here in aged prefrontal cortices and hippocampus might be implicated in their susceptibility to neurodegenerative disorders.

We can suggest that the memory impairment in aged rats, as evaluated by an inhibitory avoidance test, may be related to cortical TACE activity, since this parameter was reduced in prefrontal cortices of 21-and 26-month-old rats while in hippocampus this reduction was observed only in oldest rats (26-month-old rats). In accordance, Anderson et al. [19] showed the involvement of sAPPα levels in the cerebrospinal fluid (CSF) with better spatial cognitive performance in aged rats. Our data support the idea that decreases in cortical α-secretase (TACE)-mediated cleavage of APP, a non-amyloidogenic pathway, may be partly involved in the aging-related aversive memory decline.

In addition to its role on APP processing, TACE can regulate the inflammatory processes through TNF-α cleavage of its membrane-bound form [43–45]. Interestingly several studies have demonstrated that memory performance can be related to TNF-α, however, these studies are contradictory. Fiore et al. [46] observed that transgenic mice overexpressing TNF-α showed impaired inhibitory avoidance performance. In contrast, it has been suggested that TNF-α is essential for memory function in healthy aging, since mice deficient for TNF-α under non-inflammatory conditions have poor memory [47,48]. Our group did not show any correlation between TNF-α and the aversive memory performance in healthy aged rats [34]. Taken together, it is reasonable to speculate that different mechanisms related to TNF-α, such as TACE activity, can actually modulate the memory performance.

The amyloidogenic pathway represented by BACE activity was also investigated. Although rodents are widely used as aging model, little is known about the impact of healthy aging process on BACE activity in rats. In the present work, it was observed an augmented BACE activity in the oldest-old cortices (26-month-old); therefore, we can propose that Wistar rats are good animal models for human brain aging. In accordance, the BACE activity was increased in cortex of aged rats compared to young ones (4–6 months) [49]. Moreover, Fukomoto et al. [50] demonstrated a higher BACE activity in aged cortices and cerebellum of human, monkey and Tg2576 transgenic mice. Takahashi et al. [10] described increased Aβ1-42 levels in brains of 26-month-old healthy rats. These findings can be related to the higher cortical vulnerability to neurodegenerative disorders, especially AD. An interesting result that emerges from this work was related to TACE/BACE imbalance in favor of BACE activity in aged brain, specifically hippocampus and cortex. These findings may be implicated with age-related susceptibility to neurodegenerative disorders and could reflect a cellular environment that facilitates Aβ peptide production and consequently brain senile plaque formation. In addition, the aging-induced disruption of this balance was structure-dependent, since an imbalance was found

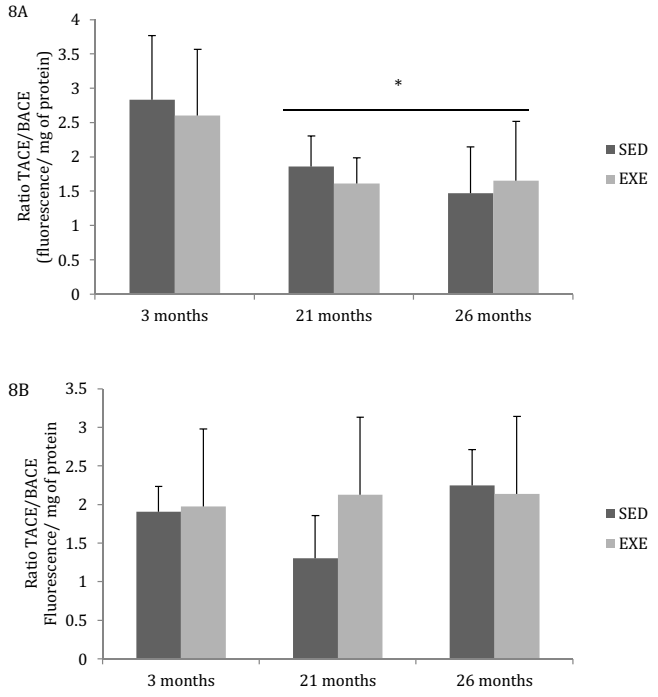


Fig. 8. Effects of aging and treadmill exercise on cortical TACE/BACE ratio in both tested time points. (Panel A) 1 h groups; 3 month-old SED (n = 7) and EXE (n = 8); 21 month-old SED and EXE (n = 7); 26 month-old SED and EXE (n = 6). (Panel B) 18 h groups; 3 month-old SED (n = 8) and EXE (n = 9); 21 month-old SED (n = 5) and EXE (n = 7); 26 month-old SED and EXE (n = 4). The columns represent the mean ± SD; two-way ANOVA followed by Tukey's test. *Values significantly different from 3-month-old groups ($p < 0.05$).

in cortices of 21-and 26- month-old rats while in the hippocampus was observed only in oldest rats. Our findings can support pharmacological BACE inhibition as a potential neuroprotective strategy; however, the efficacy profile might depend on brain structure and age-tested.

Even that a higher BACE activity was found in aged cortices, we cannot exclude other potential mechanisms involved in brain aging, since BACE-independent pathways, such as cathepsins have been described [51,52]. In addition, several processes can change the total Aβ levels in brain regions, such as Aβ oligomerization, deposition and clearance [50,53,54]. Interestingly, a decreased Aβ clearance has been suggested as a central mechanism in AD. Mawuenyega et al. [55] showed that the production rates of Aβ1-40 and Aβ1-42 were similar between individuals with symptomatic AD and age-matched cognitively normal controls while the Aβ clearance rates were decreased in AD patients. Taken together, it is possible to infer that reduced Aβ clearance can be linked to neurodegeneration in AD while non-amyloidogenic and amyloidogenic pathways, specifically of secretases, could be associated with age-related susceptibility to neurodegenerative disorders.

An important finding that emerges from this work is that secretase activities in prefrontal cortices can be modulated by the circadian rhythm in aging process. It was observed a clear temporal profile in the secretase activities in prefrontal cortices since both BACE and TACE activities were changed only in the afternoon period. On the other hand, hippocampal secretase activities were altered in both tested time points. Therefore, it is possible speculate that distinct brain areas may respond differently to circadian rhythm influence. Taken that melatonin is able to upregulate the TACE enzyme [56] and melatonin is strongly decreased with age [57] we can infer that decreased levels of melatonin are related to lesser TACE activity in cortices of aged animals. It is possible to recognize that BACE activity was increased in cortices of oldest rats in the afternoon period. Impaired rest-activity rhythms are found in AD patients as well in experimental AD model mice [58–60]. Interestingly, Blake et al. [61] using Drosophila model have showed that increased BACE expression is able to impair rest-activity rhythms especially in aged animals with a mechanism independent of Aβ production. Our data obtained with brain rodent can be related to disruption of circadian rest/activity rhythmicity present during normal aging [62,63], giving insights and might open new avenues for further studies regarding the modulation of secretase activities by circadian rhythms in aging process. Besides, taken that BACE inhibitors have been considered a promising target for AD treatment [1,10], it is possible infer that the in vivo studies of BACE inhibitors should observe times/time of day of administration. Our results might propose that the appropriate time of day for BACE inhibitors administration is afternoon in rodent models.

Another remarkable point for discussion is that our neuroprotective exercise protocol, daily running during two weeks, was unable to modulate BACE and TACE activities in prefrontal cortices and hippocampi of Wistar rats in both evaluated time points after exercise. Consequently, neither transitory nor delayed effects of our exercise protocol were observed in all tested ages. It was demonstrated that this exercise protocol transiently improved aversive memory performance in young adult and aged rats concomitantly reduced hippocampal pro-inflammatory cytokines levels of aged rats, while was able to increase anti-inflammatory cytokines in young adult rats, showing a biochemical age-dependent profile [34]. Besides, our group has suggested that exercise can impact different biochemical marks in an age-dependent manner, since lower levels of histone H4 acetylation were transitorily reversed in hippocampi of aged rats, without any effect in young ones [34]. Taken together, we can suggest that exercise-induced improvements in cognitive performance were independent of non-amyloidogenic and amyloidogenic pathways, as well it is impossible to exclude that different cellular mechanisms are involved.

Although we did not observe any transient or delayed effects of this exercise protocol it is impossible to exclude that a long-term exercise protocol could impact non-amyloidogenic and amyloidogenic pathways. Recently Sinha et al. [49] reported that a long-term dietary supplementation during 18 months with antioxidants, a combination of *N*-acetylcysteine, α-lipoic and α-tocopherol, reversed the age-related alterations in amyloid beta metabolism in cortices of rats.

The aging process and the exercise protocol were unable to alter the hippocampal APP protein levels. Our results are consistent with those obtained by Solas [64] and Anderson et al. [19] with 18- and 23-month-old rats, respectively. Although Sinha et al. [49] described that both mRNA expression and protein levels of APP were increased in the cortex of aged rats; Flood et al. [65] showed that cortical APP mRNA levels remained unchanged during aging. Additionally, a recent study showed that the hippocampal APP levels of 4-month-old APP/PS1 mice were unaltered after ten weeks of treadmill training [66].

In conclusion, our data suggest that the reduced cortical TACE activity can be linked at least in part to aging-related aversive memory decline. In addition, the imbalance of non-amyloidogenic and amyloidogenic pathways can contribute to hippocampal and cortical susceptibility to neurodegenerative disorders. Furthermore, neither transitory nor delayed effects of a daily moderate exercise protocol during 2 weeks on non-amyloidogenic and amyloidogenic pathways were observed.

Conflicts of interest

The authors declare no conflicts of interest.

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