7-Chloro-4-(Phenylselanyl) Quinoline with Memory Enhancer Action in Aging Rats: Modulation of Neuroplasticity, Acetylcholinesterase Activity, and Cholesterol Levels

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Abstract

This study investigated the effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) to restore the cognitive impairment caused by aging in male Wistar rats. Moreover, modulation of neuroplasticity markers, acetylcholinesterase (AChE) activity, and cholesterol levels was performed. Aged rats were intragastrically treated with 4-PSQ (5 mg/kg) for 7 days. Animals were tested in behavioral tasks, and then plasma (to determine cholesterol levels), hippocampus, and cerebral cortex (to determine neural cell adhesion molecule (NCAM) and polysialyltransferase (PST) levels, and AChE activity) were removed. Our findings demonstrated that treatment of aged rats with 4-PSQ restored short-term and long-term memories in the object recognition tests. 4-PSQ treatment did not restore exploratory activity (rearings) but partially restored locomotor activity (crossings) reduced by aging in the open-field test. Moreover, the compound restored the reduction in the NCAM and PST levels, and AChE activity in cerebral structures, as well as the increase in the plasma cholesterol levels, caused by aging in rats. In conclusion, 4-PSQ restored cognitive impairment caused by aging in rats by modulating synaptic plasticity, cholinergic system, and cholesterol levels.

Introduction

Nowadays, the elderly are the fastest-growing segment of the population. This assumes an increase in life expectancy and a greater development of the aging process. This process occurs dynamically and progressively, and several changes determine the individual's ability to adapt to the environment, causing greater vulnerability and a higher incidence of pathological processes [1, 2].

Neural networks are particularly vulnerable to aging. Aging impairs the functional integrity of neurons [3] associated with cognition and structural plasticity [4]. In fact, a

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reduction of structural and functional plasticity in brain regions, such as the hippocampus and the prefrontal cortex, causes a cognitive impairment, which is related to aging [5].

The neural cell adhesion molecule (NCAM) plays a key role in neural development and has been implicated in synaptic plasticity and cognitive processes in adulthood [6, 7]. Some studies showed that a decrease of NCAM expression was associated with poor performance in spatial learning and memory tasks, as well as reduced levels of polysialylated neural cell adhesion molecule (PSA-NCAM) in the hippocampus and prefrontal cortex of aging rodents [8].

Additionally, some authors show that a deficit of cognition is caused, at least in part, by damage to cholinergic projections from the basal forebrain to the cortex [9, 10]. One of the most ubiquitous enzymes present in the cholinergic synapses is acetylcholinesterase (AChE) [11]. Janeczek et al. [12] inferred that a decrease in AChE activity in normal elderly people reflects the neuroplasticity that optimizes the capacity of memory by the impact of presynaptically released acetylcholine.

Moreover, an increase in the risk of developing neurodegenerative diseases, including dementia and Alzheimer's disease, has been associated with aging [13]. Along these lines, abnormalities in cholesterol metabolism are important in the pathogenesis of Alzheimer's disease, which contribute to cognitive decline among the aged [14].

In this context, the pharmaceutical industry has developed therapeutic strategies to minimize or even slow down the effects of aging on the human brain, promoting an improvement in quality and life expectancy [15, 16]. Selenium is a trace element, which significantly influences the cognitive functions [17] and different neurological disorders [18]. As an alternative to incorporate selenium into organic molecules, synthetic approaches have been developed for the synthesis of various organoselenium derivatives. Organoselenium compounds had an effect in improving memory and learning processes in adult [19] and old [20–22] animals.

7-Chloro-4-(phenylselanyl) quinoline (4-PSQ) is an organoselenium compound and a quinoline derivative, which has several pharmacological properties already described in the literature [23–30]. Thus, quinoline derivatives have received the attention of researchers because of their important pharmacological actions [31, 32].

Based on the aforementioned facts and on our interest in applying the findings of the pharmacology of 4-PSQ, the purpose of the present study was to investigate the possible effect of 4-PSQ in the treatment of the cognitive impairment caused by aging in rats. In addition, we examined the effect of the compound on the expression of NCAM and two polysialyltransferases (STX and PST) involved in the neuroplasticity and in the AChE activity in the hippocampus and cerebral cortex, as well as plasma cholesterol levels of aged rats.

## Material and Methods

Animals

Male young adult (3 months old, 10% lifespan completed, weighing 170–250 g) and aged (23 months old, 76.6% lifespan completed, weighing 400–700 g) Wistar rats were obtained from a local breeding colony. The animals were housed in cages with free access to food and water. They were kept in a separate air-conditioned (22 ± 2 °C) room, on a 12-h light/12-h dark cycle, with lights on at 7:00 a.m. Commercial diet and water were supplied ad libitum. Animal care and all experimental procedures were conducted in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publications no. 80-23, revised in 1996) [33] and in accordance with the Committee on Care and Use of Experimental Animal Resources, Federal University of Pelotas, Brazil (CEEA 4599-2016). All efforts were made to minimize the number of animals used and their suffering.

Drugs

4-PSQ (Fig. 1) was prepared and characterized in our laboratory by the method previously described by Duarte et al. [25]. Analysis of the ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of 4-PSQ (99.9%) was determined by gas chromatography-mass spectrometry (GC/MS). The compound was dissolved in canola oil. All other chemicals were of analytical grade and obtained from standard commercial suppliers.

Experimental Protocol

Rats were randomly divided into three experimental groups (10 animals/group) (Table 1). The animals belonging to young adult and aged groups received the canola oil (1 ml/kg) and the animals of the aged + 4-PSQ group received the compound (5 mg/kg). Treatments with canola oil or 4-PSQ were performed for 7 days, daily, by intragastric (i.g.) route, via
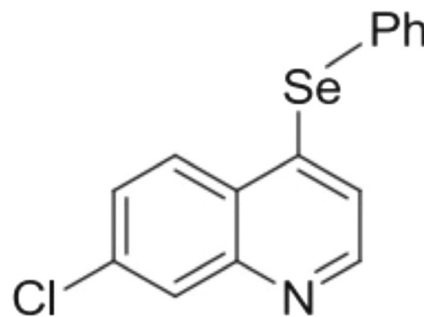
Fig. 1 Chemical structure of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ)

Table 1 Experimental groups

Group	Treatment
Young adult	Canola oil (1 ml/kg, i.g.)
Aged	Canola oil (1 ml/kg, i.g.)
Aged + 4-PSQ	4-PSQ (5 mg/kg, i.g)

The animals were intragastrically (i.g.) treated with 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) or canola oil, daily, for 7 days. The number of rats tested was 10/group

gavage. The 4-PSQ dose was chosen based on previous studies by our group [24, 30].

Figure 2 illustrates the experimental protocol of this study. After 7 days of treatment, animals were tested in behavioral tasks: the open-field (OFT) and object recognition (ORT) tests. Subsequent to behavioral tests, animals were allowed to rest until the 14th day in order to avoid behavioral stress, when they were euthanized by inhalation of isoflurane for ex vivo assays. The experimental protocol agreed with Bortolatto et al. [20].

Behavioral Tasks

OFT

The OFT was performed on the 7th day of the experimental protocol. The open field was a 40×45-cm arena surrounded by 50-cm-high plywood walls. The floor of the open field was divided into 9 equal squares (3×3). Each animal was placed at the center of the open field and observed for 4 min to record the locomotor (number of segments crossed with the four paws) and exploratory (number of rearings on the hind limbs) activities [34]. The arena floor was cleaned between experiments and the test was carried out in a temperature- and light-controlled room. The 4-min session of OFT was also useful to familiarize the rats with the arena, as a context habituation trial for the recognition memory task.

ORT

Twenty-four hours after OFT (on the 8th day of the experimental protocol), animals were trained in the same arena in order to perform a novel ORT as previously described [35]. ORT, a non-spatial memory task, is used as a measure to evaluate the short-term (STM) and long-term (LTM) memories. Four objects were used in this task: A_1, A_2, B, and C. The A_1 and A_2 objects were two identical balls, the B object was a cube and the C object was a square. Each object presented the following color pattern: blue, red, and yellow. All objects were made of plastic material, measuring 10×10 cm (length $\times$ height). On the day of the task, training was conducted by placing individual rats in the center of the arena, in which two identical objects (objects A_1 and A_2) were positioned in two adjacent corners, slightly away from the walls. Animals were allowed to explore the objects for 5 min. Exploration was defined as sniffing or touching the object with the nose and/or forepaws. Periods in which the rat moved around, climbed over, or sat on the objects were not recorded. The STM of rats was evaluated 1.5 h after training in the presence of a familiar object (A_1) and a novel object (B), and the total time spent in exploring each object was determined during 5 min as a measure of learning and recognition memory. After STM, the rats were housed in our boxes. The LTM was performed 24 h after training, on the 9th day of the experimental protocol, in which rats were placed to explore a familiar object (A_1) and a novel object (C) for 5 min and the total time spent in exploring each object was determined. To prevent olfactory cues, the objects and the field were cleaned with an ethanol/water solution after each trial. The results were expressed as exploratory preference (%) according to the following formula: [time spent on the novel object (time spent on the familiar object + time spent on the novel object)] × 100.

Ex Vivo Assays

On the 14th day of the experimental protocol, rats were anesthetized with isoflurane and blood samples were collected

Fig. 2 The experimental protocol. Aged rats were intragastrically (i.g.) treated for 7 days with 4-PSQ (5 mg/kg) or canola oil (1 ml/kg). Young adult animals received the canola oil (1 ml/kg). Open-field test (OFT) and object recognition test (ORT) were performed on the 7th, 8th, and 9th days, respectively. On the 14th day, the animals were euthanized by inhalation of isoflurane for ex vivo assays
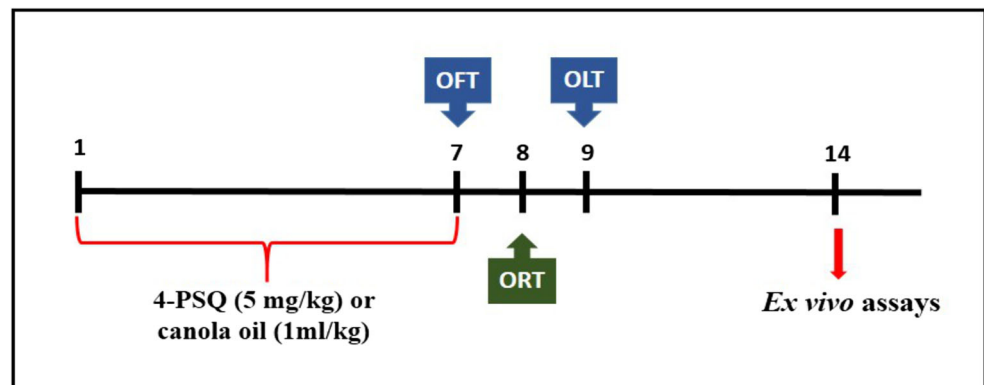
from the heart ventricle, using heparin as anticoagulant to obtain plasma. Plasma was obtained by centrifugation (900×g for 15 min) to determine the cholesterol levels.

Cerebral structures (cortex and hippocampus) were immediately removed and dissected. The cerebral cortex and hippocampus were separated and washed with cold saline solution (0.9%) to determine AChE activity and expression of NCAM, STX, and PST.

AChE Activity

Cerebral cortex and hippocampus samples were homogenized in 0.25 M sucrose buffer (1/10, weight/volume) and centrifuged at 900×g at 4 °C for 15 min. AChE activity was performed according to the method of Ellman et al. [36], using acetylthiocholine as substrate. Enzyme activity was measured by spectrophotometry at 412 nm and expressed as micromole of acetylcholine (ACh) per hour per milligram of protein. The protein concentration was measured according to the Bradford method [37].

Expression of NCAM, STX, and PST

RNA Extraction, cDNA Synthesis, and Quantitative Real-time Polymerase Chain Reaction

Total mRNA was extracted in the hippocampus and cerebral cortex using TRIzol reagent (Invitrogen™, Carlsbad, USA) followed by DNase treatment with DNase I Amplification Grade (Invitrogen™, Carlsbad, USA) in order to ensure minimum DNA contamination of the samples. The total RNA isolated was quantified and its purity (260/280 and 260/230 ratios) was examined by the spectrophotometer NanoVue (GE, Fairfield, CT, USA). The cDNA synthesis was performed using a High Capacity cDNA Reverse Transcription Kit (AppliedBiosystems™, UK) according to the manufacturer's protocol. For reverse transcription, 1 μg of total RNA was used in a reaction volume of 20 μl. The amplification was performed with GoTaq® qPCR Master Mix (Promega, Madison, WI) using the Agilent Mx3005P QPCR System (Agilent Technologies Inc., Santa Clara, CA) and the sequence of primers used is indicated in Table 2. The qPCR conditions were as follows: 10 min at 95 °C to activate the hot-start Taq polymerase, followed by 35 cycles of denaturation for 15 s at 95 °C, primer annealing for 60 s at 60 °C, and extension for 30 s at 72 °C (fluorescence signals were detected at the end of every cycle). Baseline and threshold values were automatically set by the Stratagene MxPro software. The number of PCR cycles required to reach the fluorescence threshold in each sample was defined as the cycle threshold (Ct) value, and each sample was analyzed in duplicate to obtain an average Ct for each sample. The $2^{-\Delta\Delta CT}$ method was used to normalize the fold change in gene expressions, using the beta-actin (ACTB) as housekeeping gene.

Table 2 Primers used for quantitative real-time polymerase chain reaction

Primer name	Sequence
NCAM forward	GGAGTTCCCATCCTCAAATACAA
NCAM reverse	TACCACTTGGAATGCCATGCT
STX forward	CAACTCAGGAGTCTTGCTCAACA
STX reverse	CCGGGCATACTCCTGAACTG
PST forward	CAGGACTGAGGAGCACCAAGA
PST reverse	TGTGTCATATCCTGAACATGCATTT
ACTB forward	AAGTCCCTCACCCTCCCAAAAG
ACTB reverse	AAGCAATGCTGTCACCTTCCC

The forward and reverse primer sequences used to amplify each target gene as well as the ACTB endogenous control are listed

Cholesterol Levels

The cholesterol levels were determined in the plasma of rats using an automated kinetic colorimetric method by Cobas Integra 400 plus. Cholesterol esters are cleaved by the action of cholesterol esterase and produce free cholesterol and fatty acids. Cholesterol oxidase catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen. In the presence of peroxidase, the hydrogen peroxide formed affects the oxidative coupling of phenol and 4-aminoantipyrine, forming a quinone-imine red dye. The color intensity of the dye formed is directly proportional to the concentration of cholesterol. It is determined by measuring the increase in absorbance at 512 nm.

Statistical Analysis

Data are expressed as mean ± standard error of the mean (S.E.M.). Data were analyzed by Graphpad Prism® 5; the normality of data was evaluated by the D'Agostino and Pearson omnibus normality test. Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by the Newman-Keuls test when appropriate. Values of $p < 0.05$ were considered statistically significant. The Pearson correlation coefficient was used for correlation analysis.

Results

OFT

The number of rearings and crossings in the OFT is presented in Fig. 3a and b, respectively. The one-way ANOVA revealed a significant difference in the number of rearings ($F_{(2,27)} = 10.09$, $p < 0.001$) and crossings ($F_{(2,27)} = 30.40$, $p < 0.0001$) of rats. Aged rats had a reduction in the number of rearings
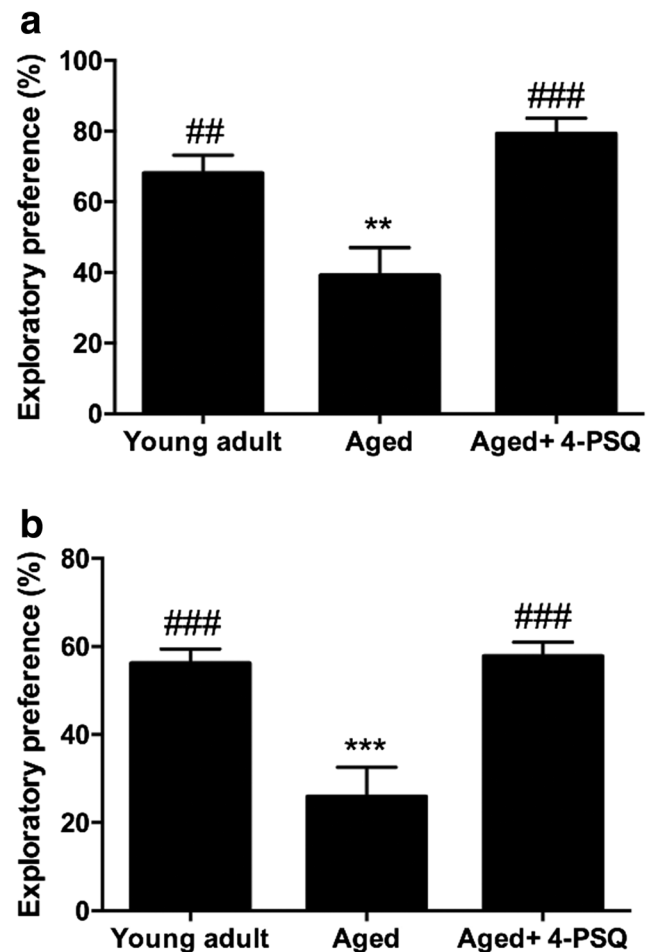
a

b
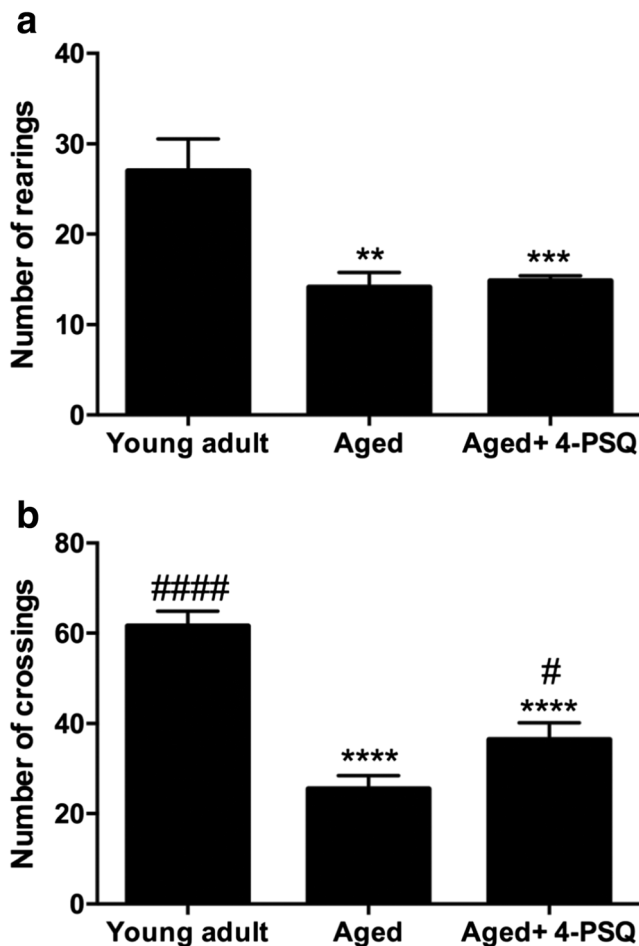
Fig. 3 Effect of 4-PSQ treatment on the exploratory (**a**) and locomotor (**b**) behaviors of rats in the open-field test (OFT). Values are expressed as mean ± standard error of the mean ($n = 10$ rats/group). **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ as compared with the young adult group. #$p < 0.05$, ####$p < 0.0001$ as compared with the aged group (one-way analysis of variance/Newman-Keuls test)

a

b

Fig. 4 Effect of 4-PSQ treatment on short-term (**a**) and long-term (**b**) memories in the object recognition test (ORT). Values are expressed as mean ± standard error of the mean ($n = 10$ rats/group). **$p < 0.01$, ***$p < 0.001$ as compared with the young adult group. ##$p < 0.01$, ###$p < 0.001$ as compared with the aged group (one-way analysis of variance/Newman-Keuls test)

(around 58%) (Fig. 3a) and crossings (around 48%) (Fig. 3b), when compared with the young adult rats. Treatment with 4-PSQ was not effective in reversing the number of rearings (Fig. 3a), but it partially restored the number of crossings (Fig. 3b).

ORT

There was no significant difference among groups in the percentage of exploratory preference during the familiarization phase ($F_{(2,27)} = 1.576$, $p > 0.05$) (data not shown).

In STM, the one-way ANOVA revealed a significant effect on the percentage of exploratory preference for a novel object in rats ($F_{(2,27)} = 11.61$, $p < 0.001$). Aged rats had a reduction (around 43%) in the exploratory preference for the new object, when compared with young adult rats (Fig. 4a). The treatment of aged rats with 4-PSQ reversed this memory parameter in aged rats, reaching the levels of young adult rats (Fig. 4a).

In LTM, the one-way ANOVA had a significant effect on the exploratory preference percentage for a novel object in rats ($F_{(2,27)} = 14.61$, $p < 0.0001$). Statistical analysis showed that the exploratory preference was reduced (around 54%) in the aged rats, when compared with the young adult rats (Fig. 4b). 4-PSQ treatment enhanced the percentage of preference for a novel object in aged rats, reaching values similar to those of young adult rats (Fig. 4b).
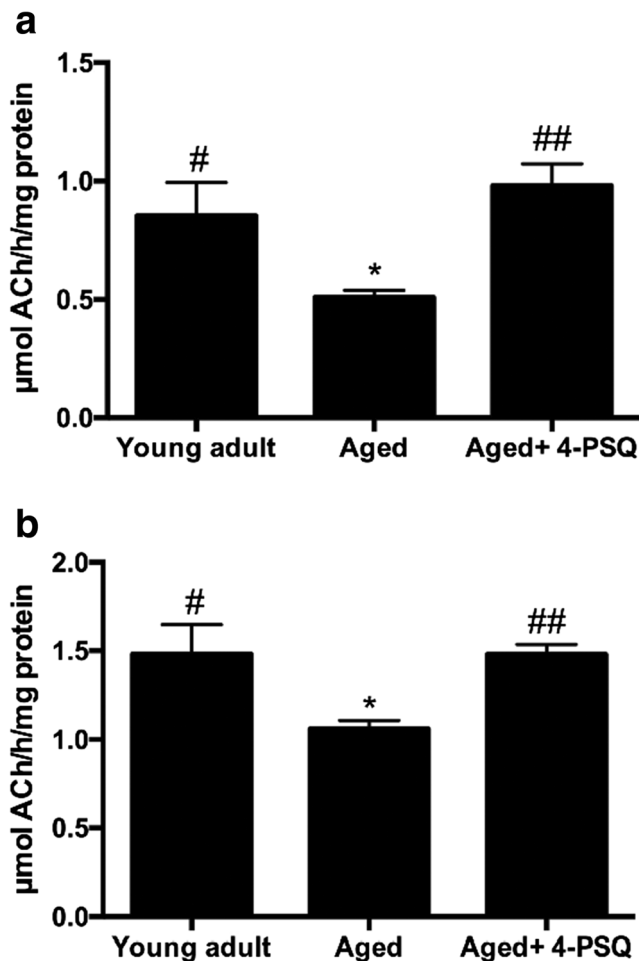
AChE Activity

The one-way ANOVA showed a significant difference in the AChE activity in the rat cerebral cortex ($F_{(2,27)} = 6.102$, $p < 0.01$) and hippocampus ($F_{(2,27)} = 5.371$, $p < 0.05$). The enzymatic activity was reduced in both brain structures of aged rats (around 40% for the cerebral cortex and 28% for the hippocampus), when compared with the young adult rats (Fig. 5a and b, respectively). Treatment with 4-PSQ was

effective to reverse the inhibition on the AChE activity in the cerebral cortex (Fig. 5a) and hippocampus (Fig. 5b) of aged rats.

The correlation analysis (Pearson's correlation coefficient) revealed a positive correlation between the AChE activity in both cerebral structures and STM ($r = 0.533$; $p < 0.05$ for the cerebral cortex and $r = 0.527$; $p < 0.05$ for the hippocampus). Moreover, the correlation analysis (Pearson's correlation coefficient) demonstrated a positive correlation between the AChE activity in both cerebral structures and LTM ($r = 0.478$; $p < 0.05$ for the cerebral cortex and $r = 0.527$; $p < 0.05$ for the hippocampus).

Expression of NCAM, STX, and PST

The one-way ANOVA of NCAM expression revealed significant changes in the cerebral cortex ($F_{(2,18)} = 5.256$, $p < 0.05$)
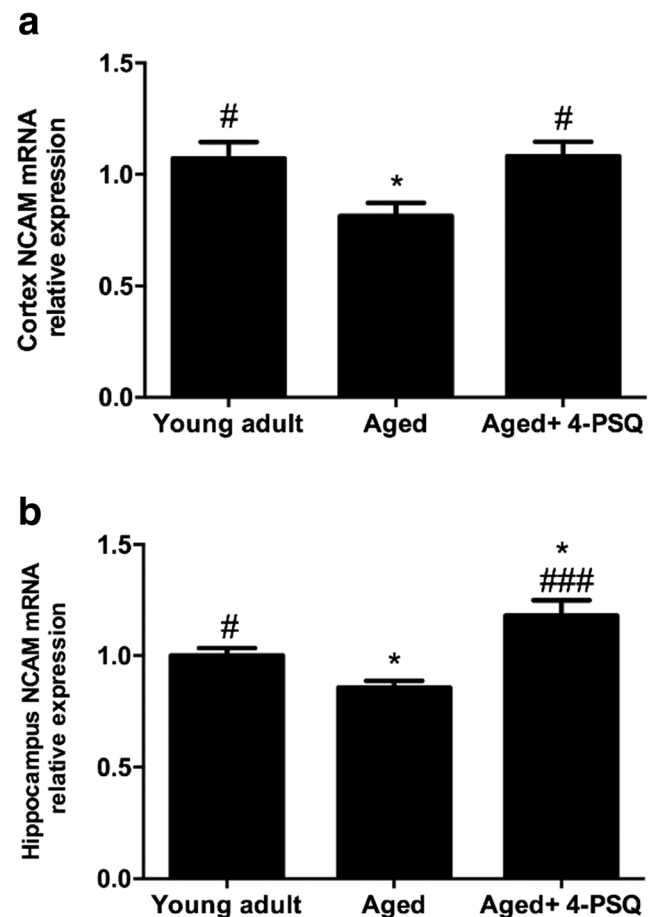
and hippocampus ($F_{(2,18)} = 11.26$, $p < 0.001$) of rats. Aged rats had a reduction in cerebral cortex and hippocampus NCAM expression (around 24 and 14%, respectively) compared with young adult rats (Fig. 6a and b, respectively). Treatment of aged rats with 4-PSQ reversed the reduction of NCAM expression in both cerebral structures (Fig. 6a and b).

The one-way ANOVA of PST expression showed a significant difference in the cerebral cortex ($F_{(2,18)} = 11.25$, $p < 0.001$) and hippocampus ($F_{(2,18)} = 5.554$, $p < 0.05$) of rats. PST expression was reduced in both cerebral structures (around 32% for the cerebral cortex and 54% for the hippocampus) of aged rats, when compared with the young adult rats (Fig. 7a and b). 4-PSQ treatment was effective to restore the reduction of the PST expression in the cerebral cortex (Fig. 7a) and hippocampus (Fig. 7b) of aged rats.

The one-way ANOVA demonstrated changes in STX expression in the cerebral cortex ($F_{(2,18)} = 6.000$, $p < 0.05$), but there was no alteration in the hippocampus ($F_{(2,18)} = 0.6449$, $p > 0.05$) of rats. STX expression did not change in the

Fig. 5 Effect of 4-PSQ treatment on the acetylcholinesterase (AChE) activity in the cerebral cortex (**a**) and hippocampus (**b**) of rats. Values are expressed as mean ± standard error of the mean ($n = 10$ rats/group). *$p < 0.05$ as compared with the young adult group. #$p < 0.05$, ##$p < 0.01$ as compared with the aged group (one-way analysis of variance/Newman-Keuls test)

Fig. 6 Effect of 4-PSQ treatment on the neural cell adhesion molecule (NCAM) levels in the cerebral cortex (**a**) and hippocampus (**b**) of rats. Values are expressed as mean ± standard error of the mean ($n = 7$ rats/group). *$p < 0.05$ as compared with the young adult group. #$p < 0.05$, ###$p < 0.001$ as compared with the aged group (one-way analysis of variance/Newman-Keuls test)
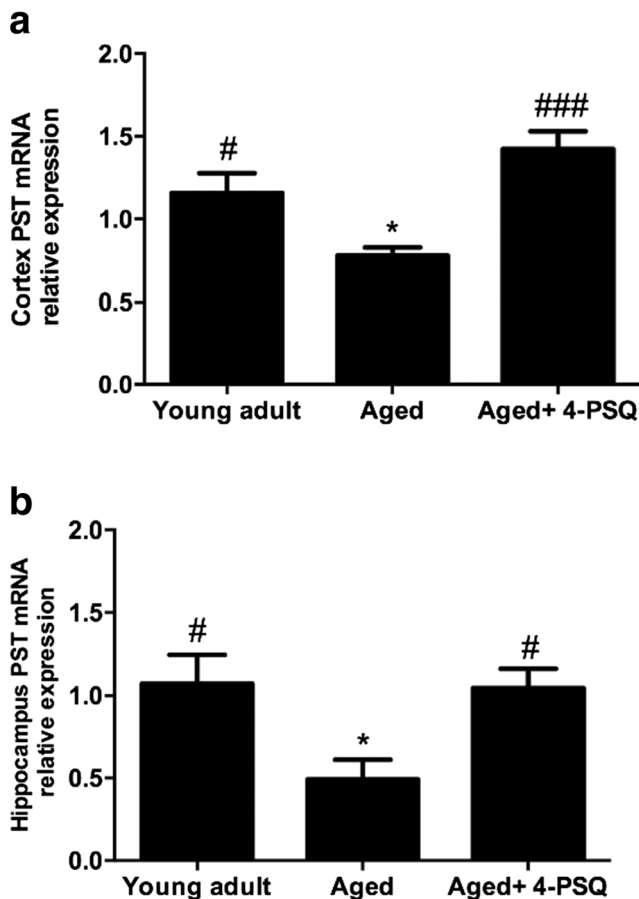
Fig. 7 Effect of 4-PSQ treatment on the polysialyltransferase PST levels in the cerebral cortex (**a**) and hippocampus (**b**) of rats. Values are expressed as mean ± standard error of the mean ($n = 7$ rats/group). $*p < 0.05$ as compared with the young adult group. $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ as compared with the aged group (one-way analysis of variance/Newman-Keuls test)

Fig. 8 Effect of 4-PSQ treatment on the polysialyltransferase STX levels in the cerebral cortex (**a**) and hippocampus (**b**) of rats. Values are expressed as mean ± standard error of the mean ($n = 7$ rats/group). $*p < 0.05$ as compared with the young adult group. $^{\#\#}p < 0.01$ as compared with the aged group (one-way analysis of variance/Newman-Keuls test)
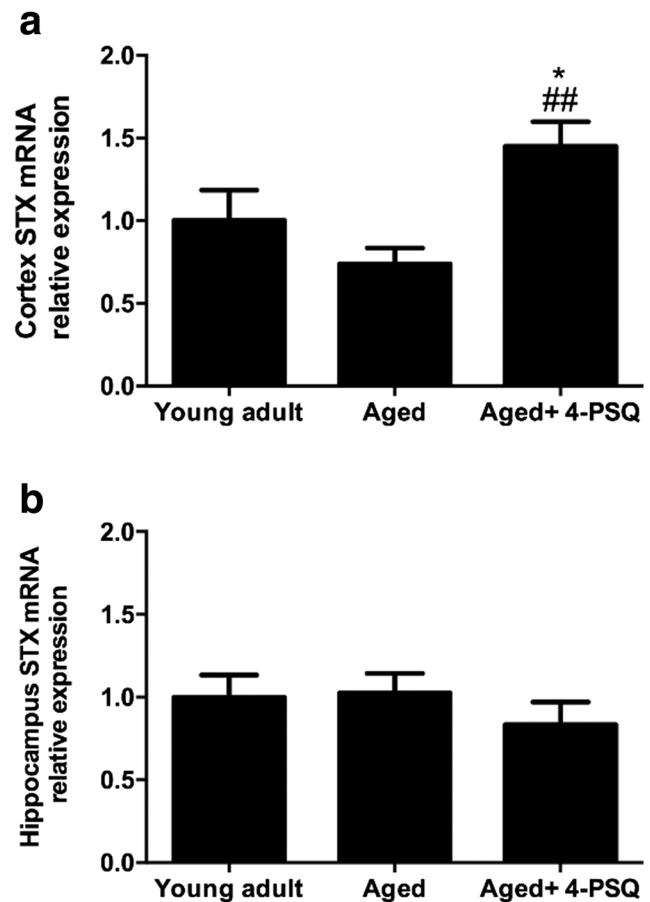
cerebral cortex and hippocampus of aged rats compared with young adult rats (Fig. 8a and b, respectively). The treatment of aged rats with 4-PSQ increased (around 45%) the STX expression in the cerebral cortex, when compared with aged and young adult rats (Fig. 8a), but there was no alteration in the hippocampus (Fig. 8b).

Cholesterol Levels

The one-way ANOVA revealed a significant difference in cholesterol levels in the plasma of rats ($F_{(2,18)} = 6.249$, $p < 0.01$). Cholesterol levels increased (around 54%) in the plasma of aged rats and treatment with 4-PSQ was effective to restore this biochemical marker (Fig. 9).

Discussion

In the present study, we provided evidence for the effect of 4-PSQ on the age-related cognitive impairment in rats. These

results are strongly related to the improvement of synaptic plasticity, cholinergic system, and cholesterol levels of aged rats. Moreover, this study also demonstrated the influence of age on locomotor and exploratory activities.

Aging is associated with a decline in cognitive function, including learning and memory [38]. In the current study, we analyzed recognition memory by ORT, a non-spatial memory type based on the natural tendency of rodents to explore novel objects, which mainly relies on the function of the perirhinal cortex and hippocampus [39]. Our results demonstrated that aged rats had an age-related deficit in STM and LTM, as evidenced in ORT.

The most important finding of this study was that treatment with 4-PSQ restored cognitive deficits impaired by age in rats, enhancing STM, LTM, and spatial memory, since it caused an increase of the exploratory preference by the novel object and by location of the object in aging rats. The compound investigated in this study, 4-PSQ, is a quinoline derivative with an organoselenium substituent. Indeed, quinoline compounds have been widely studied and have presented promising
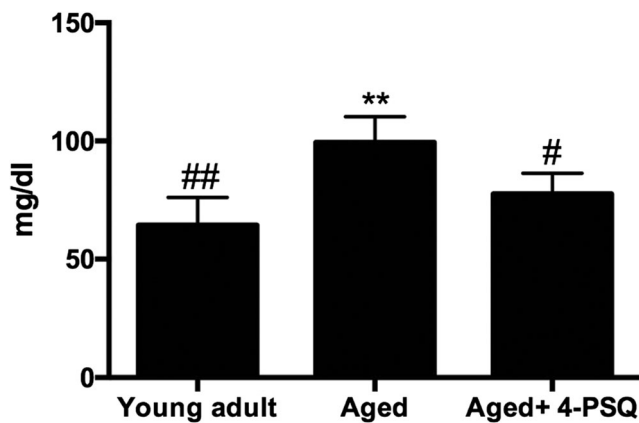
Fig. 9 Effect of 4-PSQ treatment on the plasma cholesterol levels of rats. Values are expressed as mean ± standard error of the mean ($n = 7$ rats/group). $**p < 0.01$ as compared with the young adult group. $^#p < 0.05$, $^{##}p < 0.01$ as compared with the aged group (one-way analysis of variance/Newman-Keuls test)

biological activities, being found in pharmacologically active natural products, and extensively used in clinical practice [40]. In addition, it is known that the selenium status decreases with age and may contribute to impairment of neuropsychological functions [41], such as cognitive functions [17]. Thus, incorporating selenium into organic molecules is a good alternative to supplement with this element. In this context, studies have demonstrated that organoselenium compounds are good alternatives to improve the cognitive deficit caused by aging in rats [42]. Therefore, 4-PSQ is a promising molecule, since it combines the properties of quinoline derivatives with organic selenium compounds. In fact, our research group has demonstrated a significant number of pharmacological properties of this compound, such as being antioxidant, antinociceptive, anti-inflammatory, and anxiolytic-like [24, 26, 28–30].

In addition, the possibility of a biological and/or clinical connection between movement, cognition, and emotion has been investigated in the aging process [43]. In the present study, crossing and rearing behaviors of aged rats in the OFT were reduced compared with young adult rats, indicating that advanced age could cause an impairment in the locomotor and exploratory activities of the animals. Indeed, studies have shown that aging causes a reduction in these behaviors [20, 42]. Treatment with 4-PSQ for 7 days did not protect against the impairment in the locomotor and exploratory activities caused by aging. In accordance, other studies have shown the failure in the treatment of locomotor and exploratory disorders in aged rats [20, 42].

Cognitive processes, mediated through the hippocampus and the prefrontal cortex, are affected by aging [3, 5]. In the present study, we measured the AChE activity in the cerebral cortex and hippocampus of aged rats in an attempt to relate cognitive functions to the cholinergic system. Here, a decline in the AChE activity in aged rats as compared with the young adult rats was observed in both regions of the brain. In fact, a

reduction on the AChE activity is an important marker of age-related cholinergic neurotransmission dysfunction. The central cholinergic system is constituted by neurotransmitter acetylcholine, which is involved in the regulation of cognitive functions [44]. In this way, studies have shown that a decrease of AChE activity by aging is due to acetylcholine content in the brain, since acetylcholine synthesis and release are diminished in aged animals [45]. Moreover, Richter et al. [46] in 2017 showed a negative correlation between older people and AChE activity.

Hence, the cholinergic system might be recommended as a cognitive dysfunction treatment strategy target [47]. Importantly, in the current study, treatment with 4-PSQ reversed the inhibition of AChE activity in both cerebral structures caused by aging. This finding suggests that an increase in AChE activity caused by 4-PSQ might be related to improvement obtained in behavioral tests and it is useful in the treatment of age-related memory impairments.

Evidences indicated that age-related cognitive decline is more likely associated with alterations in synaptic connectivity than with neuronal loss [3, 48]. In this sense, NCAMs have played a key role in neural development and have been implicated in synaptic plasticity and cognitive and emotional processes in adulthood [6, 7]. In the present investigation, our objective was verified whether 4-PSQ alleviates age-induced alterations in NCAMs. The aging process significantly decreases NCAM mRNA expression in the cerebral cortex and hippocampus of rats. These results are in accordance with those obtained in the behavioral tests, given that the memory of aged rats was impaired. Indeed, the literature suggests that NCAMs are indispensable for the formation and consolidation of learning and memory [7]. Treatment with 4-PSQ significantly enhanced NCAM levels in the cerebral cortex and hippocampus of aged rats, suggesting that this compound could reverse the inhibition of the transcription and translation of NCAMs induced by aging. Few studies have demonstrated the effect of organoselenium compounds on the expression of NCAMs. Wang et al. [49] showed that an organoselenium compound, selenomethionine, reversed the changes of NCAMs induced by lead.

In addition, aging reduced the mRNA levels of PST in the hippocampus and cerebral cortex of rats as compared with the young adult rats; however, no change was found in the mRNA levels of STX in both cerebral structures. PST (or ST8SiaIV) and STX (or ST8SiaII) are polysialyltransferases responsible for synthetizing α-2,8-polysialic acid and adding it to the extracellular domains of NCAM [50, 51]. During embryonic development and the early postnatal period, NCAM is heavily polysialylated (PSA-NCAM) and STX is dominant in these periods [52]. In adulthood, PSA-NCAM is restricted to specific regions of the brain, such as the hippocampus and cerebral cortex, which have roles in synaptic plasticity and general cell migration, PST being the main one associated with α-2,8-polysialylation in the postnatal brain [52, 53]. Our results suggested that aging downregulated PST mRNA in the

hippocampus and cerebral cortex of rats, this being mainly found in the old ones, reducing polysialylation of NCAM, and decreasing synaptic plasticity in these cerebral regions. Importantly, treatment with 4-PSQ significantly enhanced PST levels in both cerebral structures of aged rats, and it upregulated STX levels in the cerebral cortex. These results indicated that 4-PSQ restored polysialyltransferases, mainly PST, increasing polysialylation of NCAM, restoring synaptic plasticity in cerebral structures of aged rats, improving the cognitive functions in the behavioral tasks.

In an attempt to explain the other mechanisms involved in 4-PSQ effects in the aged rats, cholesterol was measured since it plays an important role in maintaining the function of the central nervous system. In the present study, aged rats had an increase in the plasma cholesterol levels, when compared with young adult rats. Indeed, high cholesterol levels have detrimental effects on cognitive performance in animal models [54]. Additionally, there is an association between Alzheimer's disease and cholesterol levels, potentially by increasing the neuronal content of cholesterol, promoting cleavage of the amyloid protein precursor, and an impaired working memory [55], affecting directly the neurodegeneration [56]. Several studies on humans investigated a relation between plasma lipids and dementia, showing that elderly people with neurodegenerative disease, such as Alzheimer's disease, or dementia, and cognitive deficits have higher plasma total cholesterol [57]. Our results verified that 4-PSQ was able to restore the cholesterol levels in aged rats. Indeed, clinical studies suggested that statins might reduce the risk and progression of cognitive deficit in Alzheimer's disease [58]. Therefore, the hypocholesterolemic effect of 4-PSQ is beneficial to restore the cognitive impairment caused by aging.

Conclusions

In conclusion, our findings demonstrated that 4-PSQ restored the cognitive impairment caused by aging in rats. In addition, the compound modulated the synaptic plasticity, cholinergic system, and cholesterol levels of aged rats. Thus, these results strongly contribute to the research of a novel therapeutic agent for geriatric memory deficit.

Compliance with Ethical Standards

Animal care and all experimental procedures were conducted in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publications no. 80-23, revised in 1996) [33] and in accordance with the Committee on Care and Use of Experimental Animal Resources, Federal University of Pelotas, Brazil (CEEA 4599-2016). All efforts were made to minimize the number of animals used and their suffering.

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