Neuromodulatory Mechanisms of a Memory Loss Preventive Effect of Alpha-Lipoic Acid in an Experimental Rat Model of Dementia

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Research Article

Keywords: Alzheimer's type dementia, lipoic acid, neurodegeneration, acetylcholine esterase, monoamine, memory loss

Posted Date: December 29th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1201814/v1

Version of Record: A version of this preprint was published at Journal of Molecular Neuroscience on February 16th, 2022. See the published version at https://doi.org/10.1007/s12031-022-01979-y.

Abstract

This study evaluates some of the neuromodulatory mechanisms of the memory loss preventive effect of alpha-lipoic acid (ALA) in a scopolamine (Sco)-induced rat model of an Alzheimer's disease (AD) type dementia. Our results confirmed that Sco administration induces significant memory impairment, worsens exploratory behaviour and habituation; it increases acetylcholinesterase (AChE) activity and induces pathological monoamine content changes in the brain prefrontal cortex and hippocampus. ALA administration prevented to a large extent Sco-induced memory impairment; it also improved exploratory behaviour and preserved habituation; it decreased AChE activity, reversing it to Control group levels and corrected aberrant monoamine levels in the brain prefrontal cortex and hippocampus. According to the data available, this is the first time that ALA-induced changes in AChE and monoamine levels in the brain prefrontal cortex and hippocampus (brain structures related to learning and memory) have been demonstrated in a Sco-induced rat model of AD type dementia.

Introduction

Dementia is a healthcare burden of epidemic proportion and one of the leading causes of death in the elderly. Alzheimer's disease (AD) is the most common form of dementia, currently ranked as the third leading cause of death, trailing only cardiovascular disease and neoplasia (B. D. James et al. 2014). AD is associated with a decline in cognitive functions, thinking, remembering, reasoning, and behavioural abilities (Huang et al. 2016; Ray et al. 2012). One of its major neuropathological hallmarks is the deposition of Amyloid beta (Aβ)-peptide-containing senile plaques, and intracellular neurofibrillary tangles in hippocampal and cerebrocortical brain regions (González-Reyes et al. 2017). Altered calcium homeostasis, increased brain oxidative stress (OS), neuroinflammation and mitochondrial dysfunction contribute to the ongoing pathology (Mancuso et al. 2006). These alterations lead to a progressive reduction in the number of cholinergic neurons and the levels of the neurotransmitter acetylcholine (ACh) (Nordberg et al. 1986, 1987) – important correlates of AD severity. Pathological changes can also be seen in monoaminergic neurotransmission (D'Amelio and Rossini 2012; Roy et al. 2016; Scheff et al. 2006; Burns et al. 2005), having a negative effect on working memory, memory consolidation, and memory retrieval (Sara 2009; Chudasama and Robbins 2004; Sara 2017; Schicknick et al. 2019). Most of the current AD drug therapy aims to increase brain ACh levels, mainly through the use of different AChE inhibitors.

Alpha-lipoic acid (ALA) is a naturally occurring molecule with neuroprotective, anti-inflamatory, metal-chelating and strong antioxidant properties. Data also show that it exerts protective effects on mitochondrial function (Miquel 2002). Its reduced form (dihydrolipoic acid) is responsible for many of its beneficial pharmacological effects. Namely, acting as a metal chelator, reducing reactive oxygen species (ROS) production (Ferreira et al. 2009) and regenerating other low-molecular-weight antioxidants such as glutathione (GSH), coenzyme Q10, and vitamins A and C (Bilska et al. 2007). Its anti-inflamatory effect is realized via a direct effect on gene expression or an indirect effect on several cAMP-dependent signal transduction pathways (Bozhokina et al. 2015; Fiedler et al. 2018; Meng et al. 2018; Dinicola et al. 2017).

In our previous research (Tzvetanova et al. 2018), we demonstrated an antioxidant and memory protective effect of ALA in a scopolamine (Sco)-induced rat model of dementia (Tzvetanova et al. 2018). By acting as an antioxidant, and due to its neuromodulatory activity described below, ALA can interfere with and remediate dementia pathogenesis pathways, for example those in AD. There are data demonstrating that patients with mild AD, who have been treated with ALA, show a slower progression of cognitive impairment (Molz and Schröder 2017; Heneka and O'Banion 2007; Fu et al. 2014). An ability of ALA to affect cholinergic neurotransmission related cognitive function by increasing ACh production has also been reported (Fava et al. 2013).

The neuromodulatory effect of ALA on brain monoaminergic neurotransmission in neuropathological conditions has not been well-studied. Data regarding its ability to affect acetylcholinesterase (AChE) – a fundamental component of cholinergic neurotransmission – is also scarce. It has been shown that ALA treatment restores increased AChE activity in aluminum- induced AD rat model (Ahmed 2012). The ability of ALA to increase brain levels of serotonin (Sero), dopamine (DA) and noradrenaline (NA) was demonstrated in an Aβ vaccine-induced AD model in mice (Jesudason et al. 2005).

With regard to the above-mentioned, this work aims to evaluate some of the complex neuromodulatory mechanisms of the memory loss preventive effect of ALA in an experimental Sco-induced rat model of an AD type dementia.

Materials And Methods

Animals

Adult male Wistar rats weighing 160–180 g were housed 3 per cage, on a 12h/12 h light/dark cycle, with ambient temperature measuring 25 ± 3 °C. Food and water were accessible ad libitum. Before commencing the experiment, the rats were given a 5-day habituation period.

Sco-induced Rat Model of an AD Type Dementia

A Sco-induced rat model of an AD type dementia was achieved by injecting Scopolamine hydrobromide 2 mg/kg, intraperitoneally (IP), for 11 consecutive days. The dose was selected based on our previous studies (Tsvetanova et al. 2020) as well as on literature data (Lee et al. 2012; Upadhyay 2020 et al.; Shivakumar 2014 et al.).

Experimental Design

After 5 days of habituation, the rats were randomly divided into the following 3 groups (numbering 12 per group) and treated for 11 consecutive days: Control group (treated with Saline 0.5 ml/100 g, IP), Sco group (treated with Sco 2 mg/kg, IP) and Sco+ALA group (treated with Sco 2 mg/kg, IP and ALA 30 mg/kg, IP). Sco was dissolved in distilled water ex tempore before each administration. ALA (Thiogamma® Turbo-Set 600 mg/50 ml solution for injection) was diluted with saline to be properly

administered. On the 24th hour and on the 12th day after the first administration, the rats were submitted to behavioural assessment (step-through passive avoidance test and hole-board test). Behavioural observation was conducted from 9 a.m. to 12 a.m. One hour after the last test the animals were euthanised by decapitation under mild CO2 inhalation. Two important brain structures related to memory (prefrontal cortex and hippocampus) were isolated for biochemical analysis.

Behavioural Assessment

Step-through Passive Avoidance Test. – A step-through passive avoidance test was used to evaluate potential changes in the rats' short- and long-term memory, by assessing for changes in reaction time (Jarvik and Kopp 1967). The apparatus used was composed of two chambers (a darkened and lit one) separated by a guillotine door. During the acquisition phase of the test, in the darkened chamber of the apparatus, the animals were exposed to the action of the aversive stimulus (a 0.1 mA electric shock lasting for 1 sec.). The initial latency (IL), in-between placing the animals in the lit chamber and their spontaneous entrance into the darkened one, was recorded. The animals were then tested 24 h and 12 days after the acquisition session by using the same paradigm, only without applying the foot-shock. The time to transition from the lit to the darkened chamber was recorded as step-through latency (STL). When a rat did not enter the dark chamber within 180 sec., the latency was recorded as 180 sec.

Hole-board Test. – The hole-board apparatus consisted of a platform (50 x 50 cm) with 16 symmetrically arranged round holes with a diameter of 3 cm. To evaluate changes in exploratory behaviour, the rats were placed onto the platform and the number of head dips into the holes counted for 3 min (Boissier et al. 1964). To evaluate changes in habituation, the rats were placed onto the platform and the number of head dips into the holes during 1st, 2nd and 3rd minutes were counted.

Biochemical assessment

AChE Activity. – AChE activity in rat brain structures was assayed based on Ellman's method (Ellman et al. 1961), in which the thiocholine produced by the action of AChE forms a yellow colour with 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB). 10% tissue homogenate, prepared in 0.1 M phosphate buffer (pH=8; 1000 rpm) was centrifuged at 4500 rpm for 10 min. 100 µl of supernatant was incubated with the Ellman reagent 0.01M DTNB, 0.1M phosphate buffer and 0.075M freshly prepared acetylthiocholine iodide. 500 µl of the reaction mixture was injected into a Semi-auto Chemistry Analyzer and reaction kinetics were monitored for 3 min at 405 nm. Values are expressed as (U/ml/mg protein). Protein content was measured by the method of Lowry et al. (1951).

Biogenic Amines Concentration Measurement. – The concentrations of biogenic amines – NA, DA and Sero, were determined by a fluorescence reaction in tissue homogenates (Jacobowitz and Richardson 1978). NA and DA were extracted into a 0.1 M phosphate buffer and Sero into 0,1 N HCl. For the NA and DA fluorescence reaction, ethylenediaminetetraacetic acid (EDTA), iodide solution, alkaline sulfite and 5N CH3COOH were added, whereas for the Sero fluorescence reaction, o-phthalaldehyde (OPT) was added. Fluorescence was determined at λ = 385/485 nm for NA; λ

= 320/385 nm for DA and λ = 360/470 nm for Sero, respectively. Monoamine fluorescence levels were then calculated based upon standard solution fluorescence and expressed as ng/g of fresh tissue.

Chemicals

The reagents for the biochemical assay were obtained from Sigma-Aldrich (Germany). Scopolamine hydrobromide was purchased from ACROS Organics and ALA from Solupharm GmbH & Co. KG (Germany) (as Thiogamma® Turbo-Set 600 mg/50 ml solution for injection). All other chemicals were of the highest commercially available purity.

Statistical Analysis

The results are expressed as mean ± SEM. Student's t-test was used for statistical analysis of unpaired data. A multiple-sample comparison was applied to test the differences between groups (one-way ANOVA and Dunnett's as the post hoc comparison test, two-way ANOVA and Tukey's multiple comparison test). Statistical analysis was performed using GraphPad Prism. $P<0.05$ was taken to indicate a statistically significant difference. Artwork was created by using the graphics program Graph Pad 8.

Results And Discussion

In the present study, we endeavoured to expand the available information regarding the complex mechanisms of action of ALA and its effects on cognitive function in a Sco-induced rat model of an AD type dementia. AD is a progressive disorder, mainly affecting the cholinergic neurons in the prefrontal cortex and hippocampus (Arendt 2012; Serrano-Pozo 2011). Thus, it was important to measure the effects of ALA on AChE activity and monoamine levels in these brain areas, pertaining to learning and memory.

As a competitive muscarinic receptor antagonist, Sco has been traditionally used in the field of neuropsychopharmacology as a means of inducing experimental models of dementia (Ebert and Kirch 1998; Flood and Cherkin 1986). It can induce a cognitive deficit, similar to the one observed in AD disease (Spinks and Wasiak 2011; El-Sherbiny et al. 2003; Goverdhan et al. 2012; Tzvetanova et al. 2018) and can thus serve as a useful tool for investigating learning and memory impairments. In this study, the aforementioned Sco-induced cognitive deficit was successfully demonstrated using 2 behavioural tests: step-through passive avoidance test and hole-board test.

By using the step-through passive avoidance test, alterations in learning and memory performance were evaluated by observing changes in STL. This test is a fear conditioning test that creates a conflict in the rat's mind – between the instinct to prefer the darkened chamber of the apparatus and the fear of the aversive stimulus (foot shock) they would receive there (Tzvetanova et al. 2018). STL was measured on the 24^{th} hour and 12 days after the first Sco administration. We found that 11 consecutive days of Sco administration in the Sco group affected both the short-term and long-term memory of the animals, manifesting as decreased STL. This decrease was as follows (**Fig. 1**) – on the 24th hour by 64% ($P <$

0.01, n= 6), and on the 12th day by 73% (P < 0.05, n=6) when compared with controls. In contrast, we found that multiple ALA administrations in the Sco+ALA group, in the span of 11 days, resulted in around 69% higher STL (P < 0.05, n=6) compared to the Sco group – demonstrating a beneficial effect on long-term memory. These results agree with our previous studies (Dragomanova et al., 2016; Tzvetanova et al., 2018), in which a correlation between the memory-improving effect of ALA and its strong antioxidant effect was demonstrated.

graphics program: Graph pad 8

The memory-improving effect of ALA demonstrated in the step-through passive avoidance test was also confirmed by the hole-board test. For a period of 3 minutes, between-group differences in the total number of head dips (an indicator for exploratory behaviour deterioration) and changes in habituation patterns were observed. We emphasise the significance of these habituation changes as a non-associative test for learning in animals placed in a nonfamiliar environment. The number of head dips was measured on the 12^{th} day after the first Sco administration (**Fig. 2**). Compared to the controls, 11 days of Sco administration in the Sco group resulted in a significant reduction of 91,6 % (P <0.001, n = 6) in the number of head dips – an indication for exploratory behaviour deterioration. The habituation pattern was also negatively affected. Furthermore, when placed in an unfamiliar environment, the animals did not change their response to the stimulus within the observed period of 3 min – a sign of memory impairment. In the Sco+ALA group, there was a small increase in the total number of head dips when compared to the Sco group. However, the habituation pattern was preserved, mimicking the Control group habituation pattern, and in contrast to the Sco group. These results indicate a beneficial effect of ALA on exploratory activity and memory function.

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It is a well-established fact that cognitive function is directly dependent on the normal physiology of the cholinergic system – in the prefrontal cortex, hippocampus and some parts of the striatum (Braak and Braak 1996). The loss of neurons in these brain areas is one of the main pathological features of AD, leading to a severe diminishment of synaptic cleft ACh levels (Bartus et al. 1982). One way to increase this concentration is by inhibiting the activity of AChE – an enzyme responsible for the hydrolysis of ACh (Ballard et al. 2005). The ability of ALA to increase the cholinergic activity in certain brain areas is also well-documented in the literature (Ahmed 2012). For example, it has been shown that ALA treatment restores increased AChE activity in an aluminum-induced rat model of AD (Ahmed 2012). However, until now, there were no available data demonstrating the effects of ALA on AChE activity in a Sco-induced rat model of an AD type dementia. In the present study (**Fig. 3**), Sco applied for 11 consecutive days in the Sco group disrupted brain cholinergic activity by increasing the levels of AChE in the brain prefrontal cortex by 25% (P < 0.05, n=6) and in the hippocampus by 40% (P < 0.001, n=6), compared to the controls. This increase was accompanied by memory impairment, as demonstrated by the behavioural assessment and in agreement with previous research (Zaki et al. 2014). Conversely, multiple ALA administrations in the Sco+ALA group yielded an AChE activity close to Control levels in both brain

structures. In the prefrontal cortex, ALA decreased AChE activity by by 20% ($P < 0.05$, n=6) and in the hippocampus by 44% ($P < 0.001$, n=6) compared to the Sco-treated group. According to the data available, this is the first demonstration of such effects in a Sco-induced rat model of an AD type dementia. These results suggest that the protective effect of ALA on memory could be partly due to AChE inhibition.

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However, pathological brain changes in AD do not only affect cholinergic neurotransmission. The noradrenergic, dopaminergic and serotonergic neurotransmission is also damaged, as described in literature data on animal models of AD (D'Amelio and Rossini 2012; Roy et al. 2016; Scheff et al. 2006; Burns et al. 2005). The data from this study show that 11 days of consecutive Sco administration in the Sco group results in changes in the brain levels of all above-mentioned neurotransmitters (**Fig. 4**). In the prefrontal cortex, DA levels were decreased by 51% ($P < 0.001$, n=6), NA levels were not significantly affected, and Sero levels were increased by 186% ($P < 0.001$, n=6) when compared with controls. In the hippocampus, the levels of DA and NA were decreased by 84% ($P < 0.01$ and $P < 0.001$ respectively, n=6) and those of Sero by 45%. In the Sco+ALA group, ALA administration decreased Sero levels in the prefrontal cortex by 86% ($P < 0.001$, n=6) and did not significantly change DA and NA levels when compared to the Sco group. In the hippocampus, the levels of DA, NA and Sero were increased by 367% ($P < 0.05$, n=6), 700% ($P < 0.001$, n=6) and 179% ($P < 0.01$, n=6), respectively, when compared with the Sco group. Given the effects of cerebral monoaminergic neurotransmission on working memory, memory consolidation and memory retrieval (Sara 2009; Sara 2017; Schicknick 2019) - the ability of ALA to induce changes in brain DA, NA and Sero levels can partly explain its beneficial effects on memory. According to the data available, this is the first time, that ALA-induced changes in brain monoamine levels have been found in a Sco-induced rat model of an AD type dementia.

Conclusion

We can conclude that the memory-improving effect of ALA is related to its neuromodulatory capacity to affect the cholinergic and monoaminergic systems in two brain areas related to memory – brain prefrontal cortex and hippocampus. According to the data available, this is the first time that ALA-induced changes in AChE and monoamine levels in these brain areas have been demonstrated in a Sco-induced rat model of an AD type dementia. Future research can further elaborate on these effects and their clinical implications and by thus doing – to contribute to their translation into clinical practice.

Declarations

Statements and Declarations

Ethics approval and consent to participate: The experiments have been performed strictly according to the national regulations and European Communities Council Directive (86/609/EEC) also "Principles of

laboratory animal care” (NIH publication No. 85-23) concerning the protection of animals used for scientific and experimental purposes. All efforts and study design was made with purpose to minimize number of the animals and their suffering.

Consent for publication: The authors declare their consent for publication

Data availability statement: Authors can confirm that all data generated or analyzed during this study are included in this published article

Availability of data and materials: The material used during the present study are available from the corresponding author on reasonable request.

Competing Interests: The authors have no relevant financial or non-financial interests to disclose.

Funding: This work is supported by the Bulgarian Ministry of Education and Science under the National Program for Research “Young Scientists and Postdoctoral Students”.

Acknowledgements

This work is supported by the Bulgarian Ministry of Education and Science under the National Program for Research “Young Scientists and Postdoctoral Students”.

Author Contributions: Conception and design [Lyubka Tancheva], [Maria Lazarova], and [Rumen Nikolov]. Material preparation, data collection and analysis were performed by [L. Tancheva], [M. Lazarova], [Hristian Staykov], [Yozljam Hassanova], [Miroslava Stefanova]. The first draft of the manuscript was written by [M. Lazarova] and all authors comment on previous version of the manuscript. All authors read and approved the final manuscript.

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Figures

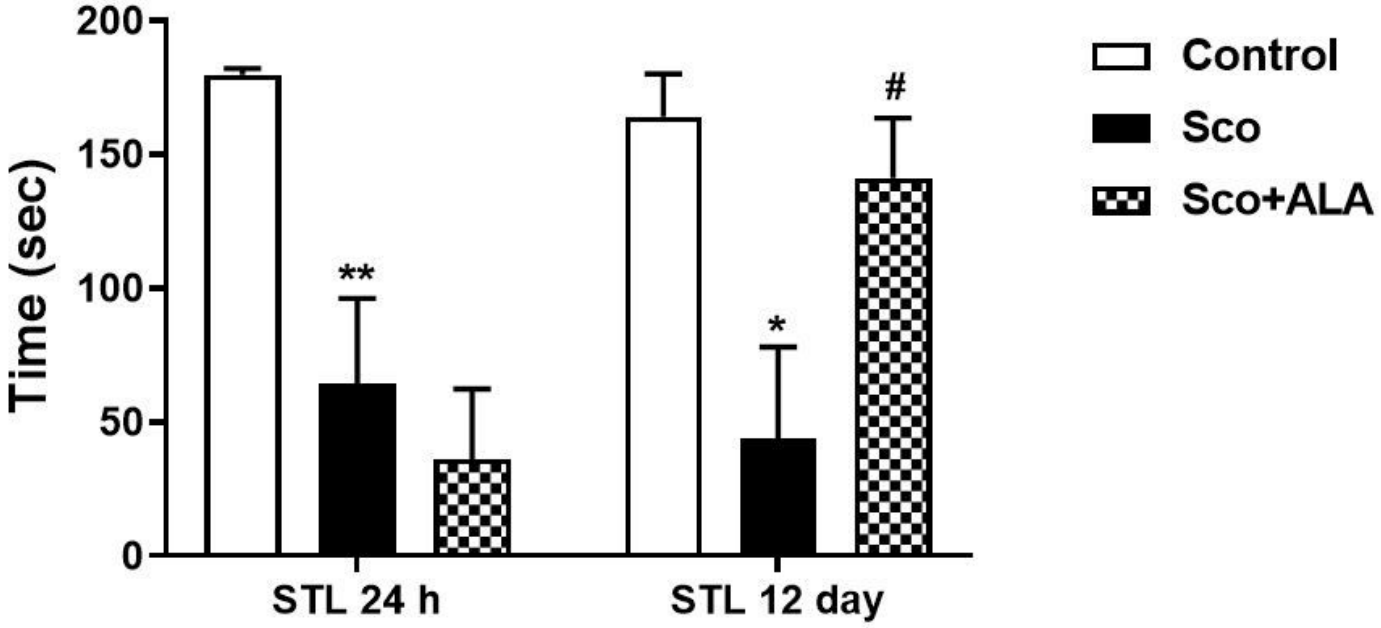

Figure 1

Effect of ALA on the rats' learning and memory performance in the Step-through Passive Avoidance Test. Results are expressed as mean ± SEM (n = 12 animals per group). Asterisk above bars indicates a significant difference: *P < 0.05, **P < 0.01. Hashtag above bars indicates a significant difference: $^{\#}$P < 0.05

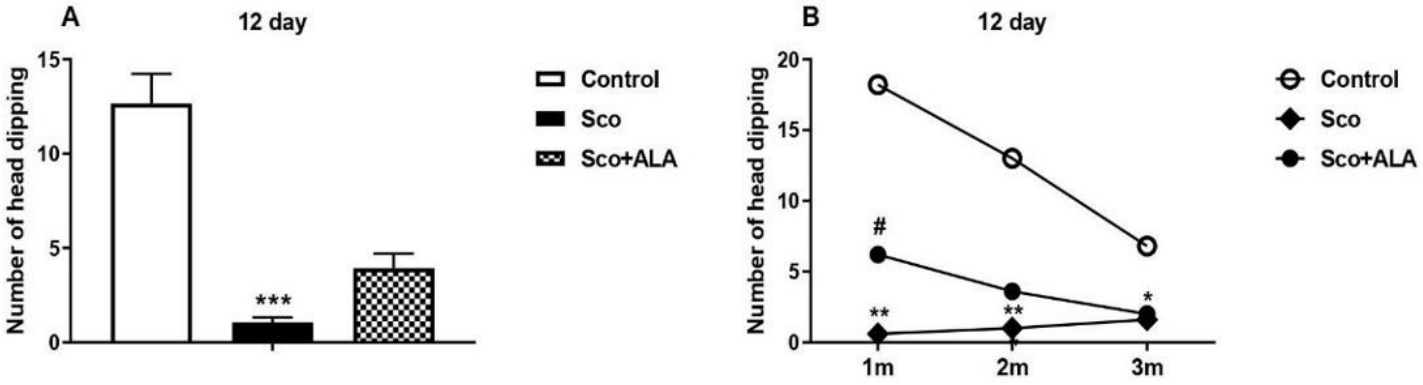

Figure 2

Effect of ALA on exploratory activity (A) and habituation (B) as measured by the Hole-board Test. Results are expressed as mean ± SEM (n = 12 animals per group). Asterisk above bars indicates a significant difference: *P < 0.05, **P < 0.01, ***P < 0.001. Hashtag above bars indicates a significant difference: $^{\#}$P < 0.05

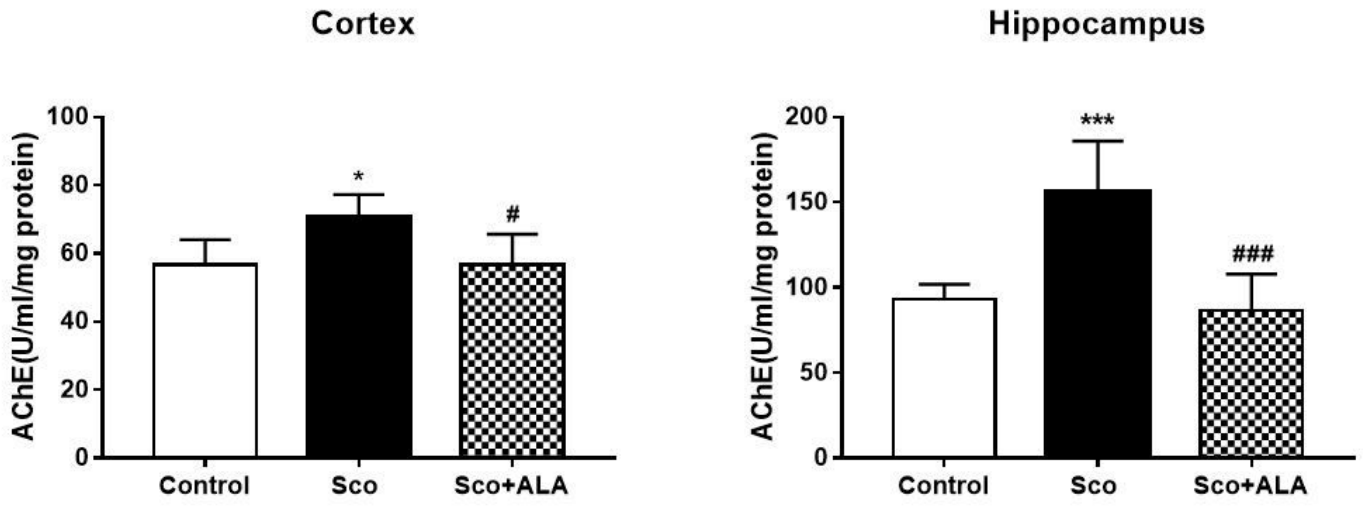

Figure 3

Effect of ALA on AChE activity in brain prefrontal cortex and hippocampus. Results are expressed as mean ± SEM (n = 12 animals per group). Asterisk above bars indicates a significant difference: *P < 0.05, ***P < 0.001. Hashtag above bars indicates a significant difference: $^{\#}$P < 0.05, $^{\#\#\#}$P < 0.001

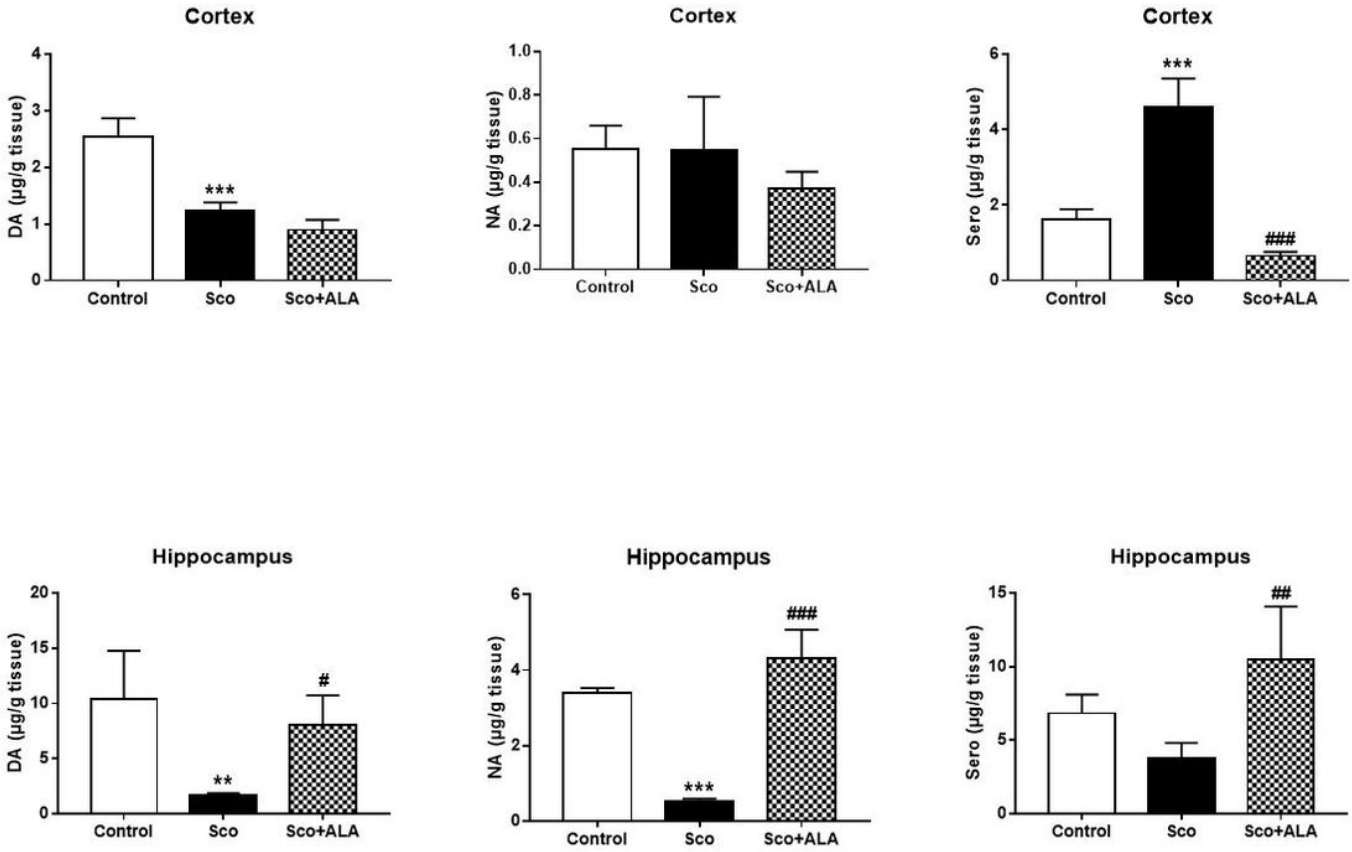

Figure 4

Effect of ALA on monoamine levels in the prefrontal cortex and hippocampus. Results are expressed as mean ± SEM (n = 12 animals per group). Asterisk above bars indicates a significant difference: **P < 0.01, ***P < 0.001. Hashtag above bars indicates a significant difference: $^{\#}$P < 0.05, $^{\#\#}$P < 0.01; $^{\#\#\#}$P < 0.001