Vitamin D3 Improves Spatial Memory and Modulates Cytokine Levels in Aged Rats

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Abstract

Background: Vitamin D deficiency is associated with an increased risk of dementia. An association between vitamin D deficiency and subjective cognitive complaints in geriatric patients has been reported.

Objective: This study aimed to evaluate the neurochemical and behavioral effects of vitamin D3 (1α-25 Dihydroxyvitamin D3) on 2-, 6-, 13-, 22-, and 31-month-old male Wistar rats.

Research Methods & Procedures: The animals were supplemented with vitamin D at doses of 42 IU/kg and 420 IU/kg for 21 days. The radial maze test was performed to evaluate spatial memory. After the behavioral test, the frontal cortex and hippocampus were dissected for the enzyme immunoassay analyses to measure cytokine levels (TNFα, IL-1β, IL-6, and IL-10).

Results: Our results showed that vitamin D supplementation was able to reverse the spatial memory impairment at the supplemented doses (42 and 420 IU/kg) in 6-, 13-, and 22-month-old animals and at a dose of 420 IU/kg in 31-month-old animals. Conclusion: Our results suggest vitamin D has a modulatory action on pro- and anti-inflammatory cytokines, since older animals showed increased cytokine levels than the 2-month-old animals. The lower dose (42 IU/kg) was able to regulate both pro- and anti-inflammatory cytokines in the hippocampus. Our results suggest that vitamin D may exert an immunomodulating effect on aging

1. Introduction

Human aging is a very complex and dynamic process that results from an environmental, genetic, epigenetic, and stochastic combinations. It is characterized by continuous remodeling and by a low-grade chronic inflammation, a phenomenon termed as *inflammaging*. (Davinelli et al., 2016; Franceschi et al., 2000; Franceschi and Campisi, 2014; Minciullo et al., 2016; Prattichizzo et al., 2016). The theory of *inflammaging*, one of the most recent on aging, is concentrated on the immune response, and takes into account the activation of chronic low-grade inflammation that occurs with aging (Davinelli et al., 2016; Minciullo et al., 2016; Prattichizzo et al., 2016).

Inflammaging is a significant risk factor for morbidity and mortality in the elderly, and it is a risk factor for most age-related diseases that share an inflammatory pathogenesis (Franceschi et al., 2000; Franceschi and Campisi, 2014). Several studies suggest that communication between the brain and the immune system is crucial to maintaining central nervous system (CNS) homeostasis. One of the most recognized effects of brain aging is the deregulation of the immune system as a result of the production of reactive oxygen species (ROS) and pro-inflammatory cytokines (Davinelli et al., 2016; Esiri, 2007).

The acute neuroinflammation is beneficial in the CNS after injury or infection by, ensuring homeostasis however, it is known that chronic neuroinflammation is harmful, in part, due to the generation of ROS (Godbout et al., 2005; Taylor et al., 2013). Pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin 1β (IL-1β), will induce elicit an adaptative immune response toon astrocytes. Monocytes chemotactic protein-1(MCP-1 / CCL2) acts in the recruitment of additional immune cells. Interleukin-6 (IL-6) plays a central role in immune responses, hematopoiesis, and acute phase reactions, as well as in neurogenesis and maturation of neuronal and glial cells under normal conditions (Heidary et al., 2014; Hubackova et al., 2012; Simpson et al., 1997).

Epidemiological studies have shown that up to one-third of adults have insufficient levels of vitamin D (Briones and Darwish, 2012; Nossov et al., 2014), and there is a strong association between low vitamin D concentration and depression (Collin et al., 2016). In addition, vitamin D has been shown to be involved in processes associated with neurogenesis and have well-known roles in calcium metabolism, bone health, the immune system (Dougherty et al., 2016; Pang et al., 2016). It has also been demonstrated an association between low concentrations of vitamin D and impairment in of cognitive functions, such as executive function, memory, and orientation, as well as with diagnosis of dementia and Alzheimer's disease (Annweiler et al., 2009; Llewellyn et al., 2009).

The level of vitamin D plays an immunomodulatory role in the CNS (Al-Harbi et al., 2017; Casaccia-Bonnefil et al., 2008). Clinical studies have been demonstrateding an association between low vitamin D levels and episodic memory disorders. It has also been shown that higher vitamin D higher levels are associated with better cognitive performance, when compared subjects with severe vitamin D deficiency to normal subjects (Soni et al., 2012). Vitamin D deficiency is also associated with an increased risk of dementia, and subjective cognitive complaints in geriatric patients (Annweiler et al., 2013; Littlejohns et al., 2014; Obermann et al., 2013; Soni et al., 2012; Tot Babberich Ede et al., 2015).

We hypothesized that vitamin D could protect against memory impairment and neuroinflammation induced by aging. Thus, the present study aimed to evaluate the effect of two doses of vitamin D on the spatial memory and cytokine levels of adult and old Wistar rats.

2. Methods

2.1 Animals

Male Wistar rats at 2-, 6-, 13-, 22-, and 31-month-old, weighing approximately 250 to 800g, were used. The 2- and 6-month-old rats were housed 5 animals per cage and 13-, 22-, and 31-month-old rats were housed 1 or 2 per cage with food and water *ad libitum* and a light/dark cycle of 12 hours (06:00 to 18:00) and temperature within 23 ± 1º C. The local ethics committee (Ethics Committee on Animal Use – CEUA of the Universidade do Extremo Sul Catarinense) approved this study (protocol 087/2016-1) supplementary material 1.

2.2 Drugs

The drug used was: vitamin D3. It was obtained from Farmasa S/A – Pharmacotherapy American Laboratory – São Paulo – Brazil (Adera D3®-colicalciferol – 3,300 IU/ml). It was diluted in water every 3 days at doses of 42 or 420IU/kg at a volume of 1 mL/kg body weight. The doses of vitamin D3 as well as the time points were chosen based on previous studies (Briones and Darwish, 2012). Appropriate vehicle-treated group were also treated simultaneously. The control group was treated with water at a volume of 1 mL/kg body weight.

2.3 Experimental design

The animals were treated with vitamin D3 at doses of 42 or 420IU/kg for 21 days by gavage. The control group was treated with vehicle (water). The gavages were scheduled every day at 10:00 a.m.. The animals underwent behavioral tests on the 18th until 22nd. At the end of the behavioral test on the last day (22nd day), the animals underwent euthanasia. The frontal cortex and hippocampus were dissected for the biochemical analyses. The rats were randomly divided with a completely randomized design into three groups as follows (initially 13 rats in each group) for every age (2, 6, 13, 22, and 31 months) (Fig. 1):

- control (water);
- vitamin D 42IU/kg;
- vitamin D 420IU/kg).

This study had 15 experimental groups. Considering that the aging process increases mortality, we have variation in the n of experimental groups because the older the age, the higher the mortality. The 31-month-old animals had significant mortality, around 69%. A study conducted by Phillips et al. (2010) observed that 31-month-old rats had 60% of mortality, justifying our study.

2.3 Radial arm-maze task

The radial maze test was performed to evaluate the spatial memory. On the 18th day of treatment. The radial maze apparatus had 8-arms, which were numbered from 1 to 8 (48 × 12 cm) and extended radially from a central area (32 cm diameter) .It was placed 50 cm above the floor, and geometric shapes were positioned in the straight arms where the food was (visual cues). On the first day, each animal was placed in the apparatus for a total of 10 min, allowed only to explore, and then returned to its cage and were kept on a restricted diet and their body weight was maintained at 85% of their free-feeding weight over a period of one week, with water being available ad libitum. On the 2nd day the animals were placed in the apparatus, where food (chocalete cereal) had already been deposited in four of the eight arms. The food bearing arms had visual cues at the end of each arm. Over a period of 10 min, the entry into each arm (total errors to find food) and the time each animal took to find the four pieces of cereal were recorded (latency to find food). The same test was held over four consecutive days, with one trial per day (Foyet et al., 2011; Hritcu et al., 2012). The behaviors were analyzed by a trained researcher, blinded for treatments. After the last test, and 24 h after the last administration of vitamin D3, the animals were subjected to euthanasia.

2.4 Euthanasia

The animals were submitted to euthanasia on the 22nd day of the treatment by decapitation. They waited for euthanasia in the next room to prevent from smelling the blood, and the guillotine was washed in running water between each euthanasia.

2.4 Tissue preparation

The brain was rapidly removed and placed on an ice-cold cutting board and washed with saline. After removal of the meninges, hippocampus and frontal cortex were extracted, snap frozen in liquid nitrogen and stored at -80ºC until biochemical analysis. The both total hippocampi were removed. The frontal cortex was dissected according Paxinos and Watson (2004) (bregma, 2.16–5.16 mm).

2.5 Enzyme-linked immunosorbent assay

The frontal cortex and hippocampus samples were solubilized in PBS (pH 7,4). Cytokines (TNF-α, IL-1β, IL-6, and IL-10) were quantified by enzyme immunoassay kits (R & D Systems, Minneapolis, MN, USA), as recommended by the manufacturer (described below). Microtiter plates (96 flat bottom wells) were incubated overnight with the capture antibody. Subsequently, the plates were washed three times with wash phosphate buffer and then blocked with 1% phosphate buffer solution for 1 hour. Afterward, the homogenized samples and the standards, which were both diluted in buffer solution, were incubated for 2 hours. Subsequently, the plates were washed three times with wash buffer and then incubated with detection antibody for another 2 hours. After the washes, the streptavidin-conjugated peroxidase was incubated for 20 minutes followed by the substrate (hydrogen peroxide and tetramethylbenzidine, 1:1). The reaction was stopped by 2N sulfuric acid. The plates were read at 450nm in a spectrophotometer. The total protein was measured according to the method of Lowry (Lowry et al., 1951), using bovine serum albumin as standard.

2.6 Statistical analysis

Performed using Statistica software 8.0 (StatSoft Inc., Tulsa, USA). Data from the ELISA analyses were evaluated by one-way analysis of variance (ANOVA), followed by Duncan's test when the p values < 0.05. Mauchly's test of sphericity was used (assumption of violated sphericity), and repeated measurements using the one-way ANOVA test were undertaken to assess radial arm–maze task data following Duncan post hoc test. The data were reported as mean ± SEM, and p values < 0.05 were considered statistically significant.

3. Results

The results from the radial arm-maze test are shown in Figs. 2A and 2B. Figure 2A shows the results of the radial maze test as the time to find food, and Fig. 2B presents the results for the number of errors to find food. The data presented a significant difference in the ANOVA test when the latency (time) was observed to find the reward [F (3,42) = 2,19; p < 0.001)] and the total errors to find the reward [F (3,42) = 27,87; p < 0.05].

Duncan's post hoc test revealed differences in the time (Fig. 2A) to find the reward in the control groups, as well as in 2-month-old animals with dose of 42 and 420 IU / kg of vitamin D3. They were significant in the 2nd, 3rd, and 4th days; thus, the animals have a conserved spatial memory, as each day they reduced time to find the reward. However, the animals at 6 and 13 months of age presented spatial memory impairment in the control group and were reverted by the doses of 42 and 420 IU/kg of vitamin D3, but only on the 4th day of the test. Animals at age 22 and 31 months also presented spatial memory impairment in both control and treated groups with 42 IU / kg of vitamin D3; however, the dose of 420 IU / kg was able to reverse this in the 3rd and 4th day of testing at both ages (Fig. 2A).

Evaluating the total of errors (Fig. 2B), Duncan's post hoc test showed differences at the age of 2 months on the 2nd, 3rd, and 4th days when compared to the 1st day in the water group.In the group treated with 42 IU/kg of vitamin D3, only the errors on the 4th day of the test decreased, and the dose 420IU/kg obtained better performance in the 3rd and 4th days of the test. The animals with 6, 13, 22, and 31 months that received water presented memory impairment, and the number of errors on the test days was not reduced. At the age of 6 months, there was a reduction in the number of errors on the 4th day of the groups treated with 42 and 420 IU/kg of vitamin D3. Unlike this, the 13-month-old animals treated with the doses of 42 and 420 IU/kg of vitamin D3 decreased the errors on the 3rd and 4th test days when compared to the 1st day within each treated group. At the age of 22 months, the 42 UI vitamin D3 dose reduced the number of errors on the 3rd and 4th day of the test, but the group treated with 420 IU/kg only decreased the errors on the last day. However, animals aged 31 months showed cognitive impairment in all treatments, and there was no decrease in errors within any of the groups (Fig. 2B).

To measure the levels of TNF-α, IL-1β, IL-6, and IL-10 in the frontal cortex and hippocampus tissues, a one-way ANOVA variance test (one-way ANOVA) of Duncan's post hoc was applied.

Figure 3 (A and B) shows the TNF-α levels in the frontal cortex and hippocampus, respectively. It presented a significant difference for TNF-α levels in the frontal cortex (Fig. 3A) [F (1,14) = 4.28; p < 0.001]

and hippocampus (Fig. 3B) [F (1,14) = 44.98; p < 0.001]. The levels of TNF-α increased in the control group at the age of 13 months in the frontal cortex and hippocampus. The two doses of vitamin D3 (42 (p < 0.05) and 420 IU/kg (p < 0.01) reversed this effect in the frontal cortex (Fig. 3A). The dose of 42IU/kg (p < 0.01) had the same action on hippocampus (Fig. 3B). In the frontal cortex, the dose of 420 IU/kg (p < 0.05) increased TNF-α in 22-month-old animals when compared to control animals (Fig. 3A). At ages 2 (42 UI/kg p = 0.38; 420 UI/kg p = 0.16), 6 (42 UI/kg p = 0.99; 420 UI/kg p = 0.41), and 31 months (42 UI/kg p = 0.47; 420 UI/kg p = 0.50), none of the vitamin doses altered levels of TNF-α in the frontal cortex (Fig. 3A). No change was observed in the hippocampus of animals 2 (42 UI/kg p = 0.52; 420 UI/kg p = 0.09), 6 (42 UI/kg p = 0.75; 420 UI/kg p = 0.69), and 31 (42 UI/kg p = 0.30; 420 UI/kg p = 0.08)) months (Fig. 3B).

IL-1β levels showed differences in the frontal cortex (p < 0.001) (Fig. 4A) and in the hippocampus (p < 0.05) (Fig. 4B) of the 2-month-old animals treated with the dose of 420 IU/kg vitamin D3. There was a significant increase in IL-1β when compared to the control ; the dose of 42 IU/kg (p = 0.88) did not show a significant result. In the frontal cortex of the 6, 13, and 31-month-old animals, an increase in levels was observed in the control groups (Fig. 4A). The dose of 420 IU/kg (p < 0.05;p < 0.001; p < 0.001) reversed this increase in both age. The dose of 42 IU/kg had a significant effect on the reduction of IL-1β levels only at the ages of 13 (p < 0.01) and 31 (p < 0.001) months. At 22 months, there were no differences in the frontal cortex (p = 0.75;p = 0.66) (Fig. 4A) or the hippocampus (p = 0.14;p = 0.41) (Fig. 4B). IL-1β levels in the hippocampus (Fig. 4B) of the 31-month-old animals reduced at the dose of 42 IU/kg (p < 0.001), and the dose of 420 IU/kg (p < 0.001) increased those when compared to control.

Interleukin IL-6 has its levels represented in the structures of the frontal cortex [F (1,14) = 7.23; p < 0.001] and hippocampus [F (1,14) = 8.10; p < 0.001], as seen in Fig. 5 (A and B). In the frontal cortex (Fig. 5A), there was no statistical difference at the ages of 2 (42 UI/kg p = 0.49; 420 UI/kg p = 0.71), 6 (42 UI/kg p = 0.18; 420 UI/kg p = 0.16), and 13 months (42 UI/kg p = 0.66; 420 UI/kg p = 0.40). At the age of 22 months, the dose of 42 IU/kg decreased IL-6 (p < 0.05) when compared to the control ;the same did not occur at the dose of 420 IU/kg (p = 0.05) (Fig. 5A). Animals aged 31 months presented differences with the dose of 42 IU/kg (p < 0.01), which reduced IL-6 levels, while the dose of 420 IU/kg did not present difference when compared to control In the frontal cortex and hippocampus (Fig. 5A and 5B, respectively), the ages of 2 (42 UI/kg p = 0.90; 420 UI/kg p = 0.52), 6 (42 UI/kg p = 0.42; 420 UI/kg p = 0.56), and 13 months (42 UI/kg p = 0.65; 420 UI/kg p = 0.65), did not present differences in any of the groups. At 22 months, IL-6 levels increased with 42 IU/kg (p < 0.01), and the dose of 420 IU/kg (p = 0.60) showed no differences. The dose of 420 IU/kg (p < 0.05) reduced IL-6 levels in the hippocampus of the 31-month animas; however, the dose of 42 IU/kg (p = 0.44) did not present ssignificant results (Fig. 5B).

IL-10 levels in frontal cortex structures [F (1,14) = 6.63; p < 0.01] and hippocampus [F (1,14) = 37.47; p < 0.01] are represented in Fig. 6 (A and B). The ages of 2 [42 UI/kg (p = 0.31); 420 UI/kg (p = 0.07)] and 6 months [42 UI/kg (p = 0.36); 420 UI/kg (p = 0.43)] did not present differences in the frontal cortex when compared to the control (Fig. 6A). At the age of 13 [42 UI/kg (p < 0.001); 420 UI/kg (p > 0.001)] and 31 months [42 UI/kg (p < 0.05); 420 UI/kg (p > 0.01)], the control group showed an increase in IL-10 levels, and the two doses of vitamin D (42 and 420 IU/kg) were able to decrease these levels at both (Fig. 6A). At

t 22 months, the dose of 42 IU/kg ($p < 0.05$) decreased IL-10 levels when compared to control ;however, the same did not occur at 420IU/kg ($p = 0.85$) (Fig. 6A). In the hippocampus (Fig. 6B), animals at 2 months showed increased IL-10 levels with both doses of 42 IU/kg ($p < 0.05$) and 420 IU/kg ($p < 0.01$) when compared with the control .At 22 months (Fig. 6B), contrary to what was observed in the frontal cortex (Fig. 6A), the dose of 42 IU/kg ($p < 0.05$) increased IL-10 levels when compared to the control ,and the dose of 420 UI/kg ($p = 0.23$) remained without significant results. In the hippocampus, the ages of 6, 13, and 31 months in both doses [42 UI/kg ($p = 0.72$; $p = 0.24$; $p = 0.13$); 420 UI/kg ($p = 0.66$; $p = 0.19$; $p = 0.12$)], respectively, did not present differences when compared to the control within each age (Fig. 6B).

Table 1 summarizes the effect of treatment with vitamin D (42 and 420 IU/kg) for 21 days in animals of 2, 6, 13, 22, and 31 months of age on spatial memory and the levels of TNF-α, IL-1β, IL-6, and IL-10 in the cerebral structures of the frontal cortex and hippocampus.

4. Discussion

The effects of vitamin D3 on the spatial memory were evaluated using the radial maze task (Dellu et al., 1997; Lee et al., 2014). In rodents, it has been reported that during natural aging, the memory and spatial functions decline (Mora-Gallegos et al., 2015). It is important to emphasize that the present study is the first to evaluate vitamin D treatment in animals at 13, 22, and 31 months old (natural aging).

The animals at 2 months old did not present spatial memory impairment, as shown by reduced time and errors to find food. The animals at 6, 13, 22, and 31 months-old did not learn the task, probably due to spatial memory impairment caused by aging. However, when treated with vitamin D (42 and 420 IU/kg), the animals at 6, 13, and 22 months old learned the task. In addition, in the rats at 31 months old, only the 420 UI/kg dose was able to improve the performance by reducing the time to find food on the 3rd and 4th day of the test. The results demonstrated that vitamin D was able to improve spatial memory of these animals.

A study using transgenic F344 male rats, a lineage of accelerated aging mice, showed that vitamin D (42 IU/kg intraperitoneally) for 21 days improved the spatial memory in the water maze task in the 20-month-old rats (Briones and Darwish, 2012). In another study conducted in 2014, F344 male rats aged 11 to 13 months received diets containing low (100 IU), medium (1,000 IU), or high (10,000) IU levels of vitamin D for 5 to 6 months. The results showed that the group supplemented with high-level vitamin D performed the water maze task extremely well and reached the goal in half the time and distance compared to the other two groups (Latimer et al., 2014). In addition, another study was performed in which the 10-day Senescence Accelerated Mouse-Prone 8 (SAMP8) received 500 UI/kg of vitamin D alone or in combination with resveratrol (43mg/kg) in their diet for 24 weeks. The supplementation of vitamin D and resveratrol reversed cognitive impairment water maze task in 34-week old SAMP8 mice, and the combined vitamin D supplementation with resveratrol was more effective than isolated resveratrol intervention (Cheng et al., 2017). These data are in agreement with the results of the present study.

One of the most recognized effects of brain aging is the deregulation of the immune system as a result of the uncontrolled production of reactive oxygen species and pro-inflammatory cytokines (Davinelli et al., 2016; Esiri, 2007). With advancing age, the body develops a process known as "chronic inflammation" or *inflammaging*. (Gruver et al., 2007; Watson et al., 2017).

To investigate the vitamin D immunoprotective effect, we evaluate the TNFα, IL-1β, IL-6, and IL-10 levels. Vitamin D was able to reduce the cytokines levels at 13 months old, since TNFα, IL-1β, and IL-10 levels decreased in the frontal cortex by both doses of vitamin D, and TNFα levels decreased in the hippocampus only at the dose of 42 IU/kg. The inflammatory cytokines TNFα, IL-1β, and IL-10 increased in the frontal cortex due to aging at 13 months of age, and only TNFα increased in the hippocampus in these animals. Therefore, the immunomodulatory action of vitamin D was observed principally in the frontal cortex. in this study was shown to be a potential immunological modulator. At 22 months old, vitamin D at the dose of 42 UI/kg decreased pro-inflammatory cytokine IL-6 and anti-inflammatory IL-10 levels in the frontal cortex, and the same dose was able to increase the levels of IL-6 and IL-10 in the hippocampus when compared to the control. The 31-months-old animals that received water showed increased levels of IL-1β and IL-10, which was reversed by both doses of vitamin D. In addition, vitamin D 42 UI/kg reduced IL-6 levels in the frontal cortex. However, 42 IU/kg of vitamin D reduced IL-1β levels in the hippocampus, and the dose of 420 IU/kg increased the levels of this in the hippocampus. The dose of 42 UI/kg was able to decrease IL-6 levels in the frontal cortex, and only the dose of 420 UI/kg reduced IL-6 levels in the hippocampus.

In the present study, advanced age animals demonstrated an increase in cytokine levels, and in most cases, vitamin D treatment was able to modulate inflammatory cytokine levels and prevent impairment to memory. In a study using F344 rats aged 6 and 20 months, vitamin D injection at the dose of 42 IU/kg was able to reduce the age-related pro-inflammatory state and amyloid burden, which suggests these vitamin D effects are related to memory improvements observed in elderly rats (Briones and Darwish, 2012). This study corroborates data from the present study since vitamin D had a modulatory effect on the cytokines.

However, in the present study, we showed unexpected results, since vitamin D treatment increased some pro-inflammatory cytokines. The dose of 42 UI/kg increased IL-6 levels in the hippocampus in 22-month-old. In addition, the dose of 420 UI/kg increased IL-1β in the frontal cortex and hippocampus of 2-month-old, IL-1β in the hippocampus of 31-month-old, and TNF-α in the frontal cortex of 22-month-old. Vilela et al. (2017) observed the increase of IL-1β and TNF-α in the hippocampus of 24-month-old rats submitted to strength training, although it improved spatial memory. The authors suggested the increase of these inflammatory cytokines may be related to memory improvement (Vilela et al., 2017), since they play an active role in neuronal development and neuroplasticity (Khairova et al., 2009; Tonelli and Postolache, 2005). Nevertheless, this is difficult to confirm without additional studies.

Thus, our results suggest that vitamin D has a modulatory action in the pro- and anti-inflammatory cytokines and prevents spatial memory impairment. Therefore, our results support the hypothesis that

vitamin D may exert an immunomodulatory action on aging. It is necessary to conduct more studies since our results showed that the regulation of cytokines may be complex in the brain.

Declarations

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Conflict of Interest

No competing financial interests exist.

Availability of data and material

Not applicable

Code availability

Not applicable

Authors' contributions

All the authors contributed equaly to the manuscript.

Ethics approval

Attached in the supplementary material.

Consent to participate

Not applicable

Consent for publication

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References

1. Al-Harbi, A.N., Khan, K.M., Rahman, A., 2017. Developmental Vitamin D Deficiency Affects Spatial Learning in Wistar Rats. The Journal of nutrition 147(9), 1795-1805.
2. Annweiler, C., Allali, G., Allain, P., Bridenbaugh, S., Schott, A.M., Kressig, R.W., Beauchet, O., 2009. Vitamin D and cognitive performance in adults: a systematic review. European journal of neurology 16(10), 1083-1089.
3. Annweiler, C., Montero-Odasso, M., Llewellyn, D.J., Richard-Devantoy, S., Duque, G., Beauchet, O., 2013. Meta-analysis of memory and executive dysfunctions in relation to vitamin D. Journal of Alzheimer's disease : JAD 37(1), 147-171.
4. Briones, T.L., Darwish, H., 2012. Vitamin D mitigates age-related cognitive decline through the modulation of pro-inflammatory state and decrease in amyloid burden. Journal of neuroinflammation 9, 244.
5. Casaccia-Bonnefil, P., Pandozy, G., Mastronardi, F., 2008. Evaluating epigenetic landmarks in the brain of multiple sclerosis patients: a contribution to the current debate on disease pathogenesis. Progress in neurobiology 86(4), 368-378.
6. Cheng, J., Rui, Y., Qin, L., Xu, J., Han, S., Yuan, L., Yin, X., Wan, Z., 2017. Vitamin D Combined with Resveratrol Prevents Cognitive Decline in SAMP8 Mice. Current Alzheimer research 14(8), 820-833.
7. Collin, C., Assmann, K.E., Deschasaux, M., Andreeva, V.A., Lemogne, C., Charnaux, N., Sutton, A., Hercberg, S., Galan, P., Touvier, M., Kesse-Guyot, E., 2016. Plasma vitamin D status and recurrent depressive symptoms in the French SU.VI.MAX cohort. European journal of nutrition.
8. Correale, J., Ysrraelit, M.C., Gaitan, M.I., 2009. Immunomodulatory effects of Vitamin D in multiple sclerosis. Brain : a journal of neurology 132(Pt 5), 1146-1160.
9. Davinelli, S., Maes, M., Corbi, G., Zarrelli, A., Willcox, D.C., Scapagnini, G., 2016. Dietary phytochemicals and neuro-inflammaging: from mechanistic insights to translational challenges. Immunity & ageing : I & A 13, 16.
10. Dellu, F., Fauchey, V., Le Moal, M., Simon, H., 1997. Extension of a new two-trial memory task in the rat: influence of environmental context on recognition processes. Neurobiology of learning and memory 67(2), 112-120.
11. Dougherty, K.A., Dilisio, M.F., Agrawal, D.K., 2016. Vitamin D and the immunomodulation of rotator cuff injury. Journal of inflammation research 9, 123-131.
12. Esiri, M.M., 2007. Ageing and the brain. The Journal of pathology 211(2), 181-187.
13. Foyet, H.S., Hritcu, L., Ciobica, A., Stefan, M., Kamtchouing, P., Cojocaru, D., 2011. Methanolic extract of Hibiscus asper leaves improves spatial memory deficits in the 6-hydroxydopamine-lesion rodent model of Parkinson's disease. J Ethnopharmacol 133(2), 773-779.
14. Franceschi, C., Bonafe, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E., De Benedictis, G., 2000. Inflamm-aging. An evolutionary perspective on immunosenescence. Annals of the New York Academy of Sciences 908, 244-254.
15. Franceschi, C., Campisi, J., 2014. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. The journals of gerontology. Series A, Biological sciences and medical

sciences 69 Suppl 1, S4-9.

16. Gabrysova, L., Howes, A., Saraiva, M., O'Garra, A., 2014. The regulation of IL-10 expression. Current topics in microbiology and immunology 380, 157-190.
17. Godbout, J.P., Chen, J., Abraham, J., Richwine, A.F., Berg, B.M., Kelley, K.W., Johnson, R.W., 2005. Exaggerated neuroinflammation and sickness behavior in aged mice following activation of the peripheral innate immune system. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 19(10), 1329-1331.
18. Gruver, A.L., Hudson, L.L., Sempowski, G.D., 2007. Immunosenescence of ageing. The Journal of pathology 211(2), 144-156.
19. Heidary, M., Rakhshi, N., Pahlevan Kakhki, M., Behmanesh, M., Sanati, M.H., Sanadgol, N., Kamaladini, H., Nikravesh, A., 2014. The analysis of correlation between IL-1B gene expression and genotyping in multiple sclerosis patients. Journal of the neurological sciences 343(1-2), 41-45.
20. Hritcu, L., Cioanca, O., Hancianu, M., 2012. Effects of lavender oil inhalation on improving scopolamine-induced spatial memory impairment in laboratory rats. Phytomedicine 19(6), 529-534.
21. Hubackova, S., Krejcikova, K., Bartek, J., Hodny, Z., 2012. Interleukin 6 signaling regulates promyelocytic leukemia protein gene expression in human normal and cancer cells. The Journal of biological chemistry 287(32), 26702-26714.
22. Khairova, R.A., Machado-Vieira, R., Du, J., Manji, H.K., 2009. A potential role for pro-inflammatory cytokines in regulating synaptic plasticity in major depressive disorder. The international journal of neuropsychopharmacology 12(4), 561-578.
23. Latimer, C.S., Brewer, L.D., Searcy, J.L., Chen, K.C., Popovic, J., Kraner, S.D., Thibault, O., Blalock, E.M., Landfield, P.W., Porter, N.M., 2014. Vitamin D prevents cognitive decline and enhances hippocampal synaptic function in aging rats. Proceedings of the National Academy of Sciences of the United States of America 111(41), E4359-4366.
24. Lee, J.Y., Kho, S., Yoo, H.B., Park, S., Choi, J.S., Kwon, J.S., Cha, K.R., Jung, H.Y., 2014. Spatial memory impairments in amnestic mild cognitive impairment in a virtual radial arm maze. Neuropsychiatric disease and treatment 10, 653-660.
25. Littlejohns, T.J., Henley, W.E., Lang, I.A., Annweiler, C., Beauchet, O., Chaves, P.H., Fried, L., Kestenbaum, B.R., Kuller, L.H., Langa, K.M., Lopez, O.L., Kos, K., Soni, M., Llewellyn, D.J., 2014. Vitamin D and the risk of dementia and Alzheimer disease. Neurology 83(10), 920-928.
26. Llewellyn, D.J., Langa, K.M., Lang, I.A., 2009. Serum 25-hydroxyvitamin D concentration and cognitive impairment. Journal of geriatric psychiatry and neurology 22(3), 188-195.
27. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. The Journal of biological chemistry 193(1), 265-275.
28. Minciullo, P.L., Catalano, A., Mandraffino, G., Casciaro, M., Crucitti, A., Maltese, G., Morabito, N., Lasco, A., Gangemi, S., Basile, G., 2016. Inflammaging and Anti-Inflammaging: The Role of Cytokines in Extreme Longevity. Archivum immunologiae et therapiae experimentalis 64(2), 111-126.

29. Mora-Gallegos, A., Rojas-Carvajal, M., Salas, S., Saborio-Arce, A., Fornaguera-Trias, J., Brenes, J.C., 2015. Age-dependent effects of environmental enrichment on spatial memory and neurochemistry. Neurobiology of learning and memory 118, 96-104.
30. Naghavi Gargari, B., Behmanesh, M., Shirvani Farsani, Z., Pahlevan Kakhki, M., Azimi, A.R., 2015. Vitamin D supplementation up-regulates IL-6 and IL-17A gene expression in multiple sclerosis patients. International immunopharmacology 28(1), 414-419.
31. Nossov, S., Dines, J.S., Murrell, G.A., Rodeo, S.A., Bedi, A., 2014. Biologic augmentation of tendon-to-bone healing: scaffolds, mechanical load, vitamin D, and diabetes. Instructional course lectures 63, 451-462.
32. Obermann, K.R., Morris, J.C., Roe, C.M., 2013. Exploration of 100 commonly used drugs and supplements on cognition in older adults. Alzheimer's & dementia : the journal of the Alzheimer's Association 9(6), 724-732.
33. Pang, Q., Qi, X., Jiang, Y., Wang, O., Li, M., Xing, X., Dong, J., Xia, W., 2016. Clinical and genetic findings in a Chinese family with VDR-associated hereditary vitamin D-resistant rickets. Bone research 4, 16018.
34. Prattichizzo, F., De Nigris, V., La Sala, L., Procopio, A.D., Olivieri, F., Ceriello, A., 2016. "Inflammaging" as a Druggable Target: A Senescence-Associated Secretory Phenotype-Centered View of Type 2 Diabetes. Oxidative medicine and cellular longevity 2016, 1810327.
35. Simpson, R.J., Hammacher, A., Smith, D.K., Matthews, J.M., Ward, L.D., 1997. Interleukin-6: structure-function relationships. Protein science : a publication of the Protein Society 6(5), 929-955.
36. Slominski, A.T., Kim, T.K., Li, W., Yi, A.K., Postlethwaite, A., Tuckey, R.C., 2014. The role of CYP11A1 in the production of vitamin D metabolites and their role in the regulation of epidermal functions. The Journal of steroid biochemistry and molecular biology 144 Pt A, 28-39.
37. Soni, M., Kos, K., Lang, I.A., Jones, K., Melzer, D., Llewellyn, D.J., 2012. Vitamin D and cognitive function. Scandinavian journal of clinical and laboratory investigation. Supplementum 243, 79-82.
38. Spach, K.M., Pedersen, L.B., Nashold, F.E., Kayo, T., Yandell, B.S., Prolla, T.A., Hayes, C.E., 2004. Gene expression analysis suggests that 1,25-dihydroxyvitamin D3 reverses experimental autoimmune encephalomyelitis by stimulating inflammatory cell apoptosis. Physiological genomics 18(2), 141-151.
39. Taylor, J.M., Main, B.S., Crack, P.J., 2013. Neuroinflammation and oxidative stress: co-conspirators in the pathology of Parkinson's disease. Neurochemistry international 62(5), 803-819.
40. Tonelli, L.H., Postolache, T.T., 2005. Tumor necrosis factor alpha, interleukin-1 beta, interleukin-6 and major histocompatibility complex molecules in the normal brain and after peripheral immune challenge. Neurological research 27(7), 679-684.
41. Tot Babberich Ede, N., Gourdeau, C., Pointel, S., Lemarchant, B., Beauchet, O., Annweiler, C., 2015. Biology of subjective cognitive complaint amongst geriatric patients: vitamin D involvement. Current Alzheimer research 12(2), 173-178.

42. Vilela, T.C., Muller, A.P., Damiani, A.P., Macan, T.P., da Silva, S., Canteiro, P.B., de Sena Casagrande, A., Pedroso, G.D.S., Nesi, R.T., de Andrade, V.M., de Pinho, R.A., 2017. Strength and Aerobic Exercises Improve Spatial Memory in Aging Rats Through Stimulating Distinct Neuroplasticity Mechanisms. Molecular neurobiology 54(10), 7928-7937.
43. Watson, N., Ding, B., Zhu, X., Frisina, R.D., 2017. Chronic inflammation - inflammaging - in the ageing cochlea: A novel target for future presbycusis therapy. Ageing research reviews 40, 142-148.
44. Ziebell, J.M., Rowe, R.K., Muccigrosso, M.M., Reddaway, J.T., Adelson, P.D., Godbout, J.P., Lifshitz, J., 2016. Aging with a traumatic brain injury: Could behavioral morbidities and endocrine symptoms be influenced by microglial priming? Brain, behavior, and immunity.

Table 1

Table 1 is available in the Supplementary Files section.

Figures

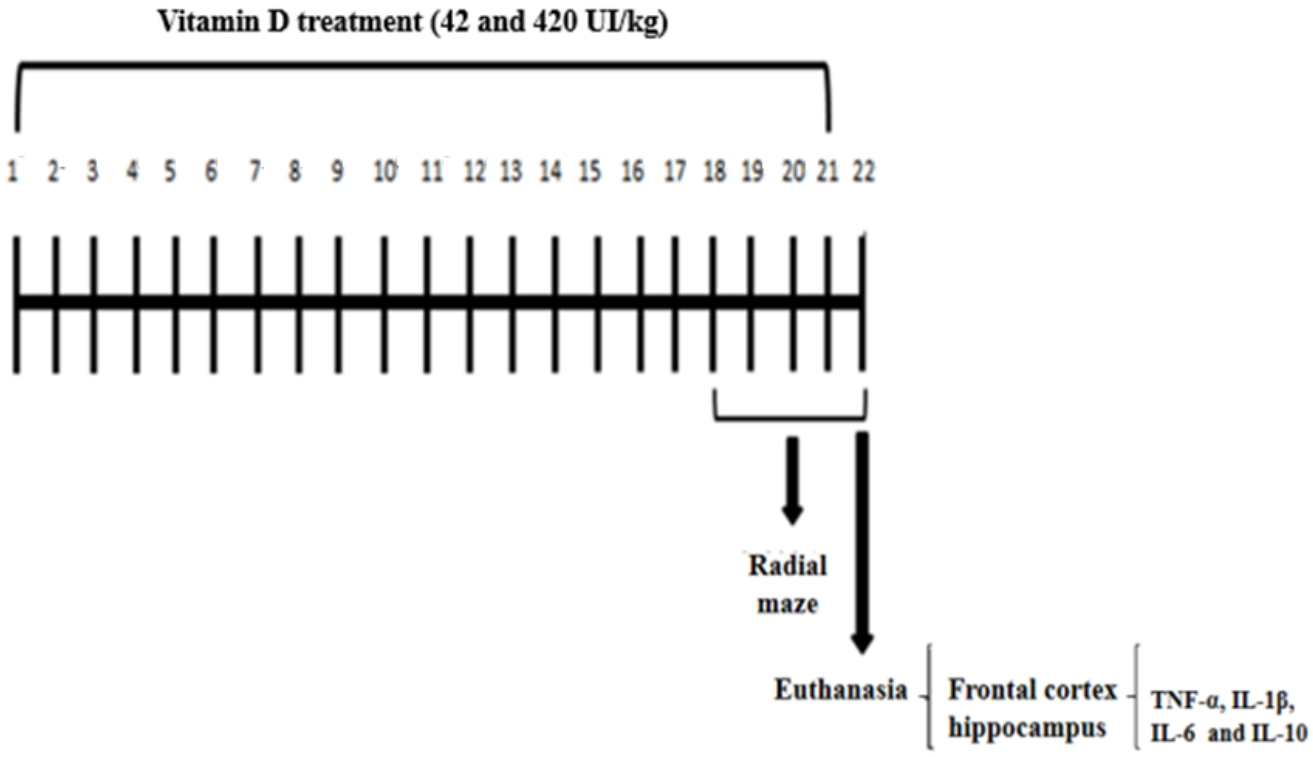

Figure 1

Experimental design.

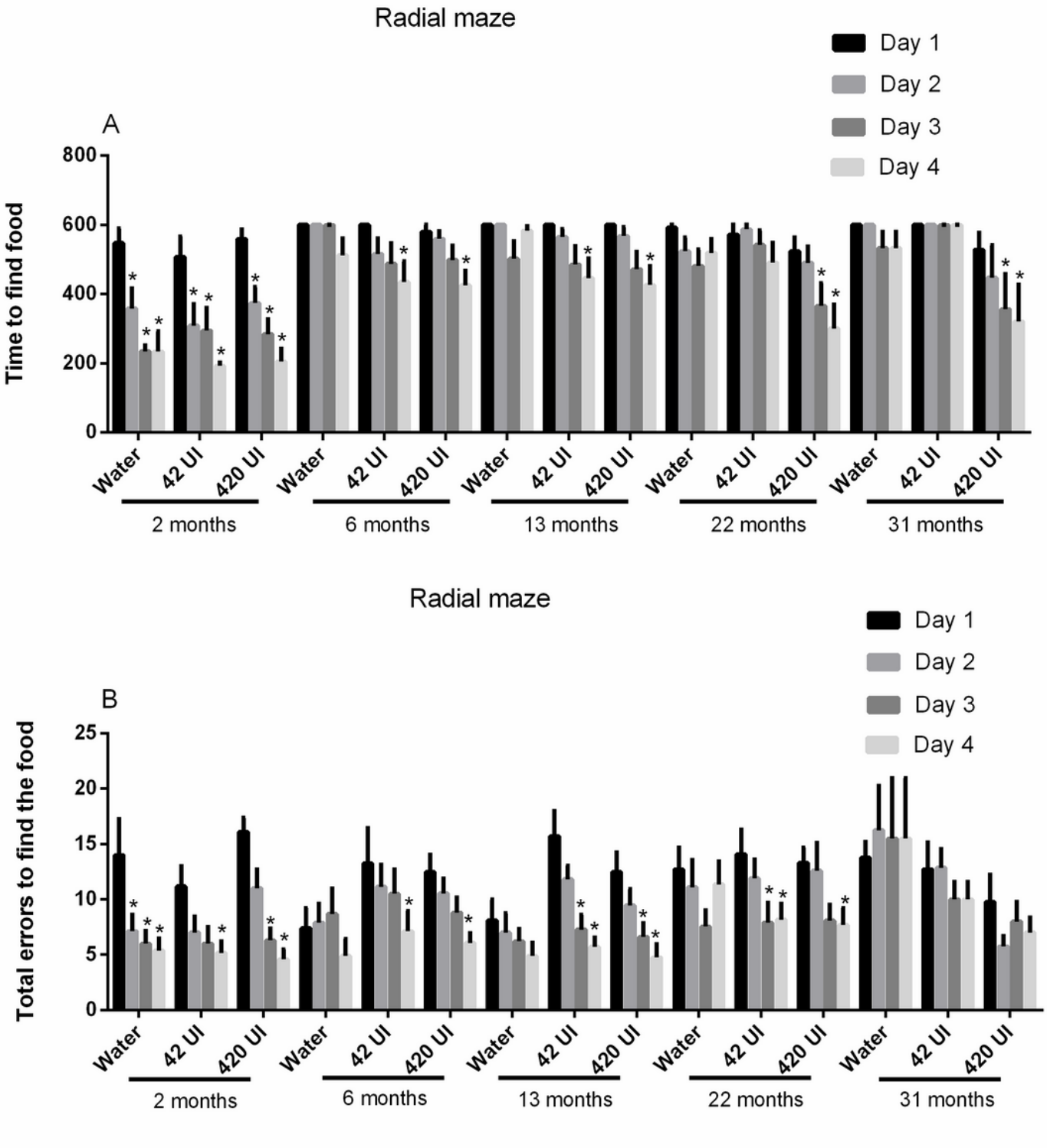

Figure 2

Effect of vitamin D3 treatment (42 and 420 IU/kg) for 21 days in animals 2, 6, 13, 22, and 31 months old in spatial memory evaluated by the radial maze task. (A) Time to find the food and (B) total errors to find the food on 1st, 2nd, 3rd and 4th test days; n = 4-13 animals per experimental group. Data are expressed as mean ± SEM. Data that show * $p < 0.05$ are considered significant when compared to the 1st day of test within the experimental group.

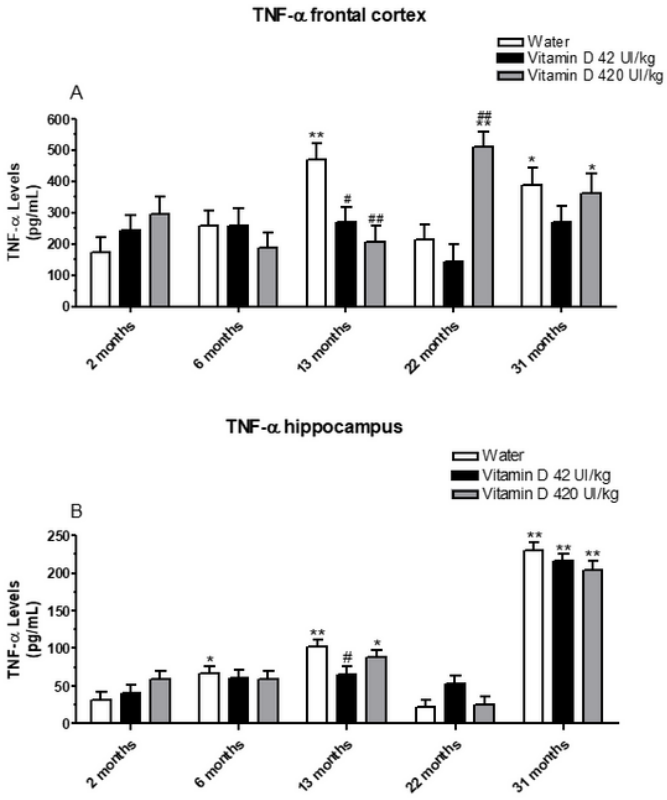

Figure 3

Effect of vitamin D3 treatment (42 and 420 UI/kg) for 21 days on TNF-α levels in brain structures of the frontal cortex and hippocampus from Wistar rats. Figure 3A shows levels of TNF-α (pg/mL) in the frontal cortex; 3B shows levels of TNF-α (pg/ml) in the hippocampus. Data are expressed as mean ± SEM of 3-6 animals per group. # $p < 0.05$; # $p < 0.01$ when compared to the controls within the same age group, and $p < 0.05$, ** $p < 0.01$ when compared to group controls of 2 months of age.

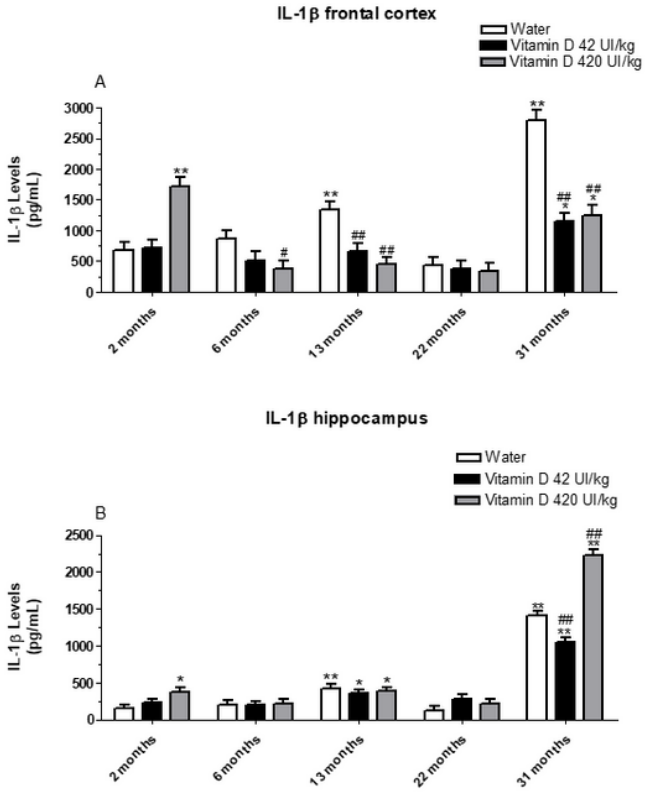

Figure 4

Effect of vitamin D3 treatment (42 and 420 UI/kg) for 21 days on IL-1β levels in brain structures of the frontal cortex and hippocampus from Wistar rats. Figure 4A shows levels of IL-1β (pg/mL) in the frontal cortex; 4B shows levels of IL-1β (pg/ml) in the hippocampus. Data are expressed as mean ± SEM of 3-6 animals per group. # $p < 0.05$, # $p < 0.01$ when compared to the water group within the same age and $p < 0.05$, ** $p < 0.01$ when compared to the water group of 2 months of age.

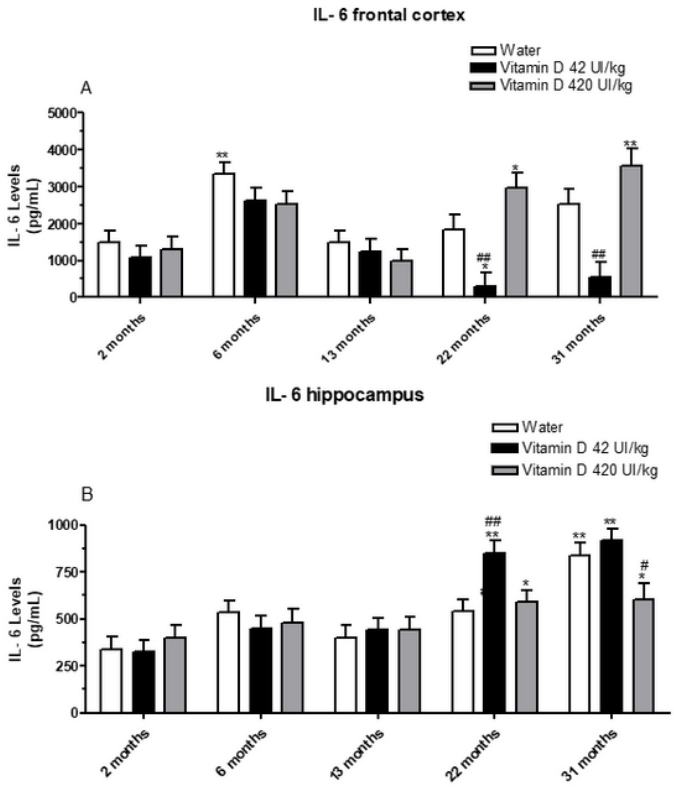

Figure 5

Effect of vitamin D3 treatment (42 and 420 UI/kg) for 21 days on IL-6 levels in brain structures of the frontal cortex and hippocampus of Wistars rats. Figure 5A shows levels of IL-6 (pg/mL) in the frontal cortex; 5B shows levels of IL-6 (pg/ml) in the hippocampus. Data are expressed as mean ± SEM of 3-6 animals per group, # $p < 0.05$, # $p < 0.01$ when compared to the water group within the same age and $p < 0.05$, ** $p < 0.01$ when compared to the water group of 2 months of age.

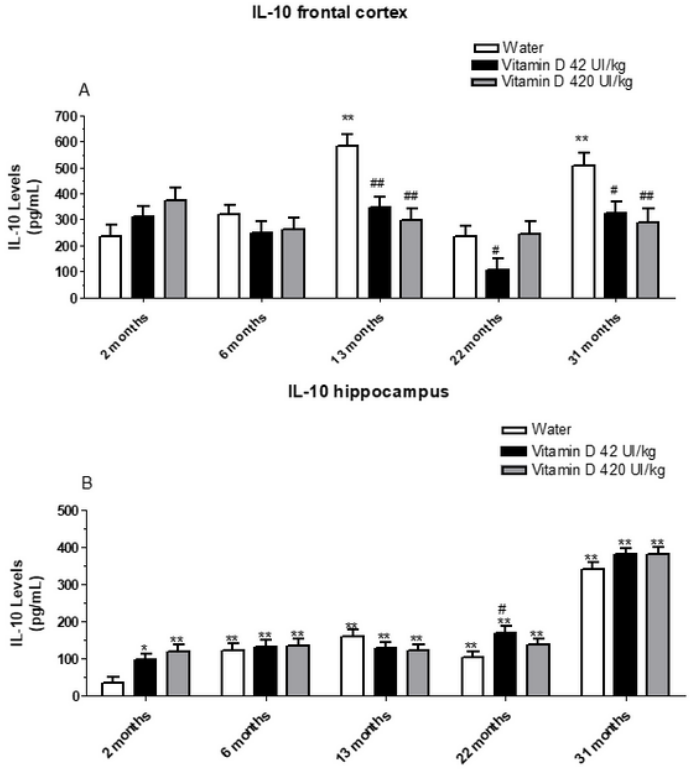

Figure 6

Effect of vitamin D3 treatment (42 and 420UI/kg) for 21 days on IL-10 levels in brain structures of the frontal cortex and hippocampus of Wistars rats. Figure 6A shows levels of IL-10 (pg/mL) in the frontal cortex; 6B shows levels of IL-10 (pg/ml) in the hippocampus. Data are expressed as mean ± SEM of 3-6 animals per group. # p < 0.05, # p < 0.01 when compared to the water group within same age and p < 0.05, ** p < 0.01 when compared to the water group of 2 months of age.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SuplementaryMaterial.docx
- Table1.docx