We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

Vitamin D_3 Modulates NF-kB/p65, 17β-Estradiol, and Vitamin D Receptors Expression at Estrogen Deficiency

Alexandra Koshkina, Olga Volkova and Julia Fedotova

Abstract

The aim of the present study was to focus on the effects of Vitamin D_3 (VD_3) supplementation (5.0 mg/kg, s.c.) on the NF-kB/p65, 17β-estradiol (17β-E_2)/VD_3 receptors expression in the hippocampus in the long-term ovariectomized (OVX) rats treated with low dose of 17β-E_2 (0.5 μg/rat, s.c.) submitted for the chronic unpredictable mild stress (CUMS) for 28 days. Sucrose preference (SPT), forced swimming (FST), and open-field (OFT) tests were conducted to estimate the anhedonia-/depression-like states. NF-kB/p65, 17β-E_2/VD_3 receptors levels in the hippocampus were evaluated by ELISA and Western blot assays. The findings demonstrated that VD_3 at high dose (5.0 mg/kg, s.c.) in a combination with low dose of 17β-E_2 decreased anhedonia in the SPT and depression-like behavior in the FST of the long-term OVX rats submitted to CUMS. VD_3 (5.0 mg/kg) resulted in significant decreased levels of hippocampal NF-kB/p65 protein expression, as well as to the normalization of hippocampal 17β-E_2/VD_3 receptors levels in long-term OVX rats treated with 17β-E_2 exposed to CUMS. In conclusion, VD_3 (5.0 mg/kg, s.c.) in a combination with low dose of 17β-E_2 had a synergic antianhedonic- and antidepressant-like effects in the adult female rats following long-term ovariectomy submitted to CUMS.

Keywords: vitamin D_3, long-term ovariectomy, chronic unpredictable mild stress, NF-kB/p65, 17β-estradiol receptor, vitamin D_3 receptors

1. Introduction

The menopausal transition is often associated with a multiplicity of manifestations, the most standard being neuropsychiatric [1, 2]. The role of ovarian hormones in affect-related disorders is of great interest for women transitioning through menopause [2, 3]. Mood disorders during menopause could partly be explained due to a loss of estrogen is known to have neuroprotective effects on brain [3]. Numerous experimental and clinical studies have documented that estrogen deficiency during menopause increases the susceptibility to mood disturbances, including anxiety [4–6]. There has been a discussion that menopausal hormonal therapy (MHT) may improve the symptoms of affective-related disorders or decrease the risk of developing these, yet some uncertainty still exist around this topic because as research has

also found that MHT does not entirely stop the development of affective-related symptoms [7].

Females going through menopause are at higher risk of developing Vitamin D (VD) deficiency due to a VD poor diet, restricted outdoor activity resulting in less sun exposure as well as a decreased capacity to produce enough calcitriol as a result of an age related decline in hydroxylation by the kidneys [8]. Our previous experimental work has confirmed that hormonal profile in ovariectomized (OVX) female rodents is also characterized by VD deficiency or insufficiency [9, 10]. Traditional methods of affective-related disorders therapy, which also include antidepressants/anxiolytics, are unfortunately of limited effectiveness [11]. Nutrient imbalance, especially VD_3 deficiency, is considered as one of the critical causes, enabling the pathophysiological mechanisms for development of psychiatric disorders [12]. In the pathophysiological mechanisms of mood disorders, many trigger factors play a role, and it is argued that one of them could be a deficiency in VD_3 [12].

VD_3 deficiency has been proven to impact on the pathogenesis of various diseases, for example, autoimmune diseases, cardiovascular diseases, infections, osteoporosis, obesity, diabetes, and certain types of cancers [13–15]. A correlation between very low VD_3 levels and numerous neuropsychiatric diseases and a correlation between an impact of VD_3 levels and normal brain functioning have also been found in recent studies [14–16]. VD receptors (VDRs) have been found present in the central nervous system [17], in the brain structures involved in processes of mood regulation (cingulate cortex, hippocampus, thalamus, and hypothalamus) [18]. In this line, it can be assumed that VD_3 likely has humoral or neurohumoral activities in these brain structures. VD_3 involves in the neurogenesis, neuroplasticity, neuroprotection, and neuroimmunomodulation [19–21]. This fact creates a neurobiological basis to propose the involvement of VD in the mechanisms of neuropsychiatric disorders [22–26].

The neuroinflammation in the central nervous system is supposed to be one of the main trigger factors for the development of affective-related disorders [27, 28]. Taking this assumption into account, mood disturbances established in menopausal women might result from complex alterations in estradiol and VD_3 levels, as well as neuroinflammation.

Nowadays, nuclear factor-kappa B (NF-κB) is postulated as the proinflammatory transcription factor that controls proinflammatory cytokines expression and is involved in the mechanisms of many inflammatory and neuroinflammatory diseases [29, 30]. NF-κB is triggered by stress and might mediate cellular responses to stressful life events, thereby critically involved in development of affective-related disorders [31–33]. The enhancement of NF-κB might induce the elevated production of proinflammatory cytokines and diminished neurohormonal stress feedback [34]. Furthermore, NF-κB pathway is involved in antidepressant action of different psychotropic drugs that used for treatment of mood disorders [35]. Clinical studies using patients with mood disorders have shown that NF-κB levels are increased in the serum of such patients [35–37]. Using genetic and environmental model of depression, it was shown that the antidepressant effect of such pharmacological treatments was dependent on NF-κB-p65 acetylation [36, 37].

The hippocampus is one of the key structures of the brain, which plays a role in affective-related disorders [38]. Both estrogen and VD_3 have been associated with the successful functioning of the hippocampus [1, 21, 25]. Basic and clinical studies have suggested that alterations in NF-kB/p65 signaling and in $17\beta\text{-}E_2/VD_3$ receptors expression in the hippocampus, as well changes of serum estradiol/VD contents are very often registered at affective-related disorders [1, 23, 39]. Animal studies have documented that the impaired behavioral profile in OVX rats is correlated with increased NF-kB/p65 levels in the brain [40, 41].

Recently, we found that VD_3 (5.0 mg/kg, s.c.) reduced anhedonia and depression-like behavior of long-term adult ovariectomized (OVX) rats exposed to the chronic unpredictable mild stress (CUMS) in the sucrose preference (SPT) and forced swimming (FST), respectively [42]. However, the therapeutic effects of VD_3 in a combination with low dose of 17β-E_2 in female rats with long-lasting decline of estrogens exposed to CUMS remain unknown. Furthermore, it is still unclear whether the antidepressant-like action of VD_3 plus 17β-E_2 application implicates NF-kB/p65 signaling pathway and modifications of 17β-E_2/VD_3 receptors expression in the hippocampus of long-term OVX adult rats with CUMS.

The current investigation was performed to clarify the antidepressant-like effect of a combination with VD_3 plus low dose of 17β-E_2 on a rat model of CUMS in the female rats with long-lasting decline of estrogens. Similar to previously published work [43], we used long-lasting estrogen deficiency caused by a post-ovariectomy period of 3 months. This animal model is widely utilized in preclinical behavioral research producing a menopausal-like state in women [44]. Such behavioral tests as sucrose preference (SPT), forced swimming (FST), and open-field (OFT) were carried out to examine the depression-like behavior. NF-kB/p65, 17β-E_2/VD_3 receptors levels in the hippocampus and serum estradiol and VD concentrations were determined to assess the possible mechanisms of the VD_3 effects on the depression-like behavior in long-term OVX rats given with low dose of 17β-E_2 subjected to CUMS.

2. Materials and methods

2.1 Animals

A total of 49 Wistar rats of 3 months age, female sex (weighing 200–220 g) were purchased in this work. Animals were divided into experimental groups with access to rat standard food and water ad libitum. The female rats were placed under a 12 light-dark scheme (light was given between 07:00 and 19:00 h) and room temperature (23 ± 2°C). All behavioral procedures and CUMS model were performed in compliance with the National research council's guide for the care and use of laboratory animals and approved by the Ethical committee for experimental studies of I.P. Pavlov Institute of Physiology (statement No.: 1095/1/25.06.2012). Stress model of depression was conducted with minimal pain for all groups of rats.

2.2 Ovariectomy

Three months before CUMS procedure, sham operation and long-term total ovariectomy with general anesthesia (ketamine 70 mg/kg and xylazine 10 mg/kg, i.p.) were performed. Long-term period (3 months) elimination of female gonadal hormones was chosen as experimental model of menopause in women [44, 45]. The removal of ovaries was carried out accordingly to our method as prescribed earlier [43]. After surgery or sham-operation (SHAM), the ovariectomized (OVX) females were placed in home cage with free access to food and water. During 12 weeks, sham-operated and OVX females had a recovery. Following 3 months of surgery, experimental rats were randomly distributed to the groups for the chronic stress procedure, except for SHAM non-stressed control rats.

2.3 CUMS model

Chronic unpredictable mild stress (CUMS) paradigm is a valid and significant animal model of depression induced by stress procedure. This behavioral model

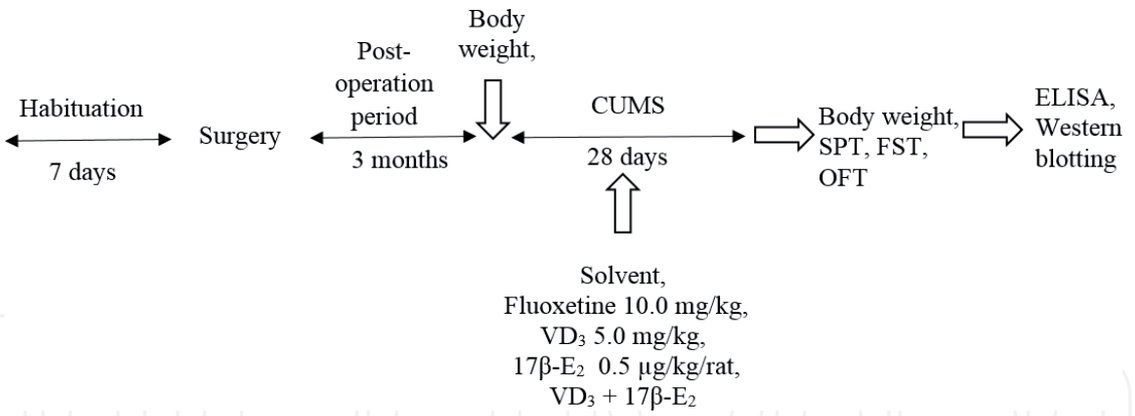

Figure 1.
Timeline of chronic treatment. Female Wistar rats were divided into 6 groups – non-CUMS SHAM rats treated with solvent (control), SHAM rats submitted to CUMS treated with solvent, long-term OVX rats exposed to CUMS given with solvent, fluoxetine as positive control (10.0 mg/kg/day), 17β-E_2 (0.5 μg/rat/day, s.c.) or VD_3 (5.0 mg/kg/day, s.c.) in a combination with low dose of 17β-E_2.

of depression state is strongly verified by both preclinical and clinical studies [46]. CUMS was made as described previously [47, 48]. The procedure included the exposure to different and unpredictable stress factors that are randomly changed during experimental days [49]. These manipulations are 24 h food deprivation, 24 h water deprivation, wet bedding overnight, titled cage overnight, unpredictable shocks (15 mA, one shocks/20s, 10 s duration, 20 min), 5 min swimming at cold water (4°C), tail hanging, 1 min, clip tail for 1 min, reversal of light/dark cycle [47, 48]. All stress triggers were performed individually and continuously. To prevent habituation and to ensure the unpredictability of the stressors, all stress manipulations randomly made accordingly to experimental scheme, repeated throughout the 4 weeks of CUMS protocol. The control SHAM females were placed in a separate room without any contact with the stressed groups of animals. These rats were maintained as undisturbed animals that are subjected only routine cage cleaning for 4 weeks. The total scheme of whole experiment is indicated on **Figure 1**.

2.4 Drugs

17β-E_2, fluoxetine hydrochloride and VD_3 as cholecalciferol were provided from Sigma Chemical Co. (St. Louis, MO, USA). The solution of female estrogen was prepared using sterile sesame oil. Vitamin D_3 was dissolved in 95% ethanol and then aliquoted and remained at −80°C. The solution of cholecalciferol for the injection into the experimental groups was diluted in sterile water, resulting in a solvent of VD_3 containing 2% ethanol. Fluoxetine hydrochloride was dissolved in sterile physiological saline. All drugs were injected subcutaneously (0.1 ml/rat) for the 4 weeks during the CUMS procedure – 30 min before the daily stressor action – and throughout the period of the behavioral tests. All behavioral measurements were made 60 min after the last drug administration.

2.5 Groups of animals

All animals were randomly assigned to the six experimental groups (n = 7 in each): non-CUMS SHAM rats treated with solvent (control), SHAM rats exposed to CUMS treated with solvent, long-term OVX rats exposed to CUMS given with solvent, fluoxetine as positive control (10.0 mg/kg/day), 17β-E_2 (0.5 μg/rat/day, s.c.) or VD_3 (5.0 mg/kg/day, s.c.) in a combination with low dose of 17β-E_2. In our preliminary studies, there were no significant differences between SHAM/OVX rats treated with physiological

saline as solvent for fluoxetine and SHAM/OVX females treated with sterile water with 2% ethanol as solvent for VD_3 or SHAM/OVX females treated with sesame oil as solvent for 17β-E_2 in behavioral trials (data are not shown). Since, we did not found any differences between these experimental groups, the sesame oil as solvent for SHAM/OVX females was used in the present work. The dose of VD_3 and dose of 17β-E_2 were based on our previous work on the behavioral effects of VD_3 on depression-like behavior of long-term OVX female rats submitted to CUMS [42, 43]. The dose of fluoxetine was utilized according to earlier studies demonstrating that the administration of fluoxetine decreases depressive-like behavior in rodents [42]. All drugs were injected subcutaneously (0.1 ml/rat) for the 4 weeks during the CUMS procedure – 30 min before the daily stressor action – and throughout the period of the behavioral tests. All behavioral measurements were made 60 min after the last drug administration.

2.6 Sucrose preference test

We performed SPT accordingly to our previous study [50, 51]. Before and after the initiation of the 4 weeks CUMS procedures, the experimental rats were subjected to the sucrose preference test (SPT) [42, 51]. This test is set up as follows: following a training trial, the rats are subjected to a 24 h deprivation of food and water. On the next day, the rats have 1 hour access to one bottle with 200 ml of water and a similar amount of sucrose solution. The experimenter measures the percentage of the consumed sucrose solution and water volumes as a measure of sucrose preference by calculating the value of the sucrose preference among all (sucrose plus water in mL) liquid consumption:

$$\%\text{sucrose preference} = \frac{\text{sucrose consumption}}{\text{sucrose consumption} + \text{water consumption}} \times 100 \qquad (1)$$

2.7 Forced swimming test

For testing of modifications of depression-like behavior, all groups of rats were submitted to the standard forced swimming test (FST) as described in earlier works (FST) [42, 43]. The three cylinders (60 cm tall and diameter 20 cm) were filled with 23–25°C water up to a 30-cm depth. On the first day, rats were pre-tested during 15 min in cylinders. Then, rats were dried with papers and placed at their home cages till the next day. On the second day (testing trial), OVX females with CUMS were examined into the apparatus for 5 min. The following parameters were registered: (1) immobility time (floating in the water with only movements necessary to keep the head above water); (2) swimming time (active swimming movements around glass cylinder); (3) climbing time (active movements with forepaws directed toward the walls). For recording of these values, a video camera was installed above the apparatus.

2.8 Open field test

The measurements of the behavioral activity in the OFT were carried out in a similar way to the method which has been published in a previous study [43]. The rats were set in the center square of the OFT and tested for 5 min. Motor activity and rearing and grooming behavior were recorded for 300 s in the OFT apparatus using a video camera, and equipment was cleaned in-between sessions.

2.9 Biochemical measurements

All rats underwent a narcosis after behavioral trials, and approximately 5 ml samples of blood were drawn from the animals to be centrifuged at 4000 *g* for

15 min at 4°C [43]. While doing so, the hippocampi of rats in the experimental group were dissected to be homogenized in cold lysis extraction buffer (0.2% sodium deoxycholate, 0.5% Triton X-100, 1% NP-40, 50 mM Tris-HCl pH 7.4, 1 mM phenylmethylsulfonyl fluoride, 1 mM N-ethyl-maleimide, and 2.5 mM phenanthroline) [43]. After that, the hippocampal samples with the cold lysis buffer were sonicated for 15 s. Then, the hippocampi were centrifuged at 12,000 *g* for 15 min at 4°C. The Bradford method was used for the normalization of hippocampal supernatants to the total protein [52]. The serum samples and hippocampal protein normalized supernatants were stored at −80°C until the ELISA assays. The serum samples were used for the measurement of the 25-hydroxyvitamin D_3 (25-OH-VD_3) and estradiol levels using a commercially available rat ELISA kits (Cusabio Biotech Co., Ltd., Wuhan, P.R. China) according to the manufacturer's instructions. The sensitivity and detection range of the 25-OH-VD_3 rat ELISA kits were 5.0 μg/l and 20–100 μg/l, respectively. The sensitivity and detection range of the estradiol rat ELISA kits were 4.0 pg/ml and 40–1500 pg/ml, respectively.

Hippocampal homogenates were used for the detection of the NF-kB/p65/p65, 17β-E_2/VD_3 receptors levels by rat ELISA kits (Cusabio Biotech Co., Ltd., Wuhan, P.R. China) according to the manufacturer's instructions. Briefly, 100 μl of hippocampal sample or standard was added to each well and incubated for 120 min at 37.0°C. Then, 100 μl of anti-NF-kB/p65/p65, anti-17β-E_2 receptor, and anti-VD_3 receptor antibodies were added to each different well and incubated for 60 min at 37.0°C. After 3 times of washing, 100 μl of HRP-avidin working solution was added to each well and incubated for 60 min at 37.0°C. Again, after 5 times of washing, 90 μl of tetramethylbenzidine solution was given to each different well and incubated for 15–30 min at 37.0°C. Then, 50 μl of stop solution was added to each well to terminate the color reaction. The NF-kB/p65/p65, 17β-E_2/VD_3 receptors levels were measured using a MC Thermo Fisher Scientific reader (Thermo Fisher Scientific Inc., Finland) with an absorbance of 450 nm. The standard curve was used for the calculation of the relationship between the optical density and the NF-kB/p65/p65, 17β-E_2/VD_3 receptors levels. The BDNF content is presented as pg/mg of tissue. The sensitivity and detection range of the NF-kB/p65 rat ELISA kit were 5.0 μg/ml and 6.0–600 μg/ml, respectively. The sensitivity and detection range of the 17β-E_2 receptor rat ELISA kit was 0.39 pg/ml and 1.56–100 pg/ml, respectively. The sensitivity and detection range of the VD_3 receptor rat ELISA kit was 5.8 pg/ml and 23.5–1500 pg/ml, respectively. The assay exhibited no significant cross reactivity with other ligands. All samples were duplicated for the assay.

2.10 Western blots

Hippocampal tissues were homogenized in cold lysis buffer containing a protease inhibitor cocktail (Sigma-Aldrich, USA) for 1 h and centrifuged at 12,000 *g* at 4°C for 20 min [42]. The protein content was evaluated by a Bio-Rad protein detector (Bio-Rad, USA), and 100 μg of total protein from each sample was denatured with buffer (6.205 mM Tris-HCl, 10% glycerol, 2% SDS, 0.01% bromophenol blue, and 50 mM 2ME) at 95°C for 5 min. The denatured proteins were separated on an SDS page (10% sodium dodecyl sulfate polyacrylamide gel) and forwarded to a nitrocellulose membrane (Amersham Biotech, USA). After that, the membranes were probed with anti-NF-kB/p65/p65, anti-17β-E_2 receptor, anti-VD_3 receptor (1:1000, Santa Cruz), and β-actin (1:1000; Sigma-Aldrich, USA) monoclonal antibodies for 2 h and secondary antirabbit antibodies (1:5000; Santa Cruz, USA) conjugated to horseradish peroxidase for 1 h. Bands were detected by 5-bromo-4-chloro-3-indolyl phosphate with a nitro blue tetrazolium kit (Abcam, China) as a chemiluminescent substrate. Signals were measured by an image analysis system (UVIdoc, Houston, TX, USA).

2.11 Statistical analysis

All experimental data are expressed as the mean ± standard deviation of the mean. The treatment effects were determined with a one-way ANOVA followed by an LSD *post hoc* test using the Statistics Package for SPSS, version 16.0 (SPSS Inc., USA). A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1 VD_3 alters the body weight in the long-term OVX rats treated with 17β-E_2 exposed to CUMS

The body weights of long-term OVX rats subjected to CUMS and treated with 17β-E_2 in a combination with all investigated doses of VD_3 are presented in **Figure 2**.

There was no difference in the initial body weight in all the experimental groups. Following 4 weeks, the body weight of SHAM rats with CUMS was significantly decreased compared to the control, non-CUMS SHAM group (**Figure 2**, $F(1,34) = 72.66$, $P < 0.001$). The body weight of long-term OVX rats with CUMS was significantly decreased compared to the non-CUMS/CUMS SHAM groups (**Figure 2**, $P < 0.001$). Administration of 17β-E_2 did not statistically enhance body weight of long-term OVX rats with CUMS compared to the non-CUMS control, CUMS OVX/SHAM groups (**Figure 2**, $P > 0.001$). However, there was a tendency to increase the body weight of long-term OVX

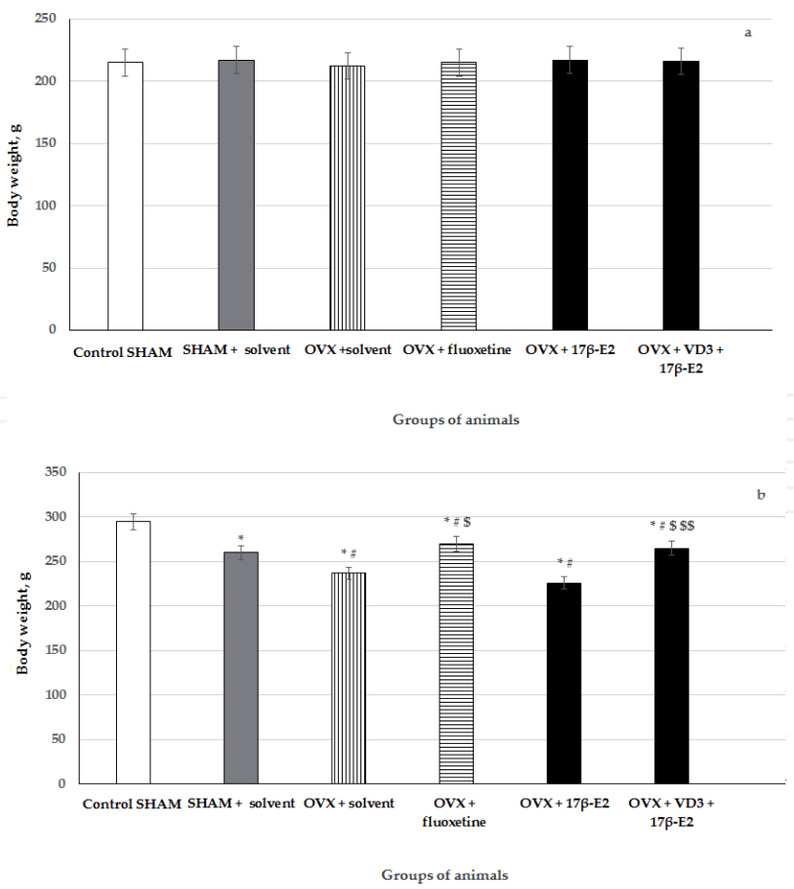

Figure 2.
*VD_3 corrects the body weight in the long-term OVX rats treated with 17β-E_2 submitted to CUMS: (a) Prior to CUMS and (b) After CUMS. * – $P < 0.05$ versus the control group, # – $P < 0.05$ versus to the SHAM group with CUMS, $ – $P < 0.05$ versus to the OVX group with CUMS, and $$ – $P < 0.05$ versus to the OVX group with CUMS treated with 17β-E_2. The data are presented as mean ± SD; n = 7 in each group.*

rats with CUMS compared to the OVX rats plus CUMS given with solvent. Supplementation with VD_3 (5.0 mg/kg) plus 17β-E_2 significantly prevented the reduction of the body weight of long-term OVX rats with CUMS ($P < 0.001$) compared to the OVX plus solvent or 17β-E_2/SHAM rats exposed to CUMS (**Figure 2**, $P < 0.001$). This effect of co-administration of VD_3 (5.0 mg/kg) plus 17β-E_2 was similar to the effect of the reference drug fluoxetine (10.0 mg/kg) in long-term OVX rats with CUMS.

3.2 VD_3 increases sucrose preference in the long-term OVX rats treated with 17β-E_2 exposed to CUMS

Before the CUMS protocol, there was no significant difference among the experimental groups in the SPT (**Figure 3**). Following 28 days of the CUMS trials, the SHAM rats exhibited a decrease in sucrose preference when compared to the control non-CUMS SHAM group ($P < 0.05$). The sucrose preference in long-term OVX rats was significantly reduced compared to the non-CUMS/CUMS SHAM rats (**Figure 3**, $F(1,34) = 56.14$, $P < 0.05$). Low dose of 17β-E_2 increased sucrose preference in long-term OVX rats with CUMS compared to the OVX group with CUMS plus solvent (**Figure 3**, $P > 0.05$). Treatment with VD_3 at dose of 5.0 mg/kg plus 17β-E_2, as well as fluoxetine, markedly increased sucrose

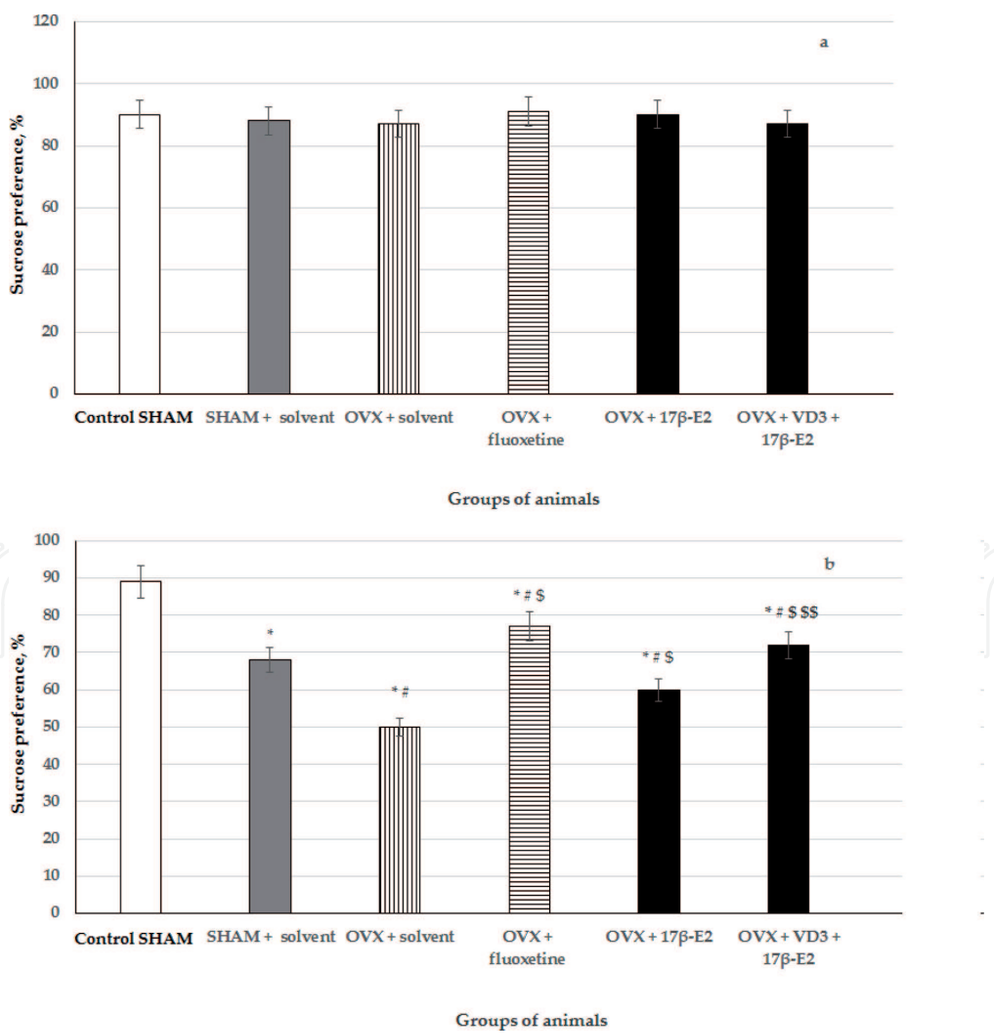

Figure 3.
*VD_3 increases sucrose preference in the long-term OVX rats treated with 17β-E_2 submitted to CUMS: (a) Prior to CUMS and (b) After CUMS. * – $P < 0.05$ versus the control group, # – $P < 0.05$ versus to the SHAM group with CUMS, $ – $P < 0.05$ versus to the OVX group with CUMS, and $$ – $P < 0.05$ versus to the OVX group with CUMS treated with 17β-E_2. The data are presented as mean ± SD; n = 7 in each group.*

consumption in the long-term OVX rats exposed to CUMS when compared to the OVX plus solvent or 17β-E_2/SHAM rats submitted to the CUMS (**Figure 3**, $P < 0.05$).

3.3 VD_3 decreases depression-like behavior in the forced swimming test of long-term OVX rats treated with 17β-E_2 exposed to CUMS

CUMS produced a significant increase of the immobility time and decrease of swimming time in the long-term OVX compared to the non-CUMS/CUMS SHAM rats (**Figure 4**, $F(1,34) = 52.84$, $F(1,76) = 68.89$, $F(1,76) = 26.12$, respectively,

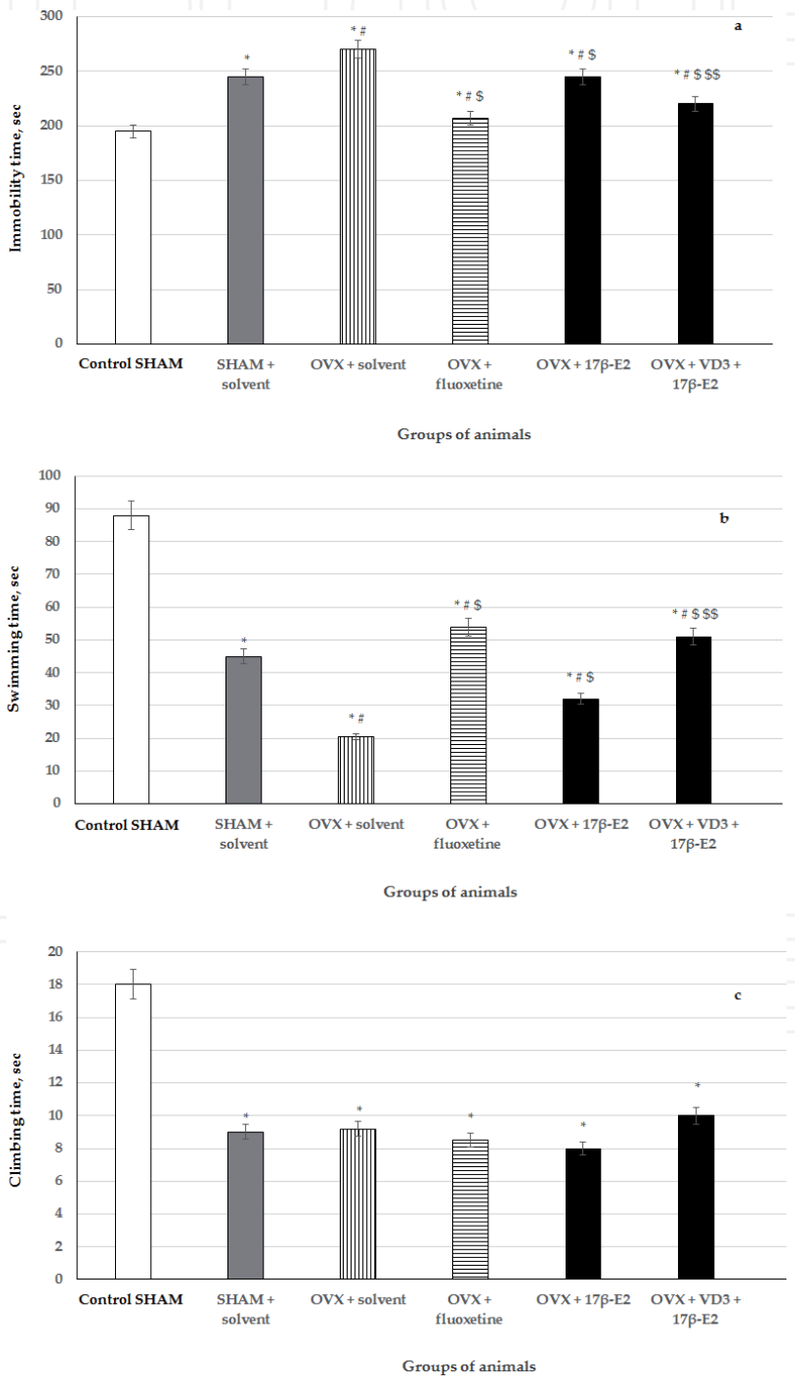

Figure 4.
*VD_3 decreased depression-like behavior in the forced swimming test of long-term OVX rats treated with 17β-E_2 submitted to CUMS: (a) immobility time, sec, (b) swimming time, and (c) climbing time, sec. * – $P < 0.05$ versus the control group, # – $P < 0.05$ versus to the SHAM group with CUMS, $ – $P < 0.05$ versus to the OVX group with CUMS, and $$ – $P < 0.05$ versus to the OVX group with CUMS treated with 17β-E_2. The data are presented as mean ± SD; n = 7 in each group.*

P < 0.05). VD_3 (5.0 mg/kg), as well as fluoxetine treatment, significantly reduced the immobility time and increased the swimming time in the long-term OVX treated with 17β-E_2 compared to the OVX plus solvent or 17β-E_2/SHAM with CUMS groups (**Figure 4**, P < 0.05).

3.4 VD_3 changes the behavior in the open field test of long-term OVX rats treated with 17β-E_2 exposed to CUMS

Following 28 days of CUMS protocol, there were no statistically significant differences for grooming activities between all the experimental groups of animals in the OFT (**Figure 5**, F(1,34) = 0.82, P > 0.05). The long-term OVX rats with CUMS

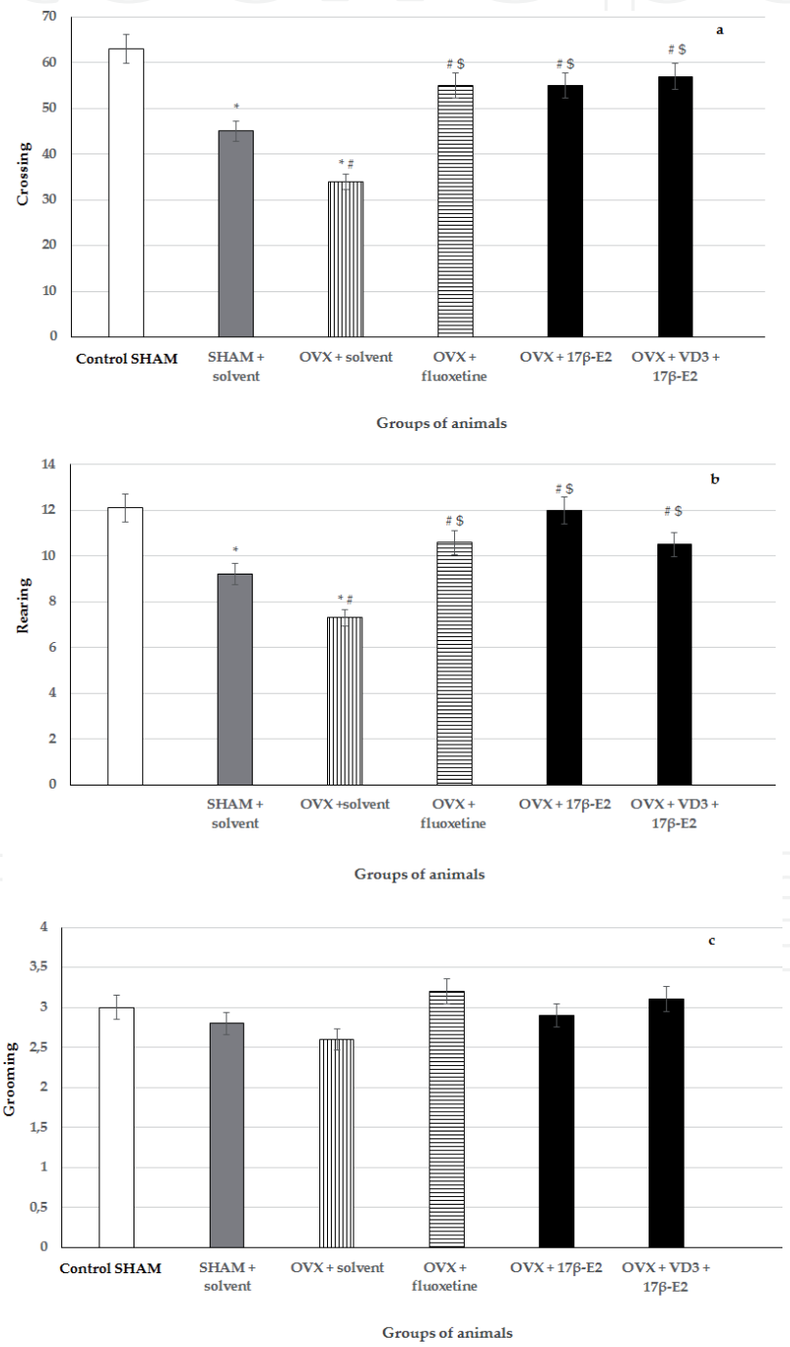

Figure 5.
*VD_3 alters the behavior in the open field test of long-term OVX rats treated with 17β-E_2 submitted to CUMS. (a) Crossing, (b) rearing, and (c) grooming. * – P < 0.05 versus the control group, # – P < 0.05 versus to the SHAM group with CUMS, $ – P < 0.05 versus to the OVX group with CUMS, and $$ – P < 0.05 versus to the OVX group with CUMS treated with 17β-E_2. The data are presented as mean ± SD; n = 7 in each group.*

showed a decreased number of rearings and crossings when they were compared to the non-CUMS/CUMS SHAM groups (**Figure 5**, $F(1,34) = 14.14$, $P < 0.05$). Administration of fluoxetine, 17β-E_2, as well as treatment with VD_3 significantly elevated the number of rearings and crossings in the long-term OVX rats with CUMS compared to the OVX/SHAM rats with CUMS plus solvent (**Figure 5**).

3.5 VD_3 alters serum estradiol and VD_3 levels in long-term OVX rats treated with 17β-E_2 exposed to CUMS

The ELISA assay revealed decreased estradiol and VD_3 concentrations in the long-term OVX rats with CUMS compared to the non-CUMS/CUMS SHAM groups (**Figure 6**, $F(1,34) = 78.56$, $F(1,34) = 56.12$, $F(1,34) = 22.21$, respectively, $P < 0.05$). Low dose of 17β-E_2 induced increase of estradiol levels in the serum blood to some extent in the long-term OVX rats with CUMS, however, it was not statistically significant ($P > 0.05$). The co-administration of VD_3 with 17β-E_2 significantly increased estradiol and VD_3 concentrations in the long-term OVX rats with CUMS compared to the OVX plus solvent or 17β-E_2/SHAM with CUMS rats (**Figure 6**, $P < 0.05$). Fluoxetine did not change the serum

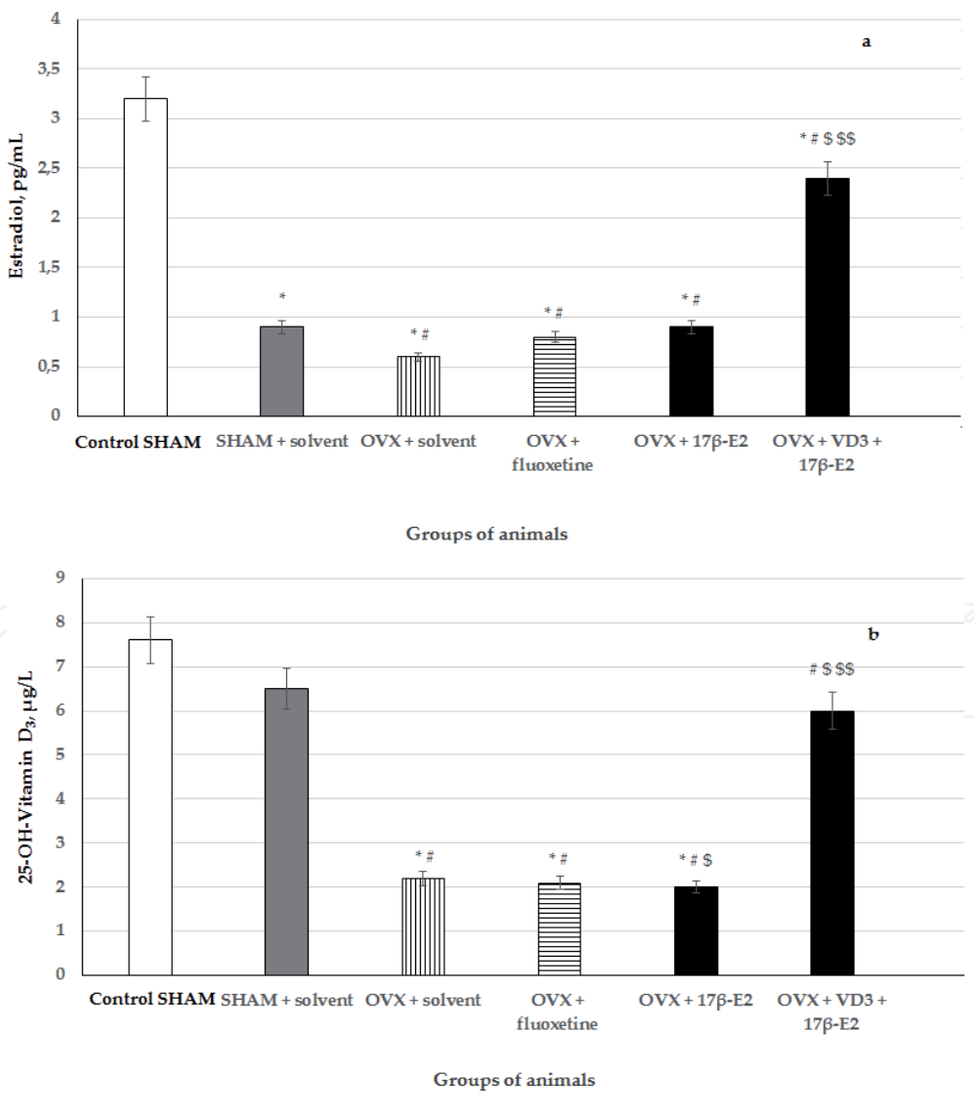

Figure 6.
*VD_3 alters serum estradiol and VD_3 levels in long-term OVX rats treated with 17β-E_2 submitted to CUMS. (a) estradiol, pg/ml and (b) 25-OH-VD3, μg/ml. * – $P < 0.05$ versus the control group, # – $P < 0.05$ versus to the SHAM group with CUMS, $ – $P < 0.05$ versus to the OVX group with CUMS, and $$ – $P < 0.05$ versus to the OVX group with CUMS treated with 17β-E_2. The data are presented as mean ± SD; n = 7 in each group.*

estradiol and VD_3 levels in the long-term OVX rats exposed to CUMS (**Figure 6**, $P > 0.05$).

3.6 VD_3 modulates hippocampal NF-kB/p65/p65 and 17β-E_2/VD_3 receptors levels in long-term OVX rats treated with 17β-E_2 exposed to CUMS

CUMS significantly increased NF-kB/p65/p65 levels and decreased 17β-E_2/VD_3 receptors concentrations in the hippocampus of SHAM rats compared to the non-CUMS control (**Figure 7**, $P < 0.05$). CUMS produced a increase of hippocampal

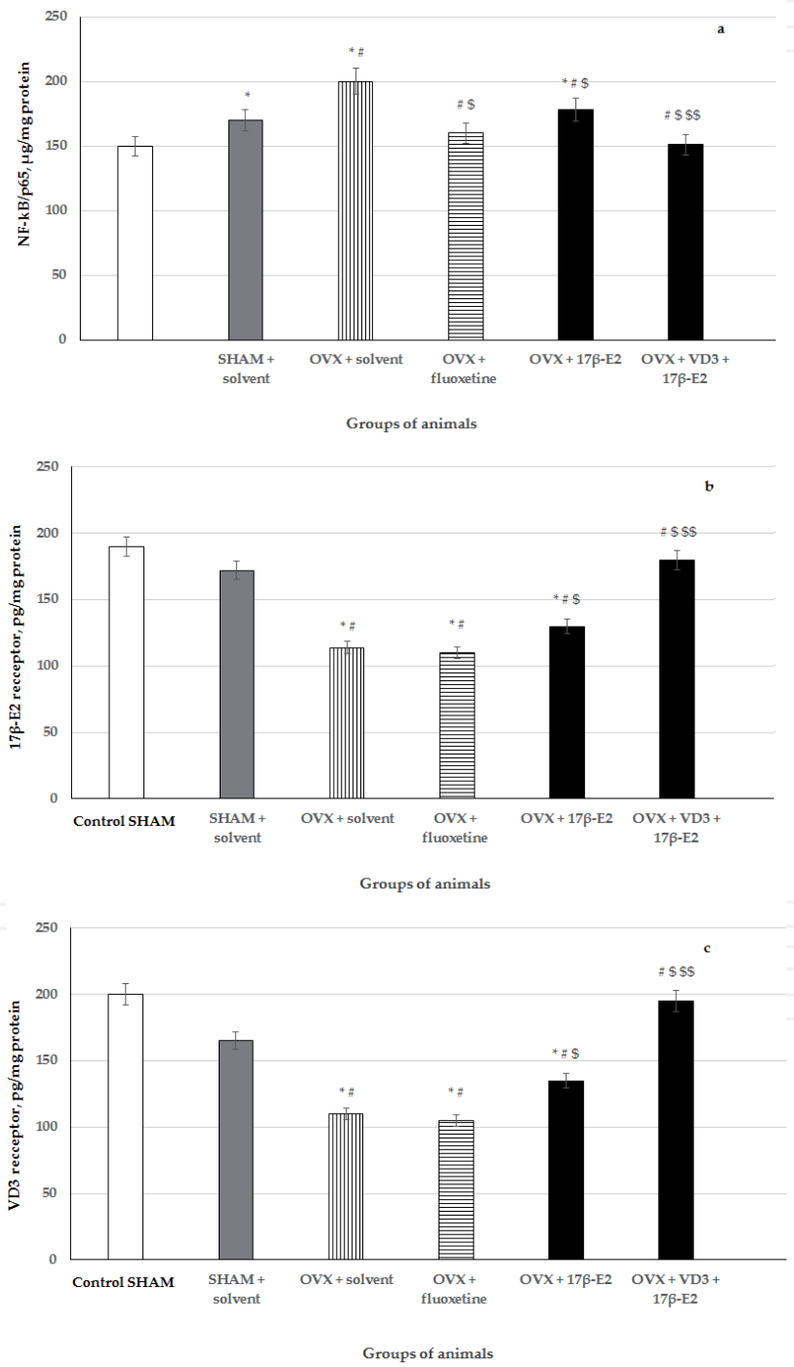

Figure 7.
*VD_3 modulates hippocampal NF-kB/p65 and 17β-E_2/VD_3 receptors levels in long-term OVX rats treated with 17β-E_2 submitted to CUMS tested by ELISA. (a) NF-kB/p65/p65, µg/ml, (b) 17β-E_2 receptor, pg/ml, and (c) VD_3 receptor, pg/ml. * – $P < 0.05$ versus the control group, # – $P < 0.05$ versus to the SHAM group with CUMS, $ – $P < 0.05$ versus to the OVX group with CUMS, and $$ – $P < 0.05$ versus to the OVX group with CUMS treated with 17β-E_2. The data are presented as mean ± SD; n = 7 in each group.*

NF-kB/p65/p65 and decrease of 17β-E_2/VD_3 receptors levels in the long-term OVX rats compared to the non-CUMS/CUMS SHAM rats (**Figure 7**, $F(1,34) = 28.44$, $P < 0.05$).

Fluoxetine (10.0 mg/kg) decreased NF-kB/p65/p65 levels in the hippocampus of long-term OVX rats treated to CUMS compared to the OVX plus solvent/SHAM rats with CUMS (**Figure 7**, $P < 0.05$). Moreover, VD_3 plus 17β-E_2 reversed 17β-E_2/VD_3 receptors levels and reduced NF-kB/p65 levels in the hippocampus of the long-term OVX rats compared to OVX plus solvent or 17β-E_2/SHAM rats with CUMS (**Figure 7**, $P < 0.05$). Fluoxetine failed to modify 17β-E_2/VD_3 receptors levels in the long-term OVX rats exposed to CUMS (**Figure 7**, $P > 0.05$).

Western blotting analysis revealed that NF-kB/p65 protein levels in the hippocampus of SHAM rats submitted to CUMS were higher compared to non-CUMS control

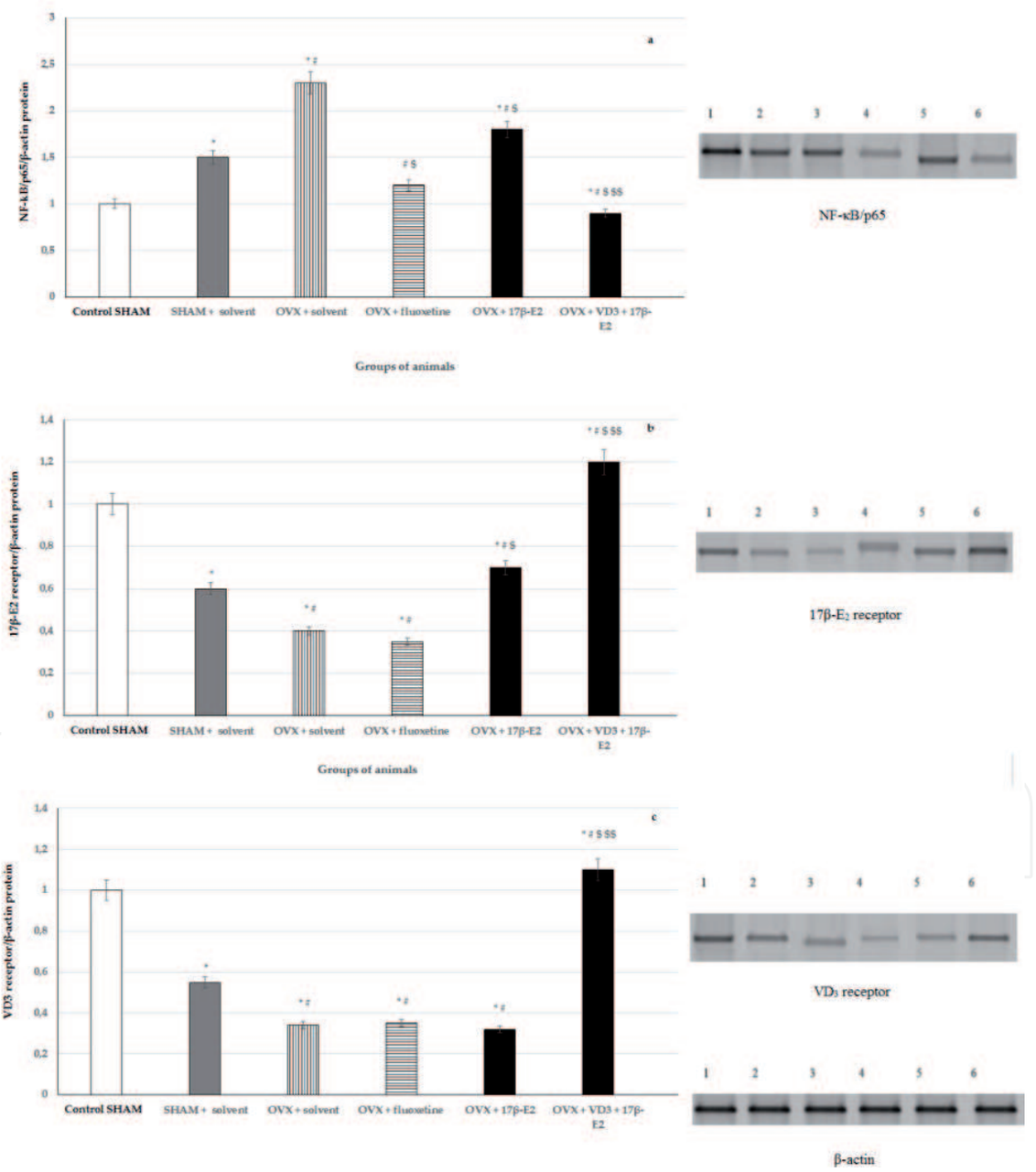

Figure 8.
*VD_3 modulates hippocampal NF-kB/p65 and 17β-E_2/VD_3 receptors expressions in long-term OVX rats with 17β-E_2 submitted to CUMS detected with western blotting treated. 1 – control SHAM, 2 – SHAM + CUMS + solvent, 3 – OVX + CUMS + solvent, 4 – OVX rats + CUMS + fluoxetine, 5 – OVX rats + CUMS + 17β-E_2, and 6 – OVX rats + CUMS + VD_3 + 17β-E_2. * – $P < 0.05$ versus the control group, # – $P < 0.05$ versus to the SHAM group with CUMS, $ – $P < 0.05$ versus to the OVX group with CUMS, and $$ – $P < 0.05$ versus to the OVX group with CUMS treated with 17β-E_2. The data are presented as mean ± SD; $n = 7$ in each group.*

females (**Figure 8**, $P < 0.05$). NF-kB/p65 levels were increased in the hippocampus of long-term OVX rats with CUMS compared to the non-CUMS/CUMS SHAM rats (**Figure 8**, $F(1,34) = 34.45$, $F(1,34) = 16.38$, respectively, $P < 0.05$). Fluoxetine (10.0 mg/kg) resulted in significant reduced levels of hippocampal NF-kB/p65 protein expression in long-term OVX with CUMS compared to the OVX plus solvent/SHAM rats with CUMS (**Figure 8**, $P < 0.05$). Co-treatment with VD_3 and 17β-E_2 decreased NF-kB/p65 protein levels and increased 17β-E_2/VD_3 receptors protein expression in the hippocampus of the long-term OVX rats compared to OVX plus solvent or 17β-E_2/SHAM rats with CUMS (**Figure 8**, $P < 0.05$). Fluoxetine did not alter 17β-E_2/VD_3 protein expression in the long-term OVX rats exposed to CUMS (**Figure 8**, $P > 0.05$).

4. Discussion

The present preclinical study analyzed the antidepressant-like effects of VD_3 (5.0 mg/kg, s.c.) in long-term adult OVX female rats given with low dose of 17β-E_2 subjected to the CUMS. A CUMS paradigm is a well-known experimental paradigm that has documented to consider as standard pathophysiological impairments in mood state linked to a clinical depressive disorders in humans [47–49]. In the present work, the implications of NF-kB/p65 signaling pathway, as well as the 17β-E_2/VD_3 receptors, in the mechanisms of VD_3 activity in depression were tested regarding to the affective-related condition of long-term adult OVX rats treated with low dose of 17β-E_2 exposed to CUMS.

The results of this study showed that in the adult long-term OVX rats undergoing CUMS, there were marked anhedonia-/depression-like behaviors, as assessed by SPT and LDT, respectively.

Moreover, long-term OVX rats exposed to CUMS exhibited decreased locomotor and rearing activities in the OFT. The ELISA assay clearly demonstrated lower estradiol and VD_3 concentrations in adult long-term OVX rats subjected to CUMS. In addition, the increased NF-kB/p65 concentration/protein expression and decreased 17β-E_2/VD_3 receptors levels were found in the hippocampus of long-term OVX rats exposed to CUMS.

Administration of 17β-E_2 failed to completely restore behavioral and biochemical parameters in the long-term OVX rats exposed to CUMS. Fluoxetine decreased anhedonia-like and depression-like states and decreased NF-kB/p65 levels in the hippocampus of the long-term OVX female rats exposed to CUMS. Data of literature have demonstrated that fluoxetine corrected depression-like profile of OVX rats in stress depression model [53]. The results of the study indicate that CUMS provokes marked behavioral, neurochemical, neurohormonal, and neuroinflammation alterations in adult OVX rats with long-lasting estrogens decline. Our data are in agreement with our recent data and other findings, which indicated that long-term estrogen deprivation in female rodents subjected to a CUMS procedure results in a profound affective-like profile [54].

The most important findings of the present study is linked to the antidepressant-like effects of VD_3 in the long-term adult OVX rats treated with low dose of 17β-E_2 under conditions of CUMS. VD_3 given with a dose of 5.0 mg/kg reversed anhedonia-like and depression-like states in the SPT/LDT paradigms in the long-term OVX rats treated with 17β-E_2 subjected to CUMS, which was similar to the effects of the fluoxetine treatment. Moreover, the VD_3 application reversed the behavioral impairments observed in the OFT in the long-term OVX rats supplemented with 17β-E_2 subjected to CUMS. Biochemical assays found that VD_3 increased the serum VD_3 and estradiol levels, as well decreased the hippocampal NF-kB/p65 content in the long-term OVX rats treated with 17β-E_2 exposed to CUMS. Additionally, VD_3

increased 17β-E_2/VD_3 receptors levels in the hippocampus of long-term OVX rats treated with 17β-E_2 subjected to CUMS. Western blot analysis revealed that VD_3 reduced NF-kB/p65 and increased 17β-E_2/VD_3 protein expression in the hippocampus of long-term OVX rats treated with 17β-E_2 subjected to CUMS. These data suggest that VD_3 attenuates the CUMS-produced behavioral impairments and normalized the serum VD_3 and estradiol levels, as well NF-kB/p65 and 17β-E_2/VD_3 production in the hippocampus of long-term OVX rats.

Inflammation is now recognized to be the one of the key components of affective-related development, with the NF-κB involved in both the early and late stages of the inflammatory processing [33, 41, 55]. NF-κB is the main transcriptional factor which controls the expression of various genes implicated in multiple cell functions and is triggered by different types of extracellular stimuli stimulated neuroinflammation [33, 34]. Deterioration of NF-κB signaling in the brain negatively influence on neuroplasticity and neuromorphology, as well as cognitive functions. However, NF-κB overstimulation is deleterious, and this detrimental effect can be reversed suppressing NF-κB signaling. That is why, NF-κB signaling is fundamental for normal brain function [36–39, 55]. The inhibition of neuroinflammation may be critical for the antidepressant action of VD_3 that was noted in our study. The increased pro-inflammatory cytokines have been found repeatedly in both animals and depressed patients [37–39]. Clinical studies indicate that inhibition of neuroinflammation by nonsteroidal antiinflammatory drugs can attenuate depression-like behaviors in depressed rodents and humans [36–39]. The inhibitory effect of VD_3 on CUMS-induced increase in pro-inflammatory cytokines in the hippocampus of the long-term OVX rats is strongly in accordance with the neuroinflammation hypothesis of depression [39]. A better comprehension of NFκB-dependent mechanisms in antidepressant action of VD_3 needs further studies.

Based on our findings, it can be assumed that VD_3 in the present study is implicated in the modulation of NF-κB signaling in long-term OVX rats with CUMS. On the other hand, VD_3 normalized 17β-E_2/VD_3 receptors levels in the hippocampus of long-term OVX rats treated with 17β-E_2 subjected to CUMS. Such complex effects of VD_3 on neuroinflammation and 17β-E_2/VD_3 receptors might promote a greater effect of combination of VD_3 plus 17β-E_2 than application only 17β-E_2. This is the first study to show the action of VD_3 in the behavioral and neuroinflammation and biochemical consequences of a CUMS in adult long-term OVX rats treated with low dose of 17β-E_2. The inhibition of NF-kB/p65 activity by VD_3 treatment is a promising fact of study for treatment of neuroinflammatory diseases that are associated with low levels of VD_3. These results suggest an anti-inflammatory role for VD_3, which may be one of the fundamental components of its activity.

Thus, the possible mechanism of VD_3 action might be explained by the stimulation of 17β-E_2/VD_3 receptors identified in the different brain structures involved in mood control [13–15]. The possible mechanisms of such action of VD_3 in the long-term OVX rats can be connected with cross-talk protein-protein interactions. Moreover, VD alters the neuroinflammation response via NF-kB/p65 signaling at the affective-related state, thereby improving depression state [39, 55]. Low VD levels appear in the majority of postmenopausal women [52, 56, 57]. Therefore, VD supplementation may be very useful for treatment of mood disorders in postmenopausal women with a low level of VD and supplemented with MHT. However, the exact role of VD supplementation in the prevention and treatment of mood disorders associated with menopausal consequences has not been completely identified.

In conclusion, the present study supports evidence for repeated administration of VD_3 in a chronic unpredictable stress model having an anti-anhedonia-like and antidepressant-like effects in long-term OVX adult rats treated with low dose of 17β-E_2. Moreover, the biochemical and western blotting assays suggest the

implications of NF-kB/p65 and 17β-E_2/VD_3 production modulation in the antidepressant-like activity of VD_3. Further studies should however explore the precise mechanism of VD_3 action, due to the necessity of an improvement of therapies focusing on mood-repair in females with long-lasting estrogen deficiency.

5. Conclusions

This study demonstrated that VD_3-induced antianhedonic- and antidepressant-like effects in the adult female rats following long-lasting estrogens decline treated with low dose of 17β-E_2 submitted to CUMS. Treatment with VD_3 modulates NF-kB/p65 concentration/protein expression and 17β-E_2/VD_3 receptors levels in the hippocampus of long-term OVX rats exposed to CUMS treated with low dose of 17β-E_2. Our study yields new knowledge into the mechanisms by which VD_3 affects to alleviate anhedonia- and depressive-like behaviors in female rodents with long-term estrogen deficiency in stress model of depression.

Acknowledgements

The reported study was funded by the Russian Science Foundation (RSF) (research project No. 16-15-10053 (extension)).

Conflicts of interest

The authors declare no conflict of interest.

Author details

Alexandra Koshkina[1], Olga Volkova[1] and Julia Fedotova[1,2*]

1 Faculty of Food and Bioengineering, ITMO University, St. Petersburg, Russia

2 Laboratory of Neuroendocrinology, I.P. Pavlov Institute of Physiology RASci, St. Petersburg, Russia

*Address all correspondence to: julia.fedotova@mail.ru

IntechOpen

© 2019 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

References

[1] Garcia-Portilla MP. Depression and perimenopause: A review. Actas Españolas de Psiquiatría. 2009;**37**(4):213-221

[2] Frey BN, Lord C, Soares CN. Depression during menopausal transition: A review of treatment strategies and pathophysiological correlates. Menopause International. 2008;**14**(3):123-128. DOI: 10.1258/mi.2008.008019

[3] Luppa M, Sikorski C, Luck T, Ehreke L, Konnopka A, Wiese B, et al. Age- and gender-specific prevalence of depression in latest-life--systematic review and meta-analysis. Journal of Affective Disorders. 2012;**136**(3):212-221. DOI: 10.1016/j.jad.2010.11.033

[4] Arevalo M-A, Azcoitia I, Garcia-Segura LM. The neuroprotective actions of oestradiol and oestrogen receptors. Nature Reviews. Neuroscience. 2015;**16**(1):17-29. DOI: 10.1038/nrn3856

[5] Lagunas N, Calmarza-Font I, Diz-Chaves Y, Garcia-Segura LM. Long-term ovariectomy enhances anxiety- and depressive-like behaviors in mice submitted to chronic unpredictable stress. Hormones and Behavior. 2010;**58**(5):786-791. DOI: 10.1016/j.yhbeh.2010.07.014

[6] Bernardi M, Vergoni AV, Sandrini M, Tagliavini S, Bertolini A. Influence of ovariectomy, estradiol and progesterone on the behavior of mice in an experimental model of depression. Physiology & Behavior. 1989;**45**(5):1067-1068. DOI: 10.1016/0031-9384(89)90238-2

[7] Su Q, Cheng Y, Jin K, Cheng J, Lin Y, Lin Z, et al. Estrogen therapy increases BDNF expression and improves post-stroke depression in ovariectomy-treated rats. Experimental and Therapeutic Medicine. 2016;**12**(3):1843-1848. DOI: 10.3892/etm.2016.3531

[8] Fedotova J, Dudnichenko T. Effects of different doses of vitamin D_3 supplementation in young perimenopausal women on depression and hormonal status. Psychoneuroendocrinology. 2017;**83**(Suppl 1):35. DOI: 10.1016/j.psyneuen.2017.07.331

[9] Fedotova JO. Vitamin D_3 treatment differentially affects anxety-like behavior in the old ovariectomized female rats treated with low dose of 17β-estradiol. BMC Medical Genetics. 2019;**20**(1):49. DOI: 10.1186/s12881-019-0774-2

[10] Lagana AS, Vitale SG, Ban Frangez H, Vrtacnik-Bokal E, D'Anna R. Vitamin D in human reproduction: The more, the better? An evidence-based critical appraisal. European Review for Medical and Pharmacological Sciences. 2017;**21**(18):4243-4251

[11] Milaneschi Y, Shardell M, Corsi AM, Vazzana R, Bandinelli S, Guralnik JM, et al. Serum 25-hydroxyvitamin D and depressive symptoms in older women and men. The Journal of Clinical Endocrinology and Metabolism. 2010;**95**(7):3225-3223. DOI: 10.1210/jc.2010-0347

[12] Fedotova JO, Pivina SG, Volkova OV. Vitamin D_3 application attenuates anxiety-like profile and increases 25-OH-VD3 levels in the serum blood of the middle-aged female rats at 12 week after overiectomy. Activitas Nervosa Superior Rediviva. 2018;**60**(2):55-56

[13] Holick MF. Vitamin D deficiency. The New England Journal of Medicine. 2007;**357**(3):266-281. DOI: 10.1056/NEJMra070553

[14] Holick MF, Chen TC. Vitamin D deficiency: A worldwide problem with health consequences. The American Journal of Clinical Nutrition. 2008;**87**(4):1080S-1086S. DOI: 10.1093/ajcn/87.4.1080S

[15] Kesby JP, Eyles DW, Burne TH, McGrath JJ. The effects of vitamin D on brain development and adult brain function. Molecular and Cellular Endocrinology. 2011;**347**(1-2):121-127. DOI: 10.1016/j.mce.2011.05.014

[16] Eyles DW, Burne TH, McGrath JJ. Vitamin D, effects on brain development, adult brain function and the links between low levels of vitamin D and neuropsychiatric disease. Frontiers in Neuroendocrinology. 2013;**34**(1):47-64. DOI: 10.1016/j.yfrne.2012.07.001

[17] Garcion E, Wion-Barbot N, Montero-Menei CN, Berger F, Wion D. New clues about vitamin D functions in the nervous system. Trends in Endocrinology and Metabolism. 2002;**13**(3):100-105. DOI: 10.1016/S1043-2760(01)00547-1

[18] Adams JS, Hewison M. Update in vitamin D. Journal of Clinical Endocrinology and Metabolism. 2010;**95**(2):471-478. DOI: 10.1210/jc.2009-1773

[19] DeLuca GC, Kimball SM, Kolasinski J, Ramagopalan SV, Ebers GC. Review: The role of vitamin D in nervous system health and disease. Neuropathology and Applied Neurobiology. 2013;**39**(5):458-484. DOI: 10.1111/nan.12020

[20] Eyles DW, Feron F, Cui X, Kesby JP, Harms LH, Ko P, et al. Developmental vitamin D deficiency causes abnormal brain development. Psychoneuroendocrinology. 2009;**34**(1):S247-S257. DOI: 10.1016/j.psyneuen.2009.04.015

[21] Obradovic D, Gronemeyer H, Lutz B, Rein T. Cross-talk of vitamin D and glucocorticoids in hippocampal cells. Journal of Neurochemistry. 2006;**96**(2):500-509. DOI: 10.1111/j.1471-4159.2005.03579.x

[22] Parker GB, Brotchie H, Graham RK. Vitamin D and depression. Journal of Affective Disorders. 2017;**208**:56-61. DOI: 10.1016/j.jad.2016.08.082

[23] Sarris J, Murphy J, Mischoulon D, Papakostas GI, Fava M, Berk M, et al. Adjunctive nutraceuticals for depression: A systematic review and meta-analyses. The American Journal of Psychiatry. 2016;**173**(6):575-587. DOI: 10.1176/appi.ajp.2016.15091228

[24] Sepehrmanesh Z, Kolahdooz F, Abedi F, Mazroii N, Assarian A, Asemi Z, et al. Vitamin D supplementation affects the beck depression inventory, insulin resistance, and biomarkers of oxidative stress in patients with major depressive disorder: A randomized, controlled clinical trial. The Journal of Nutrition. 2016;**146**(2):243-248. DOI: 10.3945/jn.115.218883

[25] Spedding S. Vitamin D and depression: A systematic review and meta-analysis comparing studies with and without biological flaws. Nutrients. 2014;**6**(4):1501-1518. DOI: 10.3390/nu6041501

[26] Chu F, Ohinmaa A, Klarenbach S, Wong ZW, Veugelers P. Serum 25Hydroxyvitamin D concentrations and indicators of mental health: An analysis of the Canadian health measures survey. Nutrients. 2017;**9**(10):E1116. DOI: 10.3390/nu9101116

[27] Miller AH, Raison CL. The role of inflammation in depression: From evolutionary imperative to modern treatment target. Nature Reviews. Immunology. 2016;**16**(1):22-34. DOI: 10.1038/nri.2015.5

[28] Song C, Wang H. Cytokines mediated inflammation and decreased neurogenesis in animal models of depression. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2011;**35**(3):760-768. DOI: 10.1016/j.pnpbp.2010.06.020

[29] Rosenblat JD, Cha DS, Mansur RB, McIntyre RS. Inflamed moods: A review of the interactions between inflammation and mood disorders. Progress in Neuro-Psychopharmacology & Biological Psychiatry. 2014;**53**:23-34. DOI: 10.1016/j.pnpbp.2014.01.013

[30] Napetschnig J, Wu H. Molecular basis of NF-kB signaling. Annual Review of Biophysics. 2013;**42**:443-468. DOI: 10.1146/annurev-biophys-083012-130338

[31] Barnes PJ. Nuclear factor-kappa B. The International Journal of Biochemistry & Cell Biology. 1997;**29**(6):867-870

[32] Lawrence T. The nuclear factor NF-kappa B pathway in inflammation. Cold Spring Harbor Perspectives in Biology. 2009;**1**(6):a001651. DOI: 10.1101/cshperspect.a001651

[33] Li Q, Verma IM. NF-kappa B regulation in the immune system. Nature Reviews. Immunology. 2002;**2**(10):725-734. DOI: 10.1038/nri910

[34] McKay LI, Cidlowski JA. Molecular control of immune/inflammatory responses: Interactions between nuclear factor-kappa B and steroid receptor-signaling pathways. Endocrine Reviews. 1999;**20**(4):435-459. DOI: 10.1210/edrv.20.4.0375

[35] Galecki P, Mossakowska-Wójcik J, Talarowska M. The anti-inflammatory mechanism of antidepressants-SSRIs, SNRIs. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2018;**80**(Pt C):291-294. DOI: 10.1016/j.pnpbp.2017.03.016

[36] Swardfager W, Rosenblat JD, Benlamri M, McIntyre RS. Mapping inflammation onto mood: Inflammatory mediators of anhedonia. Neuroscience and Biobehavioral Reviews. 2016;**64**:148-166. DOI: 10.1016/j.neubiorev.2016.02.017

[37] Wohleb ES, Delpech JC. Dynamic cross-talk between microglia and peripheral monocytes underlies stress-induced neuroinflammation and behavioral consequences. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2017;**79**(Pt A):40-48. DOI: 10.1016/j.pnpbp.2016.04.013

[38] Hanson ND, Owens MJ, NemeroffCB.Depression,antidepressants, and neurogenesis: A critical reappraisal. Neuropsychopharmacology. 2011;**36**(13):2589-2502. DOI: 10.1038/npp.2011.220

[39] Kopschina Feltes P, Doorduin J, Klein HC, Juárez-Orozco LE, Dierckx RA, Moriguchi-Jeckel CM, et al. Anti-inflammatory treatment for major depressive disorder: Implications for patients with an elevated immune profile and non-responders to standard antidepressant therapy. Journal of Psychopharmacology. 2017;**31**(9):1149-1165. DOI: 10.1177/0269881117711708

[40] Siebert C, CG B'o, Ferreira FC, de Souza Moreira D, dos Santos TM, Wyse ATS. Vitamin D partially reverses the increase in p-NF-B/p65 immunocontent and interleukin-6 levels, but not in acetylcholinesterase activity in hippocampus of adult female ovariectomized rats. International Journal of Developmental Neuroscience. 2018;**71**:122-129. DOI: 10.1016/j.ijdevneu.2018.08.008

[41] Mincheva-Tasheva S, Soler RM. NF-κB signaling pathways: Role

in nervous system physiology and pathology. Neuroscientist. 2013;**19**(2):175-194. DOI: 10.1177/1073858412444007

[42] Koshkina A, Dudnichenko T, Baranenko D, Fedotova J, Drago F. Effects of vitamin D3 in long-term ovariectomized rats subjected to chronic unpredictable mild stress: BDNF, NT-3, and NT-4 implications. Nutrients. 2019;**11**(8):E1726. DOI: 10.3390/nu11081726

[43] Fedotova J, Pivina S, Suchko A. Effects of chronic vitamin D_3 hormone administration on anxiety-like behavior in adult female rats after long-term ovariectomy. Nutrients. 2017;**9**(1):E28. DOI: 10.3390/nu9010028

[44] Bekku N, Yoshimura H. Animal model of menopausal depressive-like state in female mice: Prolongation of immobility time in the forced swimming test following ovariectomy. Psychopharmacology. 2005;**183**(3): 300-307. DOI: 10.1007/s00213-005-0179-0

[45] Bosee R, Di Paolo T. Dopamine and GABAA receptor imbalance after ovariectomy in rats: Model of menopause. Journal of Psychiatry & Neuroscience. 1995;**20**(5):364-371

[46] Banasr M, Valentine GW, Li XY, Gourley SL, Taylor JR, Duman RS. Chronic unpredictable stress decreases cell proliferation in the cerebral cortex of the adult rat. Biological Psychiatry. 2007;**62**(5):496-404. DOI: 10.1016/j.biopsych.2007.02.006

[47] Katz RJ. Animal models and human depressive disorders. Neuroscience and Biobehavioral Reviews. 1981;**5**(2):231-246. DOI: 10.1016/0149-7634(81)90004-X

[48] Willner P, Towell A, Sampson D, Sophokleous S, Muscat R. Reduction of sucrose preference bychronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. Psychopharmacologia. 1987;**93**(3): 358-364. DOI: 10.1007/bf00187257

[49] Burstein O, Franko M, Gale E, Handelsman A, Barak S, Motsan S, et al. Escitalopram and NHT normalized stress-induced anhedonia and molecular neuroadaptations in a mouse model of depression. PLoS One. 2017;**12**(11):e0188043. DOI: 10.1371/journal.pone.0188043. eCollection 2017

[50] Anisman H, Matheson K. Stress, depression, and anhedonia: Caveats concerning animal models. Neuroscience and Biobehavioral Reviews. 2005;**29**(4-5):525-546. DOI: 10.1016/j.neubiorev.2005.03.007

[51] Matthews K, Forbes N, Reid IC. Sucrose consumption as an hedonic measure following chronic unpredictable mild stress. Physiology & Behavior. 1995;**57**(2):241-248. DOI: 10.1016/0031-9384(94)00286-e

[52] Kjærgaard M, Joakimsen R, Jorde R. Low serum 25-hydroxyvitamin D levels are associated with depression in an adult Norwegian population. Psychiatry Research. 2011;**190**(2-3):221-225. DOI: 10.1016/j.psychres.2011.06.024

[53] Magni LR, Purgato M, Gastaldon C, Papola D, Furukawa TA, Cipriani A, et al. Fluoxetine versus other types of pharmacotherapy for depression. Cochrane Database of Systematic Reviews. 2013;**7**:CD004185. DOI: 10.1002/14651858.CD004185.pub3

[54] Sedaghat K, Yousefian Z, Vafaei AA, Rashidy-Pour A, Parsaei H, Khaleghian A, et al. Mesolimbic dopamine system and its modulation by vitamin D in a chronic mild stress model of depression in the rat. Behavioural Brain Research. 2019;**356**:156-169. DOI: 10.1016/j.bbr.2018.08.020

[55] Caviedes A, Lafourcade C, Claudio Soto C, Wyneken U. BDNF/NF-κB signaling in the neurobiology of depression. Current Pharmaceutical Design. 2017;**23**(21):3154-3163. DOI: 10.2174/1381612823666170111141915

[56] Bertone-Johnson ER, Powers SI, Spangler L, Brunner RL, Michael YL, Larson JC, et al. Vitamin D intake from foods and supplements and depressive symptoms in a diverse population of older women. The American Journal of Clinical Nutrition. 2011;**94**(4):1104-1112. DOI: 10.3945/ajcn.111.017384

[57] Accortt EE, Schetter CD, Peters RM, Cassidy-Bushrow AE. Lower prenatal vitamin D status and postpartum depressive symptomatology in African American women: Preliminary evidence for moderation by inflammatory cytokines. Archives of Women's Mental Health. 2016;**19**(2):373-383. DOI: 10.1007/s00737-015-0585-1

IntechOpen