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Vitamin D_3 attenuates anxiety-like behavior in long-term ovariectomized rats with unpredictable mild stress

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Abstract: The impact of various vitamin D3 (VD3) doses (1.0, 2.5, or 5 mg/kg, s.c.) in mitigating the negative consequences of chronic unpredictable mild stress (CUMS) was investigated. Adult female rats with long-term estrogen deficiency were assessed using the sucrose preference test (SPT), the elevated plus-maze (EPM), the light/dark test (LDT), and the open-field test (OFT) to measure anhedonia-like and anxiety-like behavior. The corticosterone (CS) and adrenocorticotrophic hormone (ACTH) concentrations in blood serum and the brain-derived neurotrophic factor (BDNF) expression in the hippocampus of long-term ovariectomized (OVX) rats were measured by ELISA kits and/or western blotting. Treatment with VD3 (5.0 mg/kg), similarly to fluoxetine (10.0 mg/kg), significantly reduced the anhedonia profile in the SPT and anxiety-like behavior in the EPM and LDT, and CS and ACTH levels in blood serum. It also elevated BDNF levels in the hippocampus of long-term OVX/CUMS compared to OVX/CUMS/solvent rats. Thus, these findings suggest that VD3 (5.0 mg/kg) administration might attenuate the anxiety-like profile in long-term OVX adult rats subjected to the CUMS. This might occur via activation of the BDNF signaling pathway in the hippocampus and via restoration of CS and ACTH levels in blood serum.

Introduction

Menopause involves many neuropsychiatric changes (Burger, 2008; Bromberger *et al*., 2013). The role of ovarian hormones in affect-related disorders is of great interest for women transitioning through menopause (Burger, 2008). Mood disorders during menopause could partly be explained by a loss of estrogen, as female hormones are known to have neuroprotective effects on the brain (Garcia-Portilla, 2009; Arevalo *et al*., 2015). Numerous experimental and clinical studies have documented that estrogen deficiency during menopause increases the susceptibility to mood disturbances, including anxiety (Bernardi *et al*., 1989; Rossouw *et al*., 2002; Lagunas *et al*., 2010). It has also been suggested that menopausal hormonal therapy (MHT) may improve the symptoms of, and decrease the risk of developing, affect related disorders. Yet some uncertainty still exists because research has also found that MHT does not entirely stop the development of anxiety-like symptoms (Rossouw *et al*., 2002). Females going through menopause are at higher risk of developing vitamin D (VD) deficiency due to several reasons: a diet low in VD, restricted outdoor activity resulting in less sun exposure, and a decreased capacity to produce enough calcitriol as a result of an age-related decline in hydroxylation by the kidneys (Gaugris *et al*., 2005; Kjærgaard *et al*., 2011). Our previous experiments have confirmed that the hormonal profile in ovariectomized (OVX) female rodents and menopausal women is also characterized by VD deficiency (Fedotova *et al*., 2017, 2018; Fedotova, 2019). Traditional methods of affective-related disorders therapy, which also includes antidepressants/anxiolytics, are unfortunately of limited effectiveness (Arevalo *et al*., 2015). In the pathophysiological mechanisms of mood disorders, many trigger factors play a role; it is argued that one of them could be a deficiency in vitamin D_3 (VD_3) (Gaugris *et al*., 2005; Garcia-Portilla, 2009).

VD_3 deficiency has been proven to impact the pathogenesis of various diseases, including autoimmune diseases, cardiovascular diseases, infections, osteoporosis, obesity, diabetes, and certain types of cancers (Adams and Hewison, 2010; DeLuca *et al*., 2013). A correlation between very low VD_3 levels and neuropsychiatric diseases, and also between an impact of VD_3 level and brain functioning has been observed in recent research (Kiraly *et al*., 2006; Holick, 2007; Groves *et al*., 2014; Li *et al*., 2014). Moreover, VD receptors (VDR) exist in the central nervous system (Holick and Chen, 2008; Kesby *et al*., 2011), including the very brain areas involved in affect. VD_3 is also involved in different physiological processes in the brain, such as the regulation of brain-derived neurotrophic factor (BDNF) and other neurotrophic factors, neurogenesis, neuroplasticity,

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neuroprotection, and neuroimmunomodulation (Garcion *et al.*, 2002; Eyles *et al.*, 2009; Adams and Hewison, 2010; DeLuca *et al.*, 2013), thereby playing a role in the pathophysiological mechanisms of affective-related disorders (Obradovic et al., 2006; Parker *et al.*, 2017).

Calcitriol activates the expression of tyrosine hydroxylase, which is involved in the synthesis of catecholamines and increases the production of dopamine, adrenaline, and noradrenaline (Garcion *et al.*, 2002; Groves *et al.*, 2014). Calcitriol can also enhance cholinergic neurotransmission by activating acetylcholine transferase, which is an enzyme that is involved in the synthesis of acetylcholine (Przybelski and Binkley, 2007; Harms *et al.*, 2011).

The hippocampus is one of the key brain structures involved in affective-related disorders (Hanson *et al.*, 2011). Both estrogen and VD_3 have been associated with the successful functioning of the hippocampus (Spedding, 2014; Chu *et al.*, 2017). Research on humans as well as animals showing that alterations of neurotrophic factors and their expression in the hippocampus are associated with affective-related disorders (Korte *et al.*, 1998; Levada and Cherednichenko, 2015). Moreover, animal studies have documented that impaired behavioral profiles in rats are correlated with decreased BDNF levels in the limbic system of the brain (Luellen *et al.*, 2007; Watanabe *et al.*, 2010). Clinical studies using patients with mood disorders have shown that BDNF levels are reduced in the serum of such patients (Aydemir *et al.*, 2006; Wolkowitz *et al.*, 2011; Ladea and Bran, 2013).

On the other hand, it is well known that hypothalamic-pituitary-adrenal (HPA) axis hyperactivity is one of the major trigger factors for the development of mood disorders (Bao *et al.*, 2008; Pariante and Lightman, 2008; Kino, 2015). Taking this assumption into account, mood disturbances established in menopausal women might result from complex alterations in HPA activity, and VD_3 levels, as well as BDNF production.

Our earlier work showed that VD_3 (1.0 and 2.5 mg/kg, s.c.) alone or with the addition of a low dose of 17β-estradiol, decreased anxiety-like behavior of OVX rats with long-term estrogen deficiency in the EPM and LDT (Fedotova *et al.*, 2018; Fedotova, 2019), suggesting that VD_3 exhibits an anxiolytic-like effect on rat behavioral anxiety models. However, the therapeutic effects of VD_3 on the chronic unpredictable mild stress (CUMS) models remain unknown. It is additionally still unclear whether the anxiolytic-like action of VD_3 involves BDNF and the HPA axis in long-term OVX adult rats.

The current research

The aim of the present work was to clarify the anxiolytic-like effect of VD_3 at different doses and to further study the role of the BDNF signaling pathway, as well as the HPA axis, on a rat model of CUMS. As in prior research (Fedotova *et al.*, 2017, 2018; Fedotova, 2019), we used long-term 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 (Bekku and Yoshimura, 2005). The sucrose preference test (SPT), as well as the elevated plus maze (EPM), the light-dark test (LDT) and the open-field (OFT) test, were performed to examine anxiety-like states in the rats. Serum corticosterone (CS) and adrenocorticotrophic hormone (ACTH), 25-hydroxyvitamin D_3 (25-OH-VD_3) levels, and hippocampal BDNF concentration were also tested to assess the possible mechanisms of the VD_3 effects on the anxiety-like profile in long-term OVX rats subjected to CUMS.

Materials and Methods

Animals

Adult female Wistar rats (weighing 210 ± 20 g) were purchased from the Animal Rat Center of the Rappolovo Laboratory Animal Factory (St. Petersburg, Russia). All females were maintained under standard animal vivarium conditions with a constant room temperature (22 ± 1°C), relative humidity (50 ± 10%), and a 12 h light/dark cycle (light from 07:00 to 19:00) with typical food for rodents and water ad libitum. All rats got used to the novel environment for 1 week prior to their use in this research. All stress manipulations were performed to minimize any pain and undesirable experiences in the experimental animals. The entire research procedure was approved by the Animal Care Committee of the I.P. Pavlov Institute of Physiology (Protocol No. 1095/1/25.06.2012) and carried out in compliance with the National Institute of Health guidelines for laboratory animals.

Ovariectomy

To modulate the hormonal state of the females Wistar rats, bilateral removal of the ovaries was conducted. It might be important to note that this hormonal state is considered to be similar to the menopausal period in women (Bekku and Yoshimura, 2005). As part of this procedure, narcosis was performed by means of an intraperitoneal administration of 10 mg/kg xylazine and 70 mg/kg ketamine. This was followed by the removal of both ovaries. This procedure was performed by using two standard cuts in a lateral position. After this procedure, the muscles and skin incision were restored by surgical staples. The efficacy of this surgery was validated by routine vaginal inspection and inspection of serum estradiol levels. For the sham operation, the same procedure was followed but without the amputation of the ovaries. This entire procedure was performed in line with previously published research (Fedotova *et al.*, 2017).

Following the ovariectomy or sham operation, the OVX rats were placed in their cage and were allowed to recover for a period of 12 weeks while having continuous access to food and water. After this time, each rat was randomly assigned to an experimental group for the chronic stress procedure, except for the sham-operated (SHAM), non-stressed, control rats.

CUMS model

To induce clinical depression in the rats in the experimental conditions, the CUMS procedure (Banasr *et al.*, 2007) was followed with some small alterations (Katz, 1981; Willner *et al.*, 1987). The CUMS procedure includes exposure to repeated unpredictable stressors that follow a four weeks protocol. This is meant to appear random and unpredictable to the laboratory's animals (Burstein *et al.*, 2017).

The list of stressors are: 24 h of food or water deprivation, wet bedding or tilted cage overnight, unpredictable shocks (15 mA, one shock/20 s, 10 s duration, 20 min), 5 min of

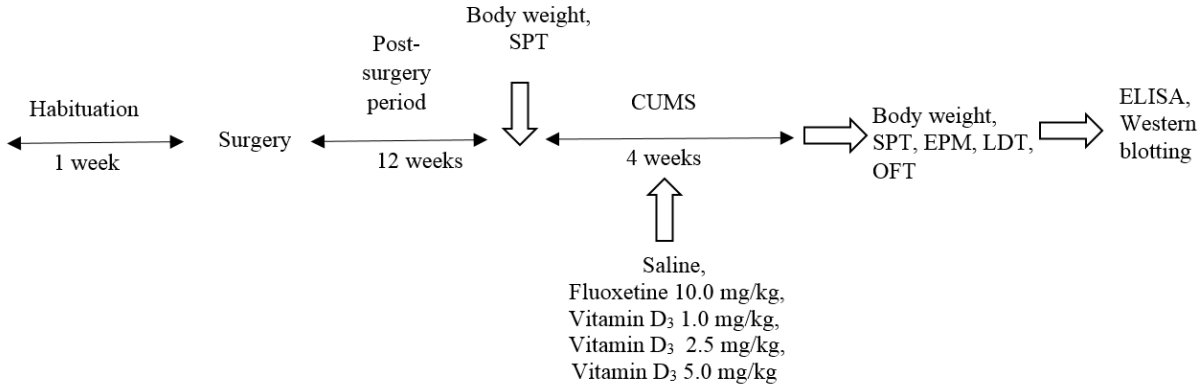

FIGURE 1. The timeline of the present study is depicted. We assessed the anxiolytic-like effects of VD_3 at different doses (1.0, 2.5, and 5.0 mg/kg, s.c.) on the depression model produced by CUMS for 28 days in the long-term OVX rats. For testing of anxiety-like state, SPT, EPM, LDT, and OFT were conducted in the present study. Serum CS/ACTH and 25-OH-VD_3 levels and hippocampal BDNF expression were determined by ELISA kits and/or western blotting to assess the possible mechanisms of the VD_3 effects on the anxiety-like profile in long-term OVX rats subjected to CUMS.

swimming in cold water (4°C), tail hanging for 1 min, clipped tail for 1 min, and a reversal of the light/dark cycle (Katz, 1981; Willner *et al.*, 1987). All the stress triggers were performed according to the daily procedure protocol.

The control, sham-operated, female rats were housed in a separate cage (and room) without any contact with the stressed groups of animals. These rats were generally undisturbed and well maintained (provided with food and routine cage cleaning). All rats were weighed before and after the CUMS period (see Fig. 1).

Drugs

Fluoxetine hydrochloride and VD_3, as cholecalciferol, were provided by Sigma Chemical Co. (St. Louis, MO, USA). VD_3 was dissolved in 95% ethanol, 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). This happened either 30 min before the daily stressor actions for the 4 weeks period during the CUMS procedure or 60 min before the final behavioral tests.

Groups of animals

The animals were randomly assigned to 1 of 7 experimental groups (n = 7 rats in each group): SHAM rats treated with saline (control), SHAM rats submitted to CUMS treated with saline, long-term OVX rats exposed to CUMS with saline, long-term OVX rats exposed to CUMS with fluoxetine as positive control (10.0 mg/kg/day, i.p.), and 3 more groups of long-term OVX rats exposed to CUMS with VD_3 at 3 different levels (1.0, 2.5, and 5 mg/kg/day, s.c.). In our preliminary studies, there were no significant differences between SHAM/OVX rats treated with physiological saline as a solvent for fluoxetine and SHAM/OVX females treated with sterile water with 2% ethanol as a solvent for VD_3 in behavioral trials (data are not shown). Since, we did not find any differences between these experimental groups, the physiological saline as a solvent for SHAM/OVX females was used in the present work. The doses of VD_3 were based on our previous studies on the behavioral effects of VD_3 on anxiety-like behavior of non-stressed long-term OVX female rats (Fedotova *et al.*, 2017). All tested doses of VD_3 were safe for animals did not induce any side effects (Fedotova *et al.*, 2017). The dose of fluoxetine was utilized according to earlier experimental data (Estrada-Camarena *et al.*, 2004). Several studies have demonstrated that the administration of fluoxetine decreases depressive-like behavior in rodents (Estrada-Camarena *et al.*, 2004; Olivares-Nazario et al., 2016). All drugs were injected as described before (Fedotova *et al.*, 2017). Furthermore, all experimental groups of rats were supervised daily by the veterinary care staff during subroutine maintenance. The rats were examined in the special animal room using a noninvasive observational assessment procedure that yielded information regarding the health state of each animal. The assessment consisted of several measurements: body condition, appearance, breathing, hydration status, posture, mobility, muscle tone, and the presence of defects in the bones, genitals, and abdomen. No rats were damaged or unhealthy during the experimental protocol.

Sucrose preference test

Before and after the initiation of the 4 weeks CUMS procedures, the experimental rats underwent the SPT (Anisman and Matheson, 2005; Wang *et al.*, 2009). 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 one-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:

Elevated plus maze

The assessment of anxiety-like behavior in the EPM was performed as in our previous studies (Fedotova *et al*., 2017; Fedotova, 2019). Briefly, the apparatus has 4 arms, 10 cm wide and 50 cm long (2 arms were opened, and another one was closed), elevated 50 cm above the floor. During a 300 s test time-period, the number of entrances and the time spent into the open/closed arms were recorded. Following each experimental trial, the maze was carefully cleaned with a cleaning solution containing 10% ethanol.

Light/Dark Test

The evaluation of anxiety-like behavior in the LDT was recently published (Fedotova *et al*., 2017; Fedotova, 2019). The LDT consists of 2 equal chambers (30 × 40 × 40 cm). One of the chambers was colored white and illuminated by a 60 W light bulb. Another chamber was colored black without any illumination. The measurable parameters in the LDT were the time spent and the number of entrances in the lighted box over a period of 300 s. Following each experimental trial, the apparatus was carefully cleaned with a cleaning solution containing 10% ethanol.

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 (Fedotova *et al*., 2018). 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.

Biochemical assay

After all the behavioral testing, all rats underwent narcosis, and approximately 5 ml samples of blood were drawn from the animals to be centrifuged at 4000 g for 15 min at 4°C. 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) (Heffner *et al*., 1980). After that, the hippocampal samples with the cold lysis buffer were sonicated for 15 s. Then, the hippocampi were centrifuged at 12000 g for 15 min at 4°C. The Bradford method was used for the normalization of hippocampal supernatants to the total protein (Bradford, 1976). 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-OH-VD_3, ACTH, and CS 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 CS rat ELISA kits were 0.1 ng/mL and 0.2-40 ng/mL, respectively. The sensitivity and detection range of the ACTH rat ELISA kits were 1.25 pg/mL and 1.25-50 pg/mL, respectively.

Rat ELISA kits were used to measure hippocampal homogenates for the detection of BDNF levels. This was done according to the manufacturer's instructions (Cusabio Biotech Co., Ltd, Wuhan, P.R. China). 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-BNDF 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 BDNF levels were measured using an 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 BDNF levels. The BDNF content is presented as pg/mg of tissue. The sensitivity and detection range of the BDNF rat ELISA kits were 0.078 ng/mL and 0.312–20 ng/mL, respectively. The assay exhibited no significant cross-reactivity with other neurotrophic factors. All samples were duplicated for the assay.

Western blotting analysis

Hippocampal tissues were homogenized in cold lysis buffer containing a protease inhibitor cocktail (Sigma-Aldrich, USA) for 1 h and centrifuged at 12000 g at 4°C for 20 min. 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-BDNF (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 BDNF 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).

Statistical analysis

All data (in the graphs) are expressed as the mean ± the standard deviation (mean ± SD). 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.

Results

VD_3 treatment reversed the body weight and increased sucrose preference in the long-term OVX rats subjected to CUMS

As shown in Fig. 2(A), there were no significant differences in the initial body weight in all the experimental groups. CUMS produced a decrease of body weight in the SHAM rats condition compared to the control, non-CUMS SHAM

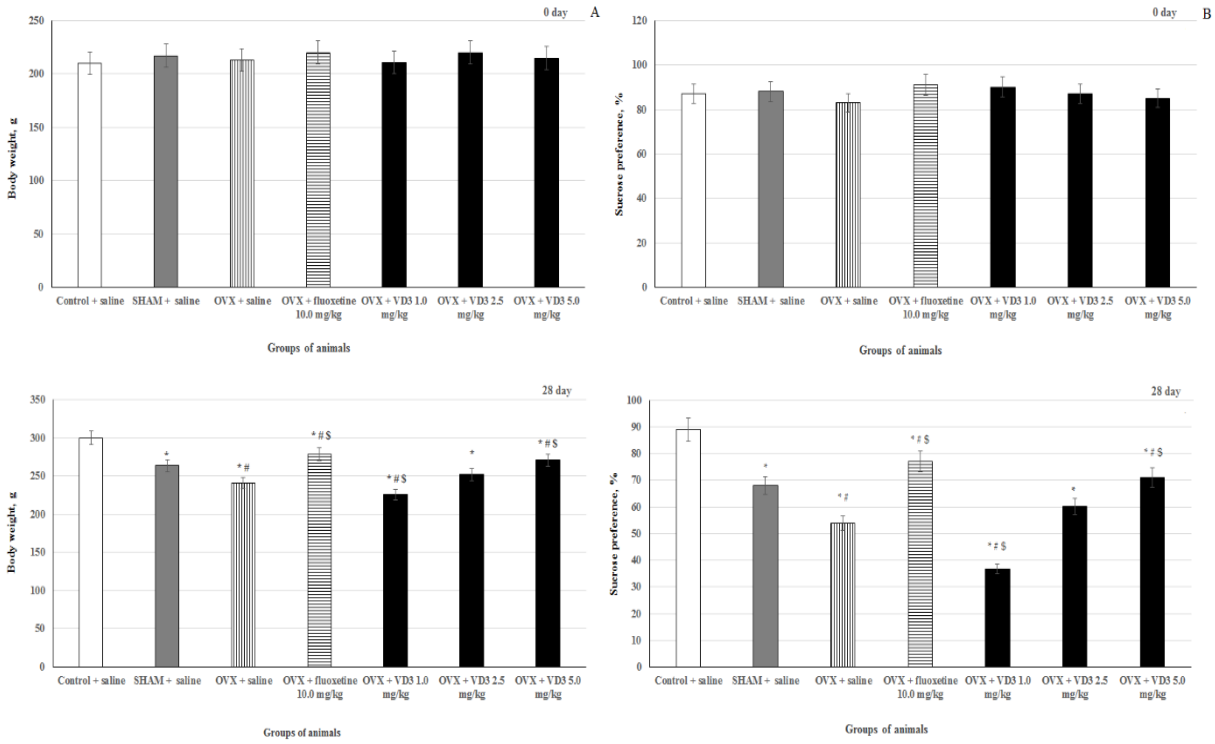

FIGURE 2. The effects of VD_3 treatment on body weight (A) and sucrose preference (B) in long-term OVX rats subjected to CUMS at day 0 (the beginning of stress protocol) and on day 28 (the end of stress protocol). * $p < 0.05$ *vs.* the non-CUMS SHAM group (control group) treated with saline, # $p < 0.05$ *vs.* the SHAM group with CUMS treated with saline, $ $p < 0.05$ *vs.* the long-term OVX group with CUMS treated with saline. The data are presented as the mean ± SD; n = 7 in each group.

group ($F(1,76) = 65.43$, $p < 0.001$) and the long-term OVX rats compared to the non-CUMS/CUMS SHAM groups (Fig. 2(A), $p < 0.001$). VD_3 (5.0 mg/kg) treatment, similarly to the fluoxetine treatment (10.0 mg/kg), significantly increased body weight ($p < 0.001$) in the long-term rats with CUMS. Administration of VD_3 (1.0 mg/kg) profoundly decreased the body weight of the long-term OVX rats compared to the OVX/SHAM rats with the CUMS group (Fig. 2(A), $p < 0.001$). VD_3 (2.5 mg/kg) failed to modify the body weight of the long-term OVX rats with CUMS (Fig. 2(A), $F(1,76) = 0.26$, $p > 0.001$).

No significant difference in the sucrose preference was observed among groups before starting the CUMS intervention (Fig. 2(B)). A significant decrease in sucrose preference of the SHAM rats with CUMS was registered when this was compared to the control non-CUMS SHAM group ($p < 0.01$).

The OVX rats with CUMS demonstrated a significant decrease in sucrose preference compared to the non-CUMS/CUMS SHAM rats (Fig. 2(B), $F(1,76) = 42.75$, $p < 0.01$). Both VD_3 (5.0 mg/kg), and fluoxetine treatments elevated sucrose consumption in the long-term OVX rats subjected to CUMS when compared to the OVX/SHAM rats with CUMS (Fig. 2(B), $p < 0.01$). The long-term OVX rats with CUMS plus VD_3 (1.0 mg/kg) showed a profoundly reduced sucrose preference compared the OVX/SHAM with CUMS rats and non-CUMS control SHAM rats (Fig. 2(B), $p < 0.01$). VD_3 (2.5 mg/kg) did not alter sucrose consumption in the long-term OVX rats with CUMS (Fig. 2(B), $F(1,76) = 1.13$, $p > 0.01$).

VD_3 treatment decreased the anxiety-like behavior of long-term OVX rats subjected to CUMS in the EPM and LDT

The CUMS decreased the time spent and the number of entries into the open arms of the maze in the long-term OVX rats compared to the non-CUMS/CUMS SHAM rats (Fig. 3(A), $F(1,76) = 34.41$ and $F(1,76) = 87.89$, respectively, $p < 0.001$). VD_3 (5.0 mg/kg), as with fluoxetine, produced an increase in the time spent and the number of entries into the open arms for the long-term OVX rats when compared to the OVX/SHAM with CUMS groups (Fig. 3(A), $p < 0.001$). However, VD_3 (1.0 mg/kg) significantly increased the extent of anxiety-like behavior in the long-term OVX rats plus CUMS compared to the long-term OVX/SHAM rats with CUMS and the non-CUMS SHAM control rats (Fig. 3(A), $p < 0.001$). VD_3 (2.5 mg/kg) administration did not change anxiety-like behavior in the EPM of the long-term OVX rats plus CUMS compared to long-term OVX rats with CUMS (Fig. 3(A), $p > 0.05$).

The time spent on and the number of entries in the light chamber were reduced after the CUMS procedure in the long-term OVX when compared to the non-CUMS/CUMS SHAM rats (Fig. 3(B), $F(1,76) = 53.94$ and $F(1,76) = 26.44$, respectively, $p < 0.001$). VD_3 (5.0 mg/kg), similarly to fluoxetine, elevated the time spent and the number of entries in the light box in the long-term OVX rats compared to the OVX/SHAM with CUMS groups (Fig. 3(B), $p < 0.001$). Treatment with VD_3 (1.0 mg/kg) significantly induced higher levels of anxiety-like behavior in the long-term OVX rats with CUMS compared to the long-term OVX/SHAM rats with CUMS and the non-CUMS SHAM control group (Fig. 3(B), $p < 0.001$). No differences were found for the VD_3 treatment at the dose of 2.5 mg/kg for the long-term OVX rats with CUMS in the LDT compared to the long-term OVX rats with CUMS (Fig. 3(B), $p > 0.05$).

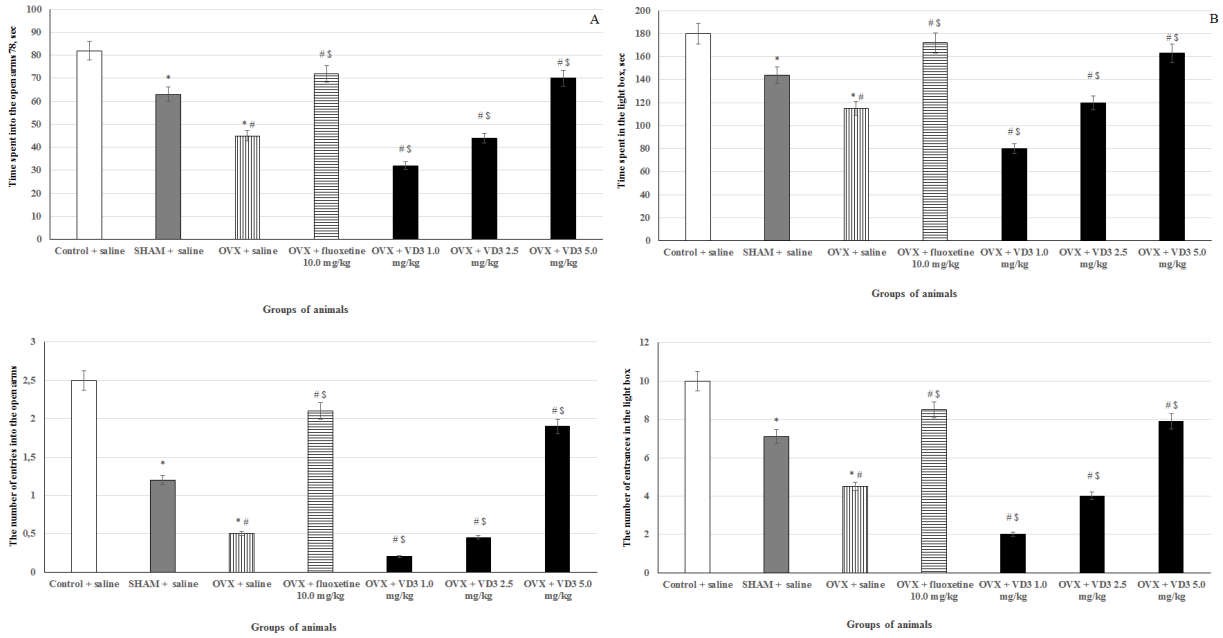

FIGURE 3. The effects of VD_3 treatment on anxiety-like behavior in long-term OVX rats subjected to CUMS in the EPM (A) and LDT (B). * $p < 0.05$ *vs.* the non-CUMS SHAM group (control group) treated with saline, # $p < 0.05$ *vs.* the SHAM group with CUMS treated with saline, $ $p < 0.05$ *vs.* the long-term OVX group with CUMS treated with saline. The data are presented as the mean ± SD; n = 7 in each group.

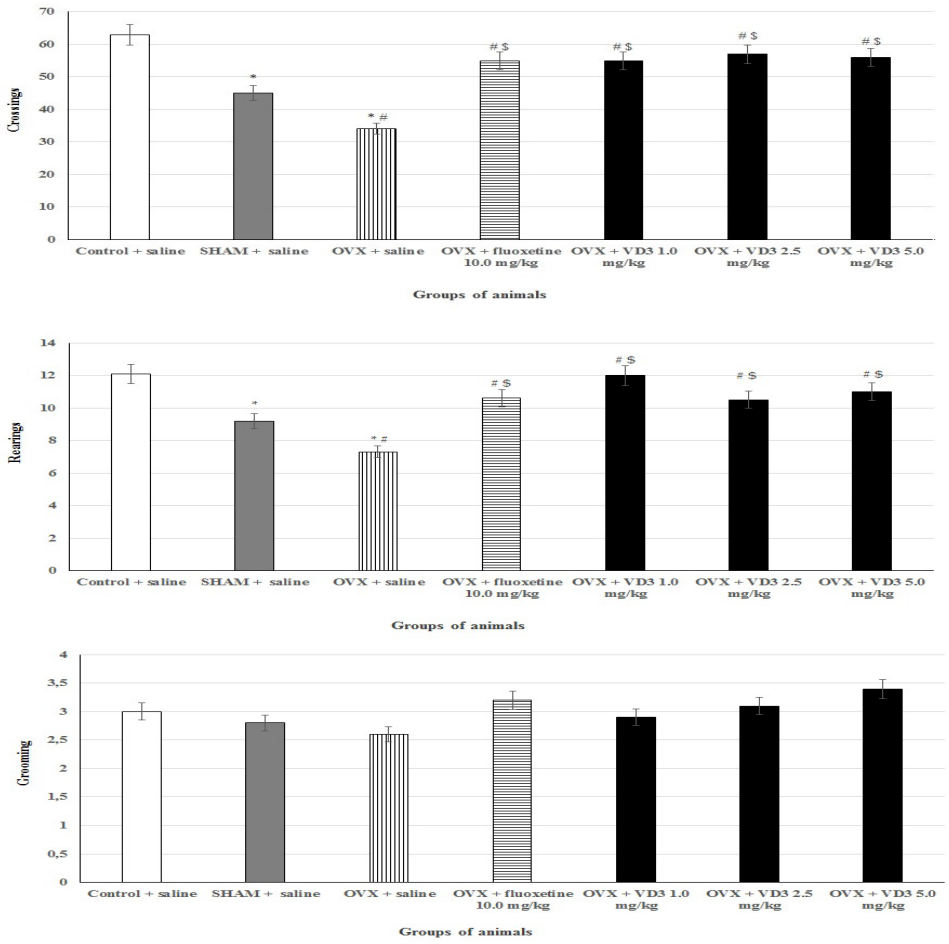

FIGURE 4. The effects of VD_3 treatment on the behavior of long-term OVX rats subjected to CUMS in the OFT. * $p < 0.05$ *vs.* the non-CUMS SHAM group (control group) treated with saline, # $p < 0.05$ *vs.* the SHAM group with CUMS treated with saline, $ $p < 0.05$ *vs.* the long-term OVX group with CUMS treated with saline. The data are presented as the mean ± SD; n = 7 in each group.

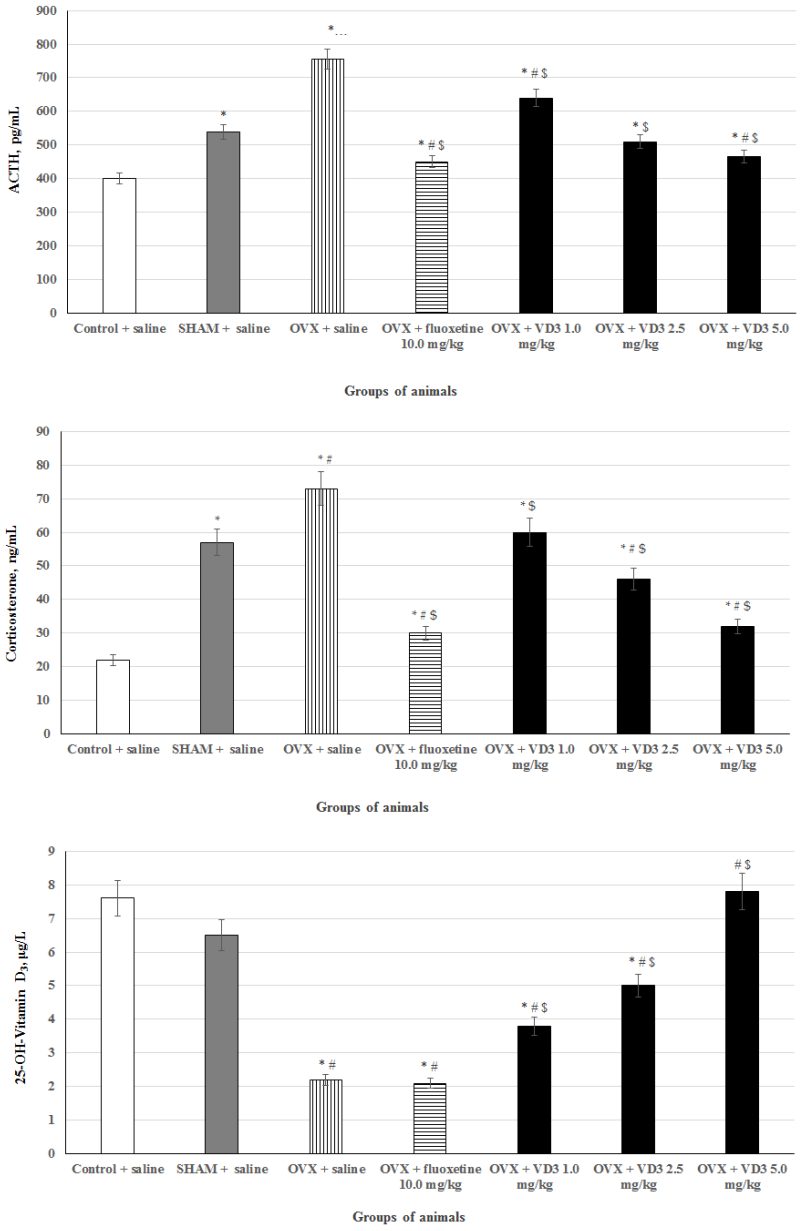

FIGURE 5. The effects of VD_3 treatment on serum corticosterone, ACTH, and 25-OH-VD_3 levels in the long-term OVX rats subjected to CUMS. * $p < 0.05$ *vs.* the non-CUMS SHAM group (control group) treated with saline, # $p < 0.05$ *vs.* the SHAM group with CUMS treated with saline, $ $p < 0.05$ *vs.* the long-term OVX group with CUMS treated with saline. The data are presented as the mean ± SD; n = 7 in each group.

VD_3 changed behavior of long-term OVX rats subjected to CUMS in the OFT

A reduced number of rearings and crossings in the long-term OVX rats with CUMS compared to the non-CUMS/CUMS SHAM groups were found (Fig. 4, $F(1,76) = 11.12$, $p < 0.05$). Fluoxetine and VD_3 in all tested doses significantly elevated the number of rearings and crossings in the long-term OVX rats with CUMS compared to the OVX/SHAM rats with CUMS (Fig. 4, $p < 0.05$).

There were no significant differences in grooming between all the experimental groups after the CUMS in the OFT (Fig. 4, $F(1,76) = 0.78$, $p > 0.05$).

VD_3 restored serum corticosterone, ACTH and VD_3 levels in long-term OVX rats subjected to CUMS

The long-term OVX rats with CUMS demonstrated a significant increase of the serum CS and ACTH levels and a decrease of 25-OH-VD_3 levels in the long-term OVX rats with CUMS when compared to the non-CUMS/CUMS SHAM groups (Fig. 5, $F(1,76) = 122.74$, $F(1,76) = 34.46$, $F(1,76) = 14.45$, respectively, $p < 0.001$). Treatment with VD_3 dose-dependently restored the pathologically enhanced CS and ACTH levels in the blood serum of the long-term OVX rats with CUMS when compared to the OVX/SHAM rats subjected to CUMS (Fig. 5, $p < 0.001$). Moreover, VD_3 dose-dependently increased serum 25-OH-VD_3 levels in the long-term OVX rats

with CUMS when compared to the OVX group exposed to CUMS (Fig. 5, $p < 0.001$). The OVX rats treated with reference drug showed a reduced serum CS/ACTH levels in the long-term OVX rats with CUMS when compared to the OVX/SHAM groups with CUMS (Fig. 5, $p < 0.001$).

VD_3 increased hippocampal BDNF levels/protein expression in long-term OVX rats subjected to CUMS

CUMS induced a decrease of hippocampal BDNF level in SHAM rats when compared to the non-CUMS control females (Fig. 6(A), $p < 0.001$). A significant reduction of hippocampal BDNF level in the long-term OVX rats with CUMS was found when compared to the non-CUMS/CUMS SHAM rats (Fig. 6(A), $F(1,76) = 49.12$, $p < 0.05$). Both VD_3 (5.0 mg/kg) or fluoxetine (10.0 mg/kg) elevated BDNF content in the hippocampus of long-term OVX rats with CUMS when compared to the OVX/SHAM rats with CUMS (Fig. 6(A), $p < 0.05$). VD_3 (1.0 mg/kg) significantly diminished hippocampal BDNF concentration in the long-term OVX rats with CUMS when compared to the OVX/SHAM with CUMS or non-CUMS SHAM groups (Fig. 6(A), $p < 0.05$). VD_3 (2.5 mg/kg) failed to modify BDNF level in the hippocampus of the long-term OVX rats with CUMS when compared to the OVX rats with CUMS (Fig. 6(A), $p > 0.05$).

CUMS reduced BDNF protein level in the hippocampus of SHAM rats when compared to non-CUMS control females (Fig. 6, $p < 0.001$). The long-term OVX rats with CUMS showed reduced BDNF levels in the hippocampus when compared to the non-CUMS/CUMS SHAM rats (Fig. 6(A), $F(1,76) = 14.84$, $p < 0.01$). Both VD_3 (5.0 mg/kg) and the reference drug significantly elevated hippocampal BDNF levels in long-term OVX rats when compared to the OVX/SHAM rats with CUMS (Fig. 6(A), $p < 0.01$). The long-term OVX rats after CUMS treated with VD_3 (1.0 mg/kg) demonstrated more significantly reduced BDNF protein levels in the hippocampus when compared to the OVX with CUMS or non-CUMS/CUMS SHAM groups (Fig. 6(B), $p < 0.01$). VD_3 at the dose of 2.5 mg/kg did not alter the hippocampal BDNF protein expression of long-term OVX rats when compared to the OVX rats with CUMS (Fig. 6(B), $p > 0.01$).

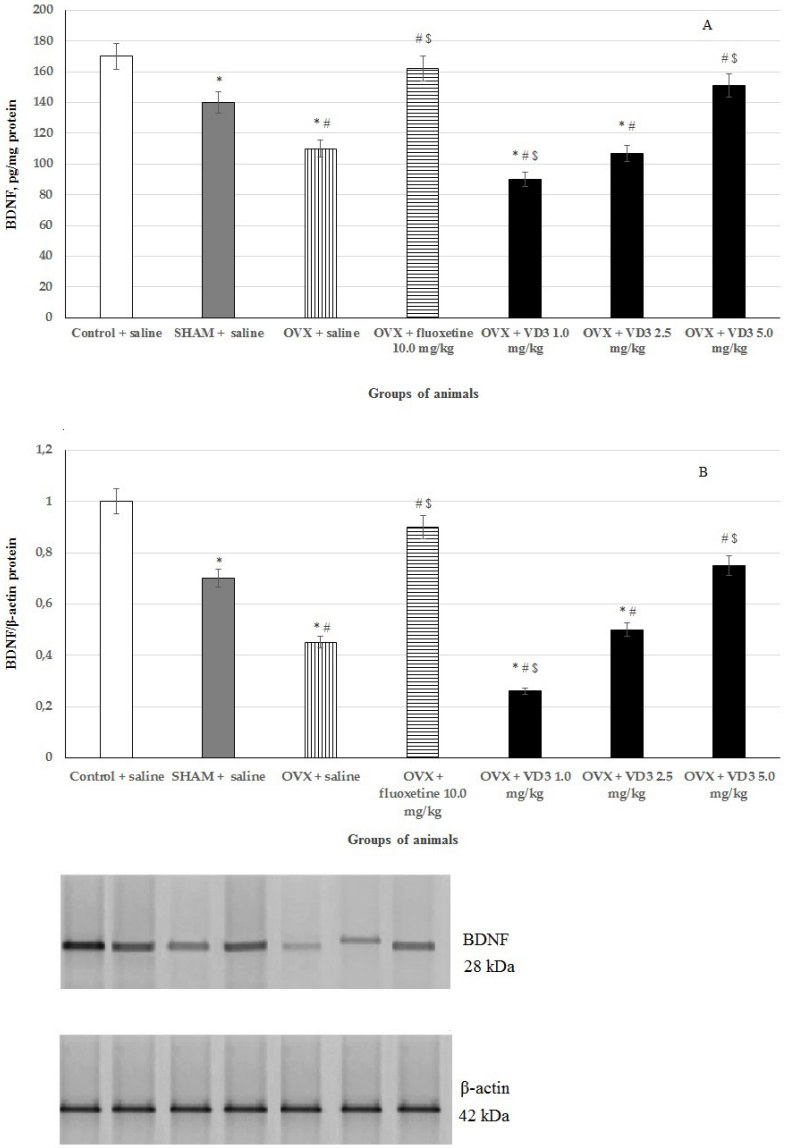

FIGURE 6. The effects of VD_3 treatment on hippocampal BDNF concentration (A) and BDNF protein expression (B) in the long-term OVX rats subjected to CUMS. 1- non-CUMS SHAM (control) rats, 2- SHAM rats with CUMS, 3- OVX rats with CUMS plus saline, 4- OVX rats with CUMS plus fluoxetine (10.0 mg/kg), 5- OVX rats with CUMS plus VD_3 (1.0 mg/kg), 6- OVX rats with CUMS plus VD_3 (2.5 mg/kg), 7- OVX rats with CUMS plus VD_3 (5.0 mg/kg). * $p < 0.05$ *vs.* the non-CUMS SHAM group (control group) treated with saline, # $p < 0.05$ *vs.* the SHAM group with CUMS treated with saline, $ $p < 0.05$ *vs.* the long-term OVX group with CUMS treated with saline. The data are presented as the mean ± SD; n = 7 in each group.

Discussion

The current experimental research explored the anxiolytic-like effects of VD_3 administered at three different doses (1.0, 2.5, and 5.0 mg/kg, s.c.) in long-term adult OVX female rats exposed to the CUMS procedure. A CUMS model is a standard animal model that has been demonstrated to reflect a typical pathological deterioration, similar to a clinical affective-related state in humans. This procedure has been proven to show high validity across studies (Katz, 1981; Willner *et al*., 1987). In the current study, the involvement of the BDNF signaling pathway, as well as the HPA axis, in the mechanisms of VD_3 action in depression, was additionally explored in relation to the affective state of long-term adult OVX rats exposed to CUMS.

The findings of this study showed that in the adult long-term OVX rats undergoing CUMS there was marked anhedonia-like and anxiety-like behavior, as evaluated by SPT and EPM/LDT, respectively. Moreover, long-term OVX rats exposed to CUMS exhibited decreased locomotor and rearing activities in the OFT. The ELISA assay clearly demonstrated elevated serum CS/ACTH levels, as well as lower VD_3 concentrations, in adult long-term OVX rats subjected to CUMS. In addition, the decreased BDNF concentrations and BDNF protein expression were revealed using rat ELISA kits or western blotting in the hippocampus of long-term OVX rats exposed to CUMS. The results of the study confirm that CUMS produces changes in behavior, neuroendocrine activity, and neuroplasticity in adult OVX rats with long-term ovarian hormone deficiency (post-ovariectomy period of 3 months). Our data are in agreement with past research, finding that long-term estrogen deprivation in female rodents subjected to a CUMS procedure resulted in a profound anxiety-like profile (Huang *et al*., 2015).

Treatment with a positive reference drug (fluoxetine) decreased anhedonia-like and depression-like states and reversed neuroendocrine impairments in the long-term OVX female rats exposed to CUMS. Preclinical findings have documented that fluoxetine might restore the functional activity of the HPA axis and BDNF expression in different structures of the brain, improving the anxiety-like profile of OVX rats with different post-ovariectomy intervals in stressed and non-stressed behavioral models (Magni *et al*., 2013).

The most important conclusions that can be based on the current research are associated with the antidepressant-like effects of VD_3 at several doses on the stress model produced by CUMS in the long-term adult OVX rats. To our knowledge, this is the first study to compare the role of VD_3 in the behavioral and neurochemical consequences of a CUMS procedure in long-term OVX rats.

VD_3 administered at a dose of 5.0 mg/kg reversed anhedonia-like and anxiety-like state in the SPT/EPM/LDT paradigms in the long-term OVX rats subjected to CUMS, which was similar to the effects of the fluoxetine treatment. Moreover, the VD_3 application (5.0 mg/kg, s.c.) restored the behavioral impairments observed in the OFT in the long-term OVX rats subjected to CUMS. Biochemical assays found that VD_3 at this dose decreased the serum CS/ACTH concentrations, increased the serum VD_3, and the hippocampal BDNF levels in the long-term OVX rats exposed to CUMS. Western blotting revealed that VD_3 (5.0 mg/kg, s.c.) enhanced the hippocampal BDNF protein expression in the long-term OVX rats with CUMS.

Thus, VD_3 at a dose of 5.0 mg/kg attenuates the CUMS-induced behavioral impairments, restored the serum CS, ACTH, VD_3, and BDNF levels in the serum/hippocampus of long-term OVX rats. The neuroendocrine and behavioral effects of VD_3 at doses of 1.0 or 2.5 mg/kg were highly different from the effects of VD_3 at a dose of 5.0 mg/kg. VD_3 at a dose of 2.5 mg/kg failed to alter the neuroendocrine and behavioral characteristics of the long-term OVX rats who were exposed to a CUMS procedure. In contrast, VD_3 supplementation at a dose of 1.0 mg/kg exacerbated the behavioral disturbances, inducing more pronounced anhedonia and anxiety-like profiles among the group of long-term OVX rats who were exposed to the CUMS procedure. The fact that the VD_3 application in all tested doses increased the rearings and crossings allows us to conclude that the effects of VD_3 in the SPT and EPM/LDT cannot possibly be attributed to behavioral changes in the OFT, but rather should be interpreted as a direct expression of the anhedonia and anxiety-like state in the long-term OVX rats exposed to CUMS. Furthermore, the VD_3 treatment (1.0 mg/kg, s.c.) significantly reduced BDNF concentrations, as well as its protein expression in the hippocampus of the long-term OVX rats with CUMS. Despite the findings that VD_3 (1.0 mg/kg, s.c.) markedly decreased CS/ACTH and increased VD_3 levels in the blood serum of the long-term OVX rats exposed to CUMS at a level similar to the action of VD_3 at a dose of 5.0 mg/kg, we were not able to register any improvements of the anxiety-like state in these groups of rats. *These findings suggest that VD_3 might involve its actions on the BDNF signaling pathway in the hippocampus rather than the HPA axis or VD_3 levels in long-term OVX rats exposed to CUMS.*

In summary, the data indicated an inversed dependence between the behavioral effects and doses of VD_3-the dose at 1.0 mg/kg exacerbated anhedonia and anxiety-like profile, 2.5 mg/kg was not effective, whereas 5.0 mg/kg reversed all the behavioral impairments in the long-term OVX rats exposed to CUMS. The main findings of the present study evidenced, for the first time, the opposite effects after treatment with VD_3 at doses of 1.0 mg/kg and 5.0 mg/kg in the EPM and LDT tests. We propose that the imbalance between different neurotransmitter systems induced by the different doses of VD_3 is responsible for the observed bidirectional effects of VD_3 on anxiety responses. Additionally, we propose that the cross-talk between different neurotransmissions is one of the main elements in the opposite effects of VD_3 treatment at different doses. Differences in signaling pathways activated by VD_3 may be responsible for these distinctions at the neurochemical or molecular levels. It can be assumed that VD_3 at different doses differentially modulates neurotransmission in the brain structures that are involved in the mechanisms of anxiety disorders. Moreover, multiple proposals must be considered in order to clarify the role of VD_3 in anxiety disorders. However, the neurobehavioral/neurochemical basis for VD_3 treatment at different doses remains unclear. The future direction of our research is to understand the role of VD_3 in the regulation of emotional disturbances and corroborate the physiological relevance of it.

Recently, we have already published our study concerning behavioral effects for the similar doses of VD_3 (1.0, 2.5, 5.0 mg/kg, s.c.) in the non-stressed long-term OVX rats (Fedotova *et al*., 2017). The data of our previous study indicated that chronic treatment with VD_3 at doses of 1.0 and 2.5 mg/kg induced anxiolytic-like effects in the EPM and LDT in female rats following long-term ovariectomy. Furthermore, this is the first study to show a beneficial effect of chronic treatment with VD_3 at doses of 1.0 and 2.5 mg/kg on anxiety-related state induced by long-term ovariectomy in female rats. We found that VD_3 treatment at a dose of 5.0 mg/kg induced an anxiogenic-like effect in the long-term, non-stressed OVX rats (Fedotova *et al.,* 2017; Fedotova *et al*., 2018; Fedotova, 2019).

Thus, the data of the present study when using long-term OVX rats subjected to CUMS are opposing to the findings of our previous study concerning the anxiolytic-like effects of VD_3 at similar doses in non-stressed long-term OVX rats (Fedotova *et al*., 2017; Fedotova, 2019). Together, the results of the present study and our previous work indicate that the behavioral effects of VD_3 are dependent on used experimental paradigms (stressed or non-stressed). This fact concerning the various effects of VD_3 on the anxiety-like state in the long-term OVX rats, which have been observed in both our studies, might provide further explanation for the controversial findings concerning the anxiolytic-like effects of VD_3 in the experimental and human studies. Further studies are needed to explore why the behavioral effects of VD_3 supplementation are completely different in non-stressed and stressed long-term OVX rats and to clarify the effects of VD_3 and the mechanisms of its action in OVX rats of various levels of stress, ages, and post-ovariectomy periods.

The behavioral effects of VD_3 in the long-term OVX rats with CUMS observed in the present study are in agreement with the existing literature. More specifically, VD_3 has been found to restore locomotor activity and anhedonic states in other research with rodent stress models (Camargo *et al*., 2018; Sedaghat *et al*., 2019). However, this is the first study showing the anxiolytic-like effect of VD_3 at a dose of 5.0 mg/kg in long-term OVX rats with CUMS. Several possible explanations exist to explain the anxiolytic-like effects of VD_3 supplementation in adult long-term OVX rats with CUMS. According to one line of research, VD_3 is implicated in the mechanisms of affective-related disorders by modulating HPA axis activity and the BDNF pathway (Kiraly *et al*., 2006; Holick, 2007; Jiang *et al*., 2013; Groves *et al*., 2014; Li *et al*., 2014). More specifically, affect-related disorders are found to be associated with the dysregulation of the HPA axis (Kino, 2015), while it has also been found that a high level of CS/ACTH promotes neuronal atrophy and decreases the expression of the BDNF protein and mRNA in the hippocampus (Swaab *et al*., 2005). Previous research also indicates that stress-related hyperactivity of the HPA axis can lead to an affective-like state that can be reversed by treatment with antidepressants (Workman *et al*., 2016). In a similar way, we found that VD_3 treatment at a dose of 5.0 mg/kg decreased serum CS/ACTH levels. This might mean that these two pharmacological treatments can restore the hyperactivity of the HPA axis in long-term OVX rats subjected to CUMS. The results of the current study do not allow us to conclude that the mechanism of the anxiolytic-like effect of VD_3 at a dose of 5.0 mg/kg is only connected with the normalization of CS/ACTH levels in the blood serum of long-term OVX rats with CUMS, as VD_3 in all investigated doses equally decreased the pathologically elevated CS/ACTH concentrations in the blood of the long-term OVX rats with CUMS in our research.

Another possible explanation for the recovery responses to VD_3 in the long-term OVX rats with CUMS is related to the action that VD_3 has on the BDNF pathway. BDNF has been considered a downstream effector relevant to neuroprotective effects (Levada and Cherednichenko, 2015) and presents a neurotrophic factor that facilitates neuronal survival and modulates the proliferation and differentiation of neurons in the brain (Aydemir *et al*., 2006). In line with this, patients with mood disorders have also found to show decreased BDNF in their plasma (Wolkowitz *et al*., 2011; Ladea and Bran, 2013). Moreover, chronic pharmacotherapy using typical antidepressants (e.g., selective serotonin reuptake inhibitors) is linked to increased BDNF expression and neurogenesis in the hippocampus in humans with mood disorders as well as in animals (Dulawa *et al*., 2004; Zhang *et al*., 2010).

It has also been found that serotonin and BDNF promote the signaling and gene expression of each other in the hippocampus (Luellen *et al*., 2007; Levada and Cherednichenko, 2015) indicating a bidirectional influence. Some research demonstrates that VD_3 promotes the secretion of monoamines, including 5-HT (Kiraly *et al*., 2006), which may partly explain its beneficial effect on anhedonia and anxiety-like symptoms in long-term OVX rats with CUMS. Future research should attempt to establish whether the effect of VD_3 on hippocampal BDNF expression is generated by the serotonergic system or by another neurobiological mechanism. The results of the current study suggest that the normalization of hippocampal concentrations and expressions of BDNF in long-term OVX rats with CUMS may be associated with certain aspects of the anxiolytic-like effects of VD_3. Several studies have reported an inverse association between plasma 25OH-VD_3 concentrations and affective-related symptoms in women (Gaugris *et al*., 2005). In line with these observations, lower mood symptoms occurred more frequently in women with VD_3 deficiency/insufficiency, and VD_3 improved the mood state in such women (Kjærgaard *et al*., 2011; Accortt *et al*., 2016). The possible mechanism of VD_3 action might be explained by the stimulation of VDR identified in the different brain structures involved in mood control (Berridge, 2017). VD_3 may affect dopaminergic and/or serotoninergic neurotransmitter systems, neurotrophic factors, and/or alter the HPA axis response at the depressive state (Mizwicki and Norman, 2009; Harms *et al*., 2011). Low VD_3 levels appear in the majority of postmenopausal women (Bertone-Johnson *et al*., 2011; Gaugris *et al*., 2005; Luk *et al*., 2012). Therefore, VD_3 supplementation may be very useful for the treatment of mood disorders in postmenopausal women with a low level of VD_3. However, the exact role of VD_3 supplementation in the prevention and treatment of mood disorders associated with menopausal consequences has not been completely established.

Conclusions

The current research provides evidence for repeated administration of VD_3 in a chronic unpredictable stress model having anti-anhedonia-like and anxiolytic-like effects in long-term OVX adult rats with CUMS. Moreover, the biochemical and western blotting assays confirm the implications of BDNF modulation in the anxiolytic-like activity of VD_3. Further studies should, however, explore the precise mechanism of VD_3 action, due to the necessity of improvement of therapies focusing on mood-repair in females with long-estrogen deficiency. Multiple neurobiological mechanisms appear to play a mediating role in the therapeutic effects of VD_3 in long-term OVX rats with CUMS. This work is hoped to inspire future researchers on this topic and play a role in inspiring practical implication which will help to promote the effective treatment of anxiety disorders in females with long-term estrogen decline.

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Conflicts of Interest

The authors declare no conflict of interest.

References

Accortt EE, Schetter CD, Peters RM, Cassidy-Bushrow AE (2016). 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* **19**: 373-383.

Adams JS, Hewison M (2010). Update in Vitamin D. *Journal of Clinical Endocrinology and Metabolism* **95**: 471-478.

Anisman H, Matheson K (2005). Stress, depression, and anhedonia: caveats concerning animal models. *Neuroscience and Biobehavioral Reviews* **29**: 525-546.

Arevalo M-A, Azcoitia I, Garcia-Segura LM (2015). The neuroprotective actions of oestradiol and oestrogen receptors. *Nature Reviews Neuroscience* **16**: 17-29.

Aydemir C, Yalcin ES, Aksaray S, Kisa C, Yildirim SG, Uzbay T, Goka E (2006). Brain-derived neurotrophic factor (BDNF) changes in the serum of depressed women. *Progress in Neuro-psychopharmacology & Biological Psychiatry* **30**: 1256-1260.

Banasr M, Valentine GW, Li XY, Gourley SL, Taylor JR, Duman RS (2007). Chronic unpredictable stress decreases cell proliferation in the cerebral cortex of the adult rat. *Biological Psychiatry* **62**: 496-504.

Bao AM, Meynen G, Swaab DF (2008). The stress system in depression and neurodegeneration: focus on the human hypothalamus. *Brain Research Reviews* **57**: 531-553.

Bekku N, Yoshimura H (2005). Animal model of menopausal depressive like state in female mice: prolongation of immobility time in the forced swimming test following ovariectomy. *Psychopharmacology* **183**: 300-307.

Bernardi M, Vergoni AV, Sandrini M, Tagliavini S, Bertolini A (1989). Influence of ovariectomy, estradiol and progesterone on the behavior of mice in an experimental model of depression. *Physiology & Behavior* **45**: 1067-1068.

Berridge MJ (2017). Vitamin D and depression: cellular and regulatory mechanisms. *Pharmacological Reviews* **69**: 80-92.

Bertone-Johnson ER, Powers SI, Spangler L, Brunner RL, Michael YL, Larson JC, Millen AE, Bueche MN, Salmoirago-Blotcher E, Liu S, Wassertheil-Smoller S, Ockene JK, Ockene I, Manson JE (2011). Vitamin D intake from foods and supplements and depressive symptoms in a diverse population of older women. *American Journal of Clinical Nutrition* **94**: 1104-1112.

Bosee R, Di Paolo T (1995). Dopamine and GABAA receptor imbalance after ovariectomy in rats: model of menopause. *Journal of Psychiatry & Neuroscience: JPN* **20**: 364-371.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**: 248-254.

Bromberger J, Kravitz H, Chang Y, Randolph JF Jr, Avis NE, Gold EB, Matthews KA (2013). Does risk for anxiety increase during the menopausal transition? Study of women's health across the nation. *Menopause* **20**: 488-495.

Burger H (2008). The menopausal transition-endocrinology. *Journal of Sexual Medicine* **5**: 2266-2273.

Burstein O, Franko M, Gale E, Handelsman A, Barak S, Motsan S, Shamir A, Toledano R, Simhon O, Hirshler Y, Chen G, Doron R (2017). Escitalopram and NHT normalized stress-induced anhedonia and molecular neuroadaptations in a mouse model of depression. *PLoS One* **12**: e0188043.

Camargo A, Dalmagro AP, Rikel L, da Silva EB, Simão da Silva KAB, Zeni ALB (2018). Cholecalciferol counteracts depressive-like behavior and oxidative stress induced by repeated corticosterone treatment in mice. *European Journal of Pharmacology* **833**: 451-461.

Chu F, Ohinmaa A, Klarenbach S, Wong ZW, Veugelers P (2017). Serum 25-Hydroxyvitamin D concentrations and indicators of mental health: an analysis of the canadian health measures survey. *Nutrients* **9**: E1116.

DeLuca GC, Kimball SM, Kolasinski J, Ramagopalan SV, Ebers GC (2013). Review: the role of vitamin D in nervous system health and disease. *Neuropathology and Applied Neurobiology* **39**: 458-484.

Dulawa SC, Holick KA, Gundersen B, Hen R (2004). Effects of chronic fluoxetine in animal models of anxiety and depression. *Neuropsychopharmacology* **29**: 1321-1330.

Estrada-Camarena E, Fernandez-Guasti A, Lopez-Rubalcava C (2004). Interaction between estrogens and antidepressants in the forced swimming test in rats *Psychopharmacology* **173**: 139-145.

Eyles DW, Feron F, Cui X, Kesby JP, Harms LH, Ko P, McGrath JJ, Burne TH (2009). Developmental vitamin D deficiency causes abnormal brain development. *Psychoneuroendocrinology* **34**: S247-257.

Fedotova JO (2019). Vitamin D_3 treatment differentially affects anxiety-like behavior in the old ovariectomized female rats treated with low dose of 17β-estradiol. *BMC Medical Genetics* **20**: 49.

Fedotova J, Pivina S, Suchko A (2017). Effects of chronic vitamin D_3 hormone administration on anxiety-like behavior in adult

female rats after long-term ovariectomy. *Nutrients* **9**: 1-17.

Fedotova JO, Pivina SG, Volkova OV (2018). Vitamin D_3 application attenuates anxiety-like profile and increases 25-OH-VD_3 levels in the blood serum of the middle-aged female rats at 12 weeks after ovariectomy. *Activitas Nervosa Superior Rediviva* **60**: 55-66.

Garcia-Portilla MP (2009). Depression and perimenopause: a review. *Actas Españolas de Psiquiatría* **37**: 213-231.

Garcion E, Wion-Barbo N, Montero-Menei CN, Berger F, Wion D (2002). New clues about vitamin D functions in the nervous system. *Trends in Endocrinology and Metabolism* **13**: 100-105.

Gaugris S, Heaney RP, Boonen S, Kurth H, Bentkover JD, Sen SS (2005). Vitamin D inadequacy among post-menopausal women: a systemic review. *QJM: Monthly Journal of the Association of Physicians* **98**: 667-676.

Groves NJ, McGrath JJ, Burne TH (2014). Vitamin D as a neurosteroid affecting the developing and adult brain. *Annual Review of Nutrition* **34**: 117-141.

Hanson ND, Owens MJ, Nemeroff CB (2011). Depression, antidepressants, and neurogenesis: a critical reappraisal. *Neuropsychopharmacology* **36**: 2589-2602.

Harms LR, Burne THJ, Eyles DW, McGrath JJ (2011). Vitamin D and the brain. *Best Practice & Research. Clinical Endocrinology & Metabolism* **25**: 657-669.

Heffner TG, Hartman JA, Seiden LS (1980). A rapid method for the regional dissection of the rat brain. *Pharmacology, Biochemistry, and Behavior* **13**: 453-456.

Holick MF (2007). Vitamin D deficiency. *New England Journal of Medicine* **357**: 266-281.

Holick MF, Chen TC (2008). Vitamin D deficiency: a worldwide problem with health consequences. *American Journal of Clinical Nutrition* **87**: 1080S-6S.

Huang H, Zhao J, Jiang L, Xie Y, Xia Y, Lv R, Dong L (2015). Paeoniflorin improves menopause depression in ovariectomized rats under chronic unpredictable mild stress. *International Journal of Clinical and Experimental Medicine* **8**: 5103-5111.

Jiang P, Zhang WY, Li HD, Cai HL, Liu YP, Chen LY (2013) Stress and vitamin D: altered vitamin D metabolism in both hippocampus and myocardium of chronic unpredictable mild stress. *Psychoneuroendocrinology* **38:** 2091-2098.

Katz RJ (1981). Animal models and human depressive disorders. *Neuroscience and Biobehavioral Reviews* **5**: 231-246.

Kesby JP, Eyles DW, Burne TH, McGrath JJ (2011). The effects of vitamin D on brain development and adult brain function. *Molecular and Cellular Endocrinology* **347**: 121-127.

Kino T (2015). Stress, glucocorticoid hormones, and hippocampal neural pro- genitor cells: implications to mood disorders. *Frontiers in Physiology* **6**: 230.

Kiraly SJ, Kiraly MA, Hawe RD, Makhani V (2006). Vitamin D as neuroactive substance: review. *Scientific World Journal* **6**: 125-139.

Kjærgaard M, Joakimsen R, Jorde R (2011). Low serum 25-hydroxyvitamin D levels are associated with depression in an adult Norwegian population. *Psychiatry Research* **190**: 221-225.

Korte M, Kang H, Bonhoeffer T, Schuman E (1998). A role for BDNF in the late-phase of hippocampal long-term potentiation. *Neuropharmacology* **37**: 553-559.

Kwiecinksi GG, Petrie GI, De Luca HF (1989). 1,25-Dihydroxyvitamin D_3 restores fertility of vitamin D-deficient female rats. *American Journal of Physiology* **256**: E483-E487.

Ladea M, Bran M (2013). Brain derived neurotrophic factor (BDNF) levels in depressed women treated with open-label escitalopram. *Psychiatria Danubina* **25**: 128-132.

Lagunas N, Calmarza-Font I, Diz-Chaves Y, Garcia-Segura LM (2010). Long-term ovariectomy enhances anxiety- and depressive-like behaviors in mice submitted to chronic unpredictable stress. *Hormones and Behaviour* **58**: 786-791.

Lee HY, Kim YK (2008). Plasma brain-derived neurotrophic factor as a peripheral marker for the action mechanism of antidepressants. *Neuropsychobiology* **57**: 194-199.

Lessmann V, Brigadski T (2009). Mechanisms, locations, and kinetics of synaptic BDNF secretion: an update. *Neuroscience Research* **65**: 11-17.

Levada OA, Cherednichenko NV (2015). Brain-derived neurotrophic factor (BDNF): neurobiology and marker value in neuropsychiatry. *Likars'ka sprava* **(3-4)**: 15-25.

Li G, Mbuagbaw L, Samaan Z, Falavigna M, Zhang S, Adachi JD, Cheng J, Papaioannou A, Thabane L (2014). Efficacy of vitamin D supplementation in depression in adults: asystematic review. *Journal of Clinical Endocrinology and Metabolism* **99**: 757-767.

Luellen BA, Bianco LE, Schneider LM, Andrews AM (2007). Reduced brain-derived neurotrophic factor is associated with a loss of serotonergic innervation in the hippocampus of aging mice. *Genes, Brain, and Behaviour* **6**: 482-490.

Luk J, Torrealday S, Neal Perry G, Pal L (2012). Relevance of vitamin D in reproduction. *Human Reproduction* **27**: 3015-3027.

Magni LR, Purgato M, Gastaldon C, Papola D, Furukawa TA, Cipriani A, Barbui C (2013). Fluoxetine versus other types of pharmacotherapy for depression. *Cochrane Database of Systematic Reviews* **7**: CD004185.

Mizwicki MT, Norman AW (2009). The vitamin D sterol-vitamin D receptor ensemble model offers unique insights into both genomic and rapid-response signaling. *Science Signaling* **2**: 4.

Obradovic D, Gronemeyer H, Lutz B, Rein T (2006). Cross-talk of vitamin D and glucocorticoids in hippocampal cells. *Journal of Neurochemistry* **96**: 500-509.

Olivares-Nazario M, Fernandez-Guasti A, Martinez-Mota L (2016). Age-related changes in the antidepressant-like effect of desipramine and fluoxetine in the rat forced-swim test. *Behavioural Pharmacology* **27**: 22-28.

Pariante CM, Lightman SL (2008). The HPA axis in major depression: classical theories and new developments. *Trends in Neurosciences* **31**: 464-468.

Parker GB, Brotchie H, Graham RK (2017). Vitamin D and depression. *Journal of Affective Disorders* **208**: 56-61.

Przybelski R, Binkley N (2007). Is vitamin D important for preserving cognition? A positive correlation of serum 25-hydroxyvitamin D concentration with cognitive function. *Archives of Biochemistry and Biophysics* **460**: 202-220.

Rossouw JF, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J (2002). Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. *Journal of the American Medical Association* **288**: 321-333.

Sedaghat K, Yousefian Z, Vafaei AA, Rashidy-Pour A, Parsaei H,

Khaleghian A, Choobdar S (2019). Mesolimbic dopamine system and its modulation by Vitamin D in a chronic mild stress model of depression in the rat. *Behavioural Brain Research* **356**: 156-169.

Spedding S (2014). Vitamin D and depression: a systematic review and meta-analysis comparing studies with and without biological flaws. *Nutrients* **6**: 1501-1518.

Swaab DF, Bao AM, Lucassen PJ (2005). The stress system in the human brain in depression and neurodegeneration. *Ageing Research Reviews* **4**: 141-194.

Wang H, Zhang ZJ, Guo YJ, Zhou H, Teng GJ, Chen BA (2009). Anhedonia and activity deficits in rats: impact of post-stroke depression. *Journal of Psychopharmacology* **23**: 295-304.

Watanabe K, Hashimoto E, Ukai W, Ishii T, Yoshinaga T, Ono T, Tateno M, Watanabe I, Shirasaka T, Saito S, Saito T (2010). Effect of antidepressants on brain-derived neurotrophic factor (BDNF) release from platelets in the rats. *Progress in Neuro-psychopharmacology & Biological Psychiatry* **340**: 1450-1454.

Willner P, Towell A, Sampson D, Sophokleous S, Muscat R (1987). Reduction of sucrose preference bychronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. *Psychopharmacology* **93**: 358-364.

Workman JL, Gobinath AR, Kitay NK, Chow C, Brummelte S, Galea LA (2016). Parity modifies the effects of fluoxetine and corticosterone on behavior, stress reactivity, and hippocampal neurogenesis. *Neuropharmacology* **105**: 443-453.

Wolkowitz OM, Wolf J, Shelly W, Rosser R, Burke HM, Lerner GK, Reus VI, Nelson JC, Epel ES, Mellon SH (2011). Serum BDNF levels before treatment predict SSRI response in depression. *Progress in Neuro-psychopharmacology & Biological Psychiatry* **35**: 1623-1630.

Zhang Y, Gu F, Chen J, Dong W (2010). Chronic antidepressant administration alleviates frontal and hippocampal BDNF deficits in CUMS rat. *Brain Research* **1366**: 141-148.