Journal of Psychiatric Research 116 (2019) 61–68

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/jpsychires

4,4′-Dichlorodiphenyl diselenide reverses a depressive-like phenotype, modulates prefrontal cortical oxidative stress and dysregulated glutamatergic neurotransmission induced by subchronic dexamethasone exposure to mice

Suélen Osório Heck[a], Vanessa Angonesi Zborowski[a], Caroline Brandão Quines[b], Cristina Wayne Nogueira[c,*]

[a] Laboratory of Synthesis, Reactivity, Pharmacological and Toxicological Evaluation of Organochalcogen Compounds, Department of Biochemistry and Molecular Biology, Center of Natural and Exact Sciences, Federal University of Santa Maria, Santa Maria, RS, 97105-900, Brazil
[b] Laboratory of Biochemistry and Toxicology of Caenorhabditis Elegans, Department of Biochemistry, Federal University of Pampa, Campus Uruguaiana, RS, Brazil
[c] Departamento de Bioquímica e Biologia Molecular, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, CEP 97105-900, RS, Brazil

ARTICLE INFO

Keywords:
Dexamethasone
Depression
Selenium
Glucocorticoids
Fluoxetine

ABSTRACT

Dexamethasone (DEX) is a synthetic agonist of glucocorticoid receptors that has been associated with neurotoxicity and neuropsychiatric diseases. $(p\text{-ClPhSe})_2$ is an organoselenium compound reported to have antioxidant, antidepressant-like, and neuroprotective actions. This study investigated whether antioxidant activity and modulation of the glutamatergic system contribute to the antidepressant-like effect of $(p\text{-ClPhSe})_2$ in mice subchronically exposed to DEX. Swiss mice received intraperitoneal injections of DEX (2 mg/kg) or saline (vehicle) once a day for 21 days. After, the mice received $(p\text{-ClPhSe})_2$ (1–10 mg/kg) or mineral oil (vehicle) by the intragastric route (i.g.) for 7 days. The mice exposed to DEX were treated with fluoxetine (20 mg/kg, i.g.) once a day for 7 days. 24 h after the last treatment, the animals performed the locomotor activity (LMA), tail suspension, and forced swimming tests. Ex vivo assays were performed in samples of prefrontal cortex (PFC). The results show that $(p\text{-ClPhSe})_2$ reversed depressive-like behavioral phenotype induced by DEX without affecting LMA. Further, $(p\text{-ClPhSe})_2$ at all doses reduced ROS levels and increased CAT activity in the PFC of DEX-exposed mice. The highest dose of $(p\text{-ClPhSe})_2$ was effective against the decrease of SOD activity in the PFC of mice exposed to DEX. $(p\text{-ClPhSe})_2$ increased the $[^3H]$ glutamate uptake/release and decreased the Na^+/K^+-ATPase activity as well as the EAAT1 and NMDA R2A protein contents in the PFC of DEX-exposed mice. Regarding the NMDA R2B levels, there was no difference among experimental groups. In conclusion, this study reveals the effectiveness of $(p\text{-ClPhSe})_2$ in reversing the depressive-like phenotype of DEX-exposed mice. In addition, $(p\text{-ClPhSe})_2$ modulated oxidative stress and glutamate neurotransmission in the PFC of mice subchronically exposed to DEX.

1. Introduction

Depression, a chronic recurring illness that affects more than 300 million people worldwide, is characterized by sadness, loss of interest or pleasure, feelings of guilt or low self-worth and disturbed sleep or appetite (WHO, 2017). Despite progress in neuroscience research over the past few decades, the etiology and pathophysiology of depression are not yet well established (Dean and Keshavan, 2017); however, a growing body of data has suggested that increased oxidative stress and dysregulated glutamatergic neurotransmission, via the N-methyl-D-aspartate (NMDA) receptor, may contribute to the neuropathology of depression (Hashimoto et al., 2013; Siwek et al., 2013).

Accumulating evidence suggests that depressive symptoms are related to high doses of synthetic glucocorticoids (GCs), such as dexamethasone (DEX), a GCs receptors agonist (Carle and Abgrall-Barbry, 2016; Fietta et al., 2009). DEX is widely prescribed in the clinic due to its powerful immunosuppressive and anti-inflammatory properties (Grodzinsky et al., 2017), and it is the standard therapy to treat some specific diseases (Donia et al., 2010; Warris et al., 2014). Although the DEX therapy is associated with several beneficial effects, it has been

* Corresponding author.
E-mail address: criswn@ufsm.br (C.W. Nogueira).

https://doi.org/10.1016/j.jpsychires.2019.05.027
Received 1 February 2019; Received in revised form 4 May 2019; Accepted 31 May 2019

related to clinically relevant sleep and mood problems (Warris et al., 2016). Moreover, findings in experimental animal models have shown that DEX induces depressive/anxiogenic-like phenotype in mice (Skupio et al., 2015), oxidative stress in the rat brain (Zidan et al., 2018), and dysregulation of glutamatergic neurotransmission, via NMDA ionotropic receptor, in adult mice after neonatal exposure (Li et al., 2014).

Considering the limitations of available treatments and the high prevalence of depression among people (Wagner et al., 2018; WHO, 2017), it is interesting the development of novel effective therapeutic alternatives to treat this condition. This way, studies have demonstrated the efficacy of synthetic organoselenium compounds in different experimental models of depression (Nogueira and Rocha, 2011; Oliveira et al., 2017; Quines et al., 2016). Among these compounds, p-chlorodiphenyl diselenide $(p\text{-ClPhSe})_2$ has been reported to have antidepressant-like and antioxidant actions (Bortolatto et al., 2012) in animal models of disease. Moreover, $(p\text{-ClPhSe})_2$ modulates the serotoninergic system in the hypothalamus of rats (Bortolatto et al., 2015) and [^{3}H]glutamate uptake in the hippocampus of mice exposed to corticosterone, which are mechanisms related to its neuroprotective action (Zborowski et al., 2016).

Because oxidative stress and glutamatergic neurotransmission play a key role in the pathophysiology of affective disorders, this study was undertaken to investigate whether antioxidant activity and modulation of the glutamatergic system contribute to the antidepressant-like effect of $(p\text{-ClPhSe})_2$ in mice subchronically exposed to DEX.

2. Material and methods

2.1. Animals

The present experimental protocol was approved by the Ethical Research Committee of Federal University of Santa Maria (register number: 7003210715/2016) affiliated to the Council for Control of Animal Experiments (CONCEA) and in accordance with the NIH Guide for the Care and Use of Laboratory Animals. The experiments were carried out using male Swiss mice (8 weeks old) weighing between 35 and 40 g from our breeding colony. Animals were housed in cages with free access to food (GUABI, RS, Brazil) and water. They were kept in a room with controlled temperature (22 ± 2 °C), on a 12/12 h light-dark cycle (with lights turned on at 7:00 a.m.).

2.2. Drugs

Dexamethasone sodium phosphate 4 mg/mL was purchased from Farmace Chemical Company (Brazil) and diluted in saline solution (SAL) to achieve the correct dose. Fluoxetine was purchased from Sigma Chemical (St. Louis, MO, USA) and diluted in water. $(p\text{-ClPhSe})_2$ was prepared and characterized in our laboratory according to the method described in the literature (Paulmier, 1986). The chemical purity (99.9%) of $(p\text{-ClPhSe})_2$ was determined by gas chromatography–mass spectrometry (GC/MS). ^{1}H and ^{13}C Nuclear Magnetic Resonance Spectroscopy analysis showed analytical and spectroscopic agreement with the assigned structure. $(p\text{-ClPhSe})_2$ was diluted in mineral oil.

2.3. Experimental design

The experimental protocol of this study was divided into three processing sets as shown in Fig. 1. In the first set (n = 8), all animals were distributed equally into nine groups to perform the behavioral tests and the PFC samples were designated to analyze parameters of oxidative stress (SAL + mineral oil, SAL + $(p\text{-ClPhSe})_2$ 1 mg/kg, SAL + $(p\text{-ClPhSe})_2$ 5 mg/kg, SAL + $(p\text{-ClPhSe})_2$ 10 mg/kg, DEX + mineral oil, DEX + $(p\text{-ClPhSe})_2$ 1 mg/kg, DEX + $(p\text{-ClPhSe})_2$ 5 mg/kg, DEX + $(p\text{-ClPhSe})_2$ 10 mg/kg, DEX + fluoxetine). In the second (n = 5) and third (n = 5) sets, the animals were subjected to the same behavioral experience; however, only the highest dose of $(p\text{-ClPhSe})_2$, the most effective, was chosen to be carried out in the ex vivo analyses, totalizing five groups (SAL + mineral oil, SAL + $(p\text{-ClPhSe})_2$ 10 mg/kg, DEX + mineral oil, DEX + $(p\text{-ClPhSe})_2$ 10 mg/kg, DEX + fluoxetine).

The animals received DEX at the dose of 2 mg/kg/body weight, or SAL (10 mL/kg), by the intraperitoneal (i.p.) route at 6.00 p.m. for 21 days. At the 22nd day, the animals performed tail suspension (TST) and forced swimming (FST) tests, in this sequence, to investigate the development of depressive-like phenotype induced by subchronic administration of DEX. The resilient phenotype was defined based on the mean values of immobility time of control group in both the TST and FST; therefore, all animals exposed to DEX that exhibited mean values of immobility time less than those of controls were characterized as resilient to these tests. The mice that exhibited resiliency to depressive-like phenotype (23%) were excluded from this experimental protocol.

The animals with depressive-like phenotype were treated with $(p\text{-ClPhSe})_2$ (1, 5 or 10 mg/kg), fluoxetine (20 mg/kg) (Zhu et al., 2016) or mineral oil (vehicle, 10 mL/kg) by the i.g. Route once a day for 7 days (day 22–28). Twenty-four h after the last treatment, in order to minimize the number of mice used, each animal was subjected to three behavior tests in the following sequence (locomotor activity test (LMA), TST, and FST) without time interval, which likely could have influenced the measured variables.

Although, all experiments have the respective control groups, we assume this as a limitation of the present study. The behavior tests were carried out between 8:00 and 12:00 a.m., whereas the i.g. treatments were administered to mice at 1:00 p.m.

At the 30th day, the animals were killed by cervical dislocation, the PFC samples from mice of the first and third sets were removed and frozen at −80 °C for a maximum time of 3 months to carry out the ex vivo assays.

2.4. Behavior tests

2.4.1. Spontaneous locomotor activity

Spontaneous locomotor activity of animals was performed for 4 min using a monitor that continuously tracks the animal's movement. The mice were placed in the center of the apparatus cage (45 cm × 45 cm × 45 cm) and allowed to freely explore this space. Number of crossings, average speed (mm/s), and total distance traveled (mm) were monitored and recorded through Insight® Monitor Activity software. Because the animals were not habituated to the locomotion apparatus before testing, the results of this test could indicate a response to a novel environment, a measure of anxiety-like behavior (Seibenhener and Wooten, 2015).

2.4.2. Tail suspension test (TST)

The TST was used to assess the mouse depressive-like behavior and was performed based on the method reported by Steru et al. (1985). Each mouse was suspended by its tail with adhesive tape in a horizontal wooden bar approximately 30 cm above the floor. The latency for the first immobility episode and total duration of immobility were evaluated for 6 min by a blinded observer using a stopwatch. The mouse was considered immobile when it hung passively and completely motionless.

2.4.3. Forced swimming test (FST)

The FST was used for assessing the mouse depressive-like behavior and the procedure was based on that previously described (Porsolt et al., 1979). Each mouse was gently placed in a cylindrical container (15 cm × 40 cm) containing 19 cm of water maintained at 25 ± 1 °C. The latency for the first immobility episode and total duration of immobility were measured for 6 min by a blinded observer using a stopwatch. The mouse was considered immobile when it ceased struggling

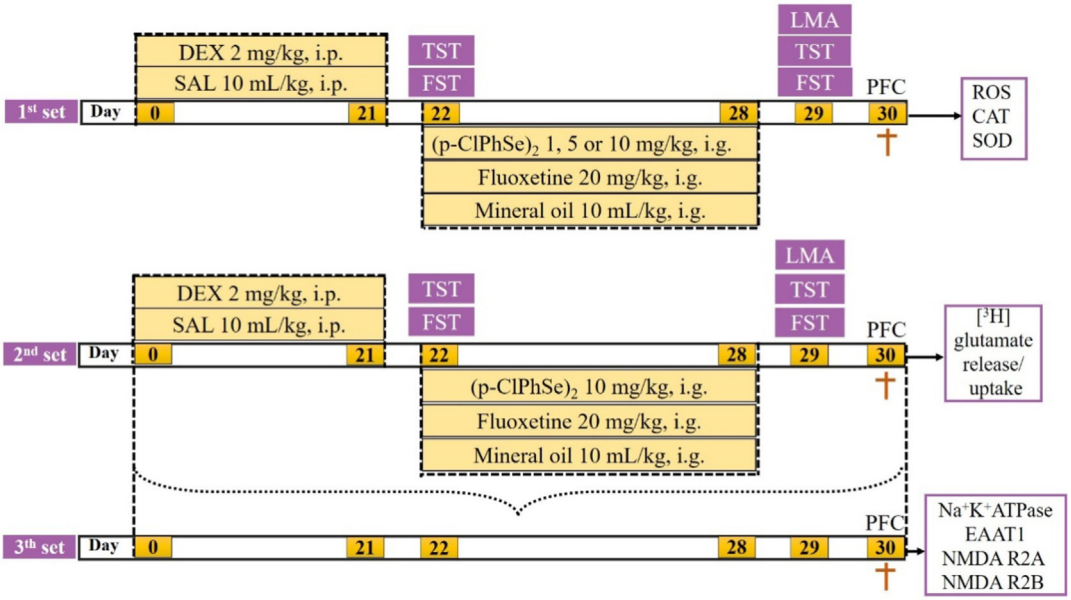

Fig. 1. Schematic representation of experimental protocol.

and remained floating motionless in the water, making only those movements necessary to keep its head above water.

2.5. Ex vivo assays

2.5.1. Parameters of oxidative stress

In order to determine the effect of $(p\text{-ClPhSe})_2$ on oxidative imbalance in the depressive-like phenotype induced by subchronic exposure to DEX, the samples of PFC from mice of the first set were collected and homogenized in 50 mM Tris–HCl, pH 7.4 (1:10, w/v). The homogenate was centrifuged at 2500 × *g* for 10 min at 4 °C, and a low-speed supernatant fraction (S1) was used for the following determinations: the levels of Reactive oxygen species (ROS) and activities of Catalase (CAT), and Superoxide dismutase (SOD).

2.5.1.1. ROS levels. The reactive oxygen species levels were determined by a spectrofluorimetric method, using 2′,7′ dichlorofluorescein diacetate (DCHF-DA) (Loetchutinat et al., 2005). The oxidation of DCHF-DA to fluorescent dichlorofluorescein (DCF) is measured for the detection of intracellular ROS. S1 (10 µL) was incubated with 10 µL of DCHF-DA (1 mM) and 3 mL of Tris–HCl (10 mM), pH 7.4. The DCF fluorescence intensity emission was recorded at 520 nm (with 480 nm excitation) 60 min after the addition of DCHF-DA to the medium. The results were expressed as arbitrary units (AU) of fluorescence.

2.5.1.2. CAT activity. CAT activity was determined by a spectrophotometric assay according to the method reported by Aebi (1984). The enzymatic reaction was performed by the addition of a S1 aliquot and H_2O_2 (substrate) at a concentration of 0.3 mM in a medium containing phosphate buffer (50 mM), pH 7.0. The enzymatic activity was expressed in units/mg of protein. 1 unit (U) decomposes 1 µmoL of H_2O_2/min at pH 7 and at 25 °C.

2.5.1.3. SOD activity. SOD activity was measured spectrophotometrically according to the method described by Misra and Fridovich (1972). This method is based on the capacity of SOD in inhibiting autoxidation of epinephrine to epinechrome. In this assay, S1 diluted 1:10 (v/v) was added in the 50 mmol/L Na_2CO_3 buffer pH 10.3, and the enzymatic reaction was initiated by adding epinephrine. The color reaction was measured at 480 nm and the results were expressed in units/mg of protein. 1 U decomposes 1 µmoL of epinephrine/min at pH 7 and at 25 °C.

2.5.2. Glutamatergic system

In order to investigate the effect of $(p\text{-ClPhSe})_2$ on the glutamatergic system of mice subchronically exposed to DEX, the PFC samples obtained from mice of the second set of experimental protocol were used to determinate [^{3}H] glutamate release and uptake. The PFC samples taken from mice of the third processing batch were designated to determine the activity of Na^+, K^+- ATPase and the protein levels, by using Western blot assay, of EAAT1 (excitatory amino acid transporter 1), NMDA R2A (N-methyl-D-aspartate receptor sub-unit 2A) and NMDA R2B (N-methyl-D-aspartate receptor subunit 2B).

2.5.2.1. [^{3}H] Glutamate release assay. The crude synaptosomes were obtained based on a previously described method (Gray and Whittaker (1962), in which the samples of PFC were firstly homogenized in homogenization solution (Sucrose 1.28 M, EDTA 4 mM and DTT 25 mM) and centrifuged at 1000 × *g* at 4 °C for 10 min. Then, the supernatants were centrifuged at 12000 × *g* at 4 °C for 20 min. After, the pellets were washed three times with Tris/HCl-buffered salt solution pH 7.4 (Tris/HCl 27 mM, NaCl 133 mM, KCl 2.4 mM, $MgSO_4$ 1.2 mM, KH_2PO_4 1.2 mM, Glucose 12 mM, $CaCl_2$ 1.0 mM) and centrifuged at 15000 × *g* at 4 °C for 15 min. At the end, the final pellet of this centrifugation was suspended in ten volumes of ice-cold sucrose solution (0.32 M, pH 7.4) and then used as a crude synaptosome preparation in the [^{3}H] glutamate release assay, that was carried out as described by Migues et al. (1999). The PFC synaptosomal suspension (Gray and Whittaker, 1962) was loaded with 0.25 µCi [3H] glutamate (Amersham, specific activity 53 mCi/mmoL, final concentration 5 µM) and pre-incubated with Tris/HCl-buffered salt solution at 37 °C for 15 min. Aliquots of crude synaptosomes (1.4 mg protein) were centrifuged at 16000 × *g* for 1 min. Supernatants were discarded, and the pellets were washed four times in Tris/HCl buffer by centrifugation at 16000 × *g* at 4 °C for 1 min. To assess the basal release of [^{3}H] glutamate, the final pellet was resuspended in Tris/HCl buffer and incubated for 1 min in this medium at 37 °C. Incubation was terminated by immediate centrifugation (16000 × *g*, 1 min, 4 °C). Radioactivity present in supernatants and pellets was separately determined in a scintillation counter. The released [^{3}H] glutamate was calculated as a percentage of the total amount of radiolabel present in the

synaptosomes at the start of the incubation.

2.5.2.2. [^{3}H] Glutamate uptake assay. The glutamate uptake of PFC slices was performed based on the method reported by Thomazi et al. (2004). The slices were washed with 1.0 mL Hank's buffered salt solution (HBSS). After 10 min of pre-incubation, the uptake assay was performed by adding 13.3 μM [^{3}H] glutamate in 300 μL HBSS at 37 °C. Incubation was terminated after 5 min by three ice-cold washes with Milli-Q water immediately followed by the addition of 0.5M NaOH, which was kept overnight. Unspecific uptake was measured using the same protocol described above, with differences in the temperature (4 °C) and medium composition (choline chloride stead of sodium chloride). Na^+-dependent uptake was considered as the difference between the total uptake and the unspecific uptake. Incorporated radioactivity was measured using a liquid scintillation counter.

2.5.2.3. Na^+/K^+-ATPase activity. The Na^+, K^+- ATPase activity was determined according to a method described by Wyse et al. (2000). For this, an aliquot of S1 was added to the reaction mixture for Na^+, K^+-ATPase activity assay containing 3 mM MgCl, 125 mM NaCl, 20 mM KCl and 50 mM Tris–HCl, pH 7.4, in a final volume of 500 μL. The reaction was initiated by the ATP addition to a final concentration of 3.0 mM. Control samples were accomplished under the same conditions with the addition of 0.1 mM ouabain. The samples were incubated at 37 °C for 30 min, and the reaction was stopped by trichloroacetic acid solution (10% TCA) with 10 mM $HgCl_2$. The Na^+, K^+-ATPase activity was calculated by the difference between the two assays. Released inorganic phosphate (Pi) was measured based on the method reported by Fiske and Subbarow (1925). The enzymatic activity was expressed as nmol Pi/min/mg protein.

2.5.2.4. Western blot assay. The protein levels of EAAT1 (excitatory amino acid transporter 1), NMDA R2A (N-methyl-D-aspartate receptor sub-unit 2A) and NMDA R2B (N-methyl-D-aspartate receptor subunit 2B) were detected. For this purpose, PFC samples were homogenized in ice-cold sucrose buffer containing 1 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 0.1 mM ethylene glycol tetraacetic acid (EGTA) and 0.1 mM phenyl methyl sulfonyl fluoride in the presence of protein inhibitor commercial cocktail (Sigma-Aldrich Company, St. Louis, Missouri, United States). The PFC extracts were diluted to a final protein concentration of 2 μg/μL. The samples (20 μg protein) and pre-stained molecular weight standards (Sigma-Aldrich Company, St. Louis, Missouri, United States) were separated on 10% resolving with 4% concentrating SDS-PAGE electrophoresis gels. Proteins were transferred to nitrocellulose membrane using Transfer-Blot® Turbo™ Transfer System (1.0 mA; 45 min).

After blocking with 3% bovine serum albumin solution, the blots were incubated overnight at 4 °C with primary antibodies (Table 1). β-actin was stained as additional control of the protein loading. After primary antibodies incubation, membranes were washed and incubated with secondary antibodies conjugated with horseradish peroxidase (Bio-Rad Laboratories, Hercules, CA, USA) for 1 h at room temperature and developed with chemiluminescence kit (Amersham, São Paulo/Brazil). Optical density (OD) of the Western blotting bands was quantified using Image J software. Each value was derived from the ratio between arbitrary units obtained by the protein band and the respective β-actin band.

Table 1
List of primary antibodies.

Antibody	Type	Company	Dilution
β-actin	Mouse	CellSignaling	1:5000
EAAT1	Rabbit	CellSignaling	1:1000
NMDA R2A	Rabbit	CellSignaling	1:1000
NMDA R2B	Rabbit	CellSignaling	1:1000

2.5.3. Protein determination

The protein concentration was measured by the Bradford method (Bradford, 1976) using bovine serum albumin as a standard.

2.6. Statistical analysis

The normality of data was analyzed using a D'Agostino and Pearson omnibus normality test. Statistical comparisons among experimental groups were performed by one-way analysis of variance (ANOVA) followed by the Newman-Keuls test. The results are presented as the mean ± standard error of the mean (SEM). Probability values less than 0.05 ($p < 0.05$) were considered as statistically significant.

3. Results

3.1. $(p\text{-ClPhSe})_2$ reverses depressive-like behavioral phenotype induced by subchronic exposure of DEX in mice

The results revealed a statistically significant increase in the immobility time of mice subchronically exposed to DEX when compared to those of the vehicle-exposed animals in the TST [$F_{(1,36)} = 7.72$; $p < 0.001$] and FST [$F_{(1,36)} = 9.11$; $p < 0.001$]. $(p\text{-ClPhSe})_2$ at the doses of 5 and 10 mg/kg was effective against the immobility time of mice increased by DEX ($p < 0.05$ and $p < 0.01$, respectively) in the TST (Fig. 2a) and FST (Fig. 2b). The lowest dose of $(p\text{-ClPhSe})_2$ was ineffective against the increase in the immobility time of mice exposed to DEX in both tests (Fig. 2a and b).

In addition, fluoxetine was effective against the increase in the immobility time induced by DEX in the TST and FST ($p < 0.001$) (Fig. 2a and b).

Regarding the latency for the first immobility episode in the TST and FST, there was no difference among groups (Fig. 2c and d). In the LMA, the number of crossings, speed, and total distance traveled were not altered in mice of all experimental groups ($p > 0.05$) (Table 2).

3.2. $(p\text{-ClPhSe})_2$ has an antioxidant action in the PFC of mice subchronically exposed to DEX

Fig. 3a depicts that $(p\text{-ClPhSe})_2$ reversed the increase in ROS levels induced by DEX in the PFC of mice. The results indicate a significant increase in ROS levels of DEX-exposed mice when compared to those of the vehicle-exposed animals ($F_{(1,36)} = 4.76$; $p < 0.01$). $(p\text{-ClPhSe})_2$ at all doses tested ($p < 0.01$) as well as fluoxetine ($p < 0.001$) decreased the ROS levels in the PFC of mice exposed to DEX.

The data revealed a significant decrease in CAT ($F_{(1,36)} = 3.77$; $p < 0.05$) and SOD ($F_{(1,36)} = 4.36$; $p < 0.05$) activities of DEX-exposed mice when compared with those of the vehicle group. $(p\text{-ClPhSe})_2$ at all doses tested was effective against the decrease in CAT activity in the PFC of mice exposed to DEX. Only at a dose of 10 mg/kg, $(p\text{-ClPhSe})_2$ was effective against the decrease of SOD activity in the PFC of mice exposed to DEX ($p < 0.05$).

$(p\text{-ClPhSe})_2$ and fluoxetine were effective against the decrease in CAT ($p < 0.05$) and SOD ($p < 0.001$) activities induced by DEX in the PFC of mice (Fig. 3b and c).

3.3. $(p\text{-ClPhSe})_2$ modulates the glutamatergic system in the PFC of mice subchronically exposed to DEX

The results show a decrease in the [^{3}H] glutamate uptake ($F_{(1,10)} = 12.07$; $p < 0.001$) and [^{3}H] glutamate release ($F_{(1,10)} = 8.63$; $p < 0.001$) in the PFC of mice exposed to DEX when compared with those of the vehicle group. $(p\text{-ClPhSe})_2$ increased the [^{3}H] glutamate uptake ($p < 0.01$) and [^{3}H] glutamate release ($p < 0.01$) in the PFC of mice exposed to DEX (Fig. 4a and b).

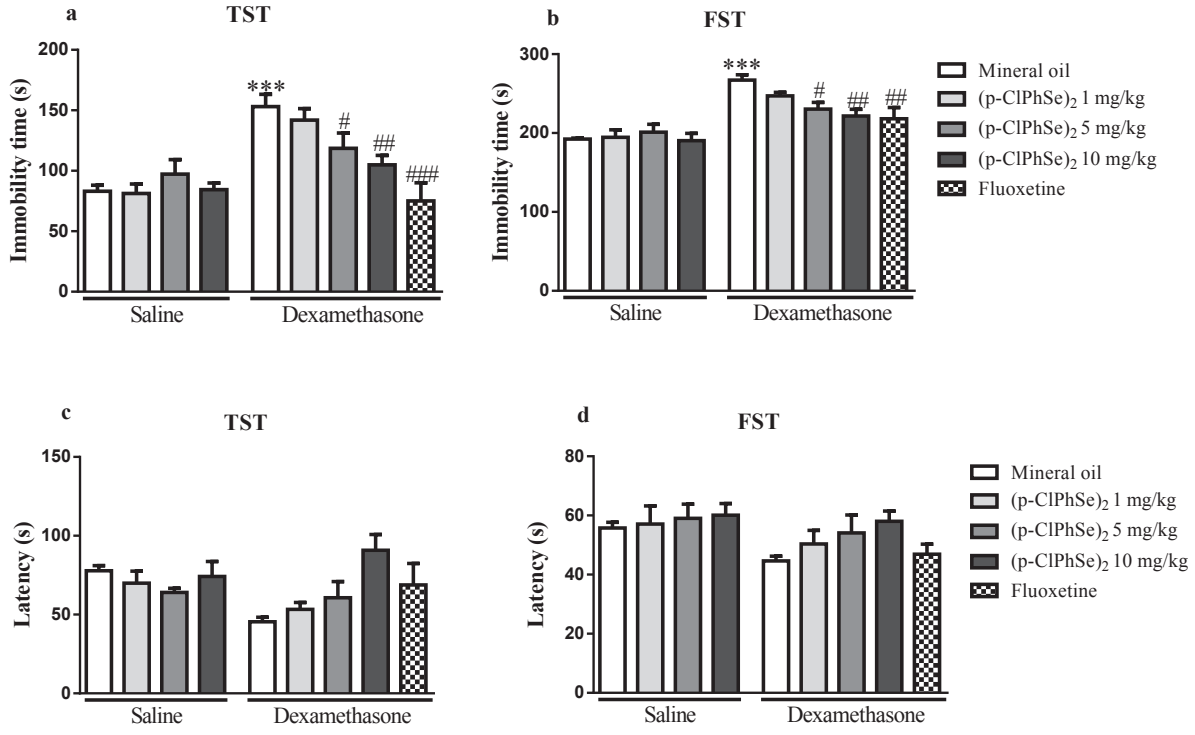

Fig. 2. Effects of $(p\text{-ClPhSe})_2$ (1–10 mg/kg) and fluoxetine (20 mg/kg) on immobility time in the TST (a) and FST (b) as well as on latency in the TST (c) and FST (d) of mice subchronically exposed to DEX. The results represent the means ± S.E.M. of 8 animals per group. Data analysis was carried out through one-way ANOVA followed by the Newman-Keuls post-test. Significance: ***$p < 0.001$ as compared with the vehicle-treated mice. #$p < 0.05$, ##$p < 0.01$ and ###$p < 0.001$ as compared with the DEX-exposed mice.

Table 2
Effects of $(p\text{-ClPhSe})_2$ and fluoxetine on locomotor activity of mice subchronically exposed to DEX.

Group/parameters	Crossings	Distance (mm)	Speed (mm/s)
Vehicle	500.5 ± 53.9	8230 ± 865	38.2 ± 2.7
$(p\text{-ClPhSe})_2$ mg/kg			
1	470.6 ± 60.8	7230 ± 595	36.5 ± 3.3
5	435.3 ± 65.3	7234 ± 1174	32.1 ± 4.1
10	431.3 ± 34.9	6790 ± 692	32.4 ± 2.9
DEX	406.6 ± 30.8	6409 ± 548	32.4 ± 2.1
DEX + $(p\text{-ClPhSe})_2$ mg/kg			
1	382.4 ± 41.2	6350 ± 597	31.3 ± 2.8
5	374.4 ± 40.9	6967 ± 660	31.8 ± 2.2
10	376.6 ± 28.8	6375 ± 734	30.9 ± 2.8
DEX + Fluoxetine 20 mg/kg	350.4 ± 33.6	6173 ± 640	27.2 ± 3.2

The results represent the means ± S.E.M. of 8 animals per group. Data analysis was carried out through one-way ANOVA followed by the Newman-Keuls post-test. DEX means dexamethasone.

However, fluoxetine was ineffective against the decrease of [^{3}H] glutamate uptake, but it reversed the decrease in [^{3}H] glutamate release ($p < 0.01$), in the PFC of mice exposed to DEX (Fig. 4a and b).

The results illustrated in Fig. 4c show that $(p\text{-ClPhSe})_2$ and fluoxetine reversed the increase of Na^+/K^+-ATPase activity induced by DEX in the PFC of mice. The data revealed a significant increase in the Na^+/K^+-ATPase activity of DEX-exposed mice when compared to those of the vehicle-exposed animals ($F_{(1,10)} = 7.93$; $p < 0.01$).

Fig. 5 shows a significant increase in the protein contents of EAAT1 ($F_{(1,10)} = 5.12$; $p < 0.01$) and NMDA R2A ($F_{(1,10)} = 8.16$; $p < 0.01$) but not of NMDA R2B in the PFC of mice exposed to DEX when compared with those of the vehicle group.

$(p\text{-ClPhSe})_2$ and fluoxetine were effective against the increase in protein contents of EAAT1 and NMDA R2A induced by DEX in the PFC of mice ($p < 0.05$).

The NMDA R2B levels in the PFC of mice exposed to DEX were not altered by $(p\text{-ClPhSe})_2$ and fluoxetine treatments.

4. Discussion

To the best of our knowledge this is the first study that shows the effectiveness of $(p\text{-ClPhSe})_2$ to reverse depressive-like behavioral phenotype induced by DEX subchronic exposure in Swiss mice. The $(p\text{-ClPhSe})_2$ antidepressant-like action was accompanied by the modulation of oxidative stress and glutamatergic neurotransmission, via the NMDA receptor, in the PFC of mice subchronically exposed to DEX, without affecting the spontaneous locomotor behavior.

The development of depressive-like phenotype of this experimental protocol was based on a pilot study, in which Swiss mice received DEX at the dose of 2 mg/kg (i.p.) for 21 days. In our previous study, we showed that this dose of DEX was sufficiently high to induce the reduction of body weight in mice from the 9th to the 21st day when compared to that of the saline group (control). These findings are also in accordance with previous studies (Sigwalt et al., 2011; Skupio et al., 2015), that indicated that DEX exposure results in loss of body weight in mice. Although we did not measure the brain weight in none of our studies, we hypothesize that the exposure to DEX can also reduce this measure, as demonstrated by others (Chang, 2014; Kanagawa et al., 2006).

The glutamate system is crucial to many brain processes, including modulation of neurogenesis/neurodegeneration and neuronal plasticity (Mattson, 2008). Growing evidence suggests that the glutamatergic signaling plays a key role in neurobiology and treatment of depressive disorders (Duric et al., 2013; Hashimoto et al., 2013). The PFC, as a significant nerve center of thinking and behavior regulation in the brain, is very associated with depression (Liu et al., 2017; Treadway et al., 2015). In the present study, subchronic exposure to DEX induced a depressive-like behavioral phenotype in mice and a dysregulation of the prefrontal cortical glutamatergic signaling. In fact, the DEX effects

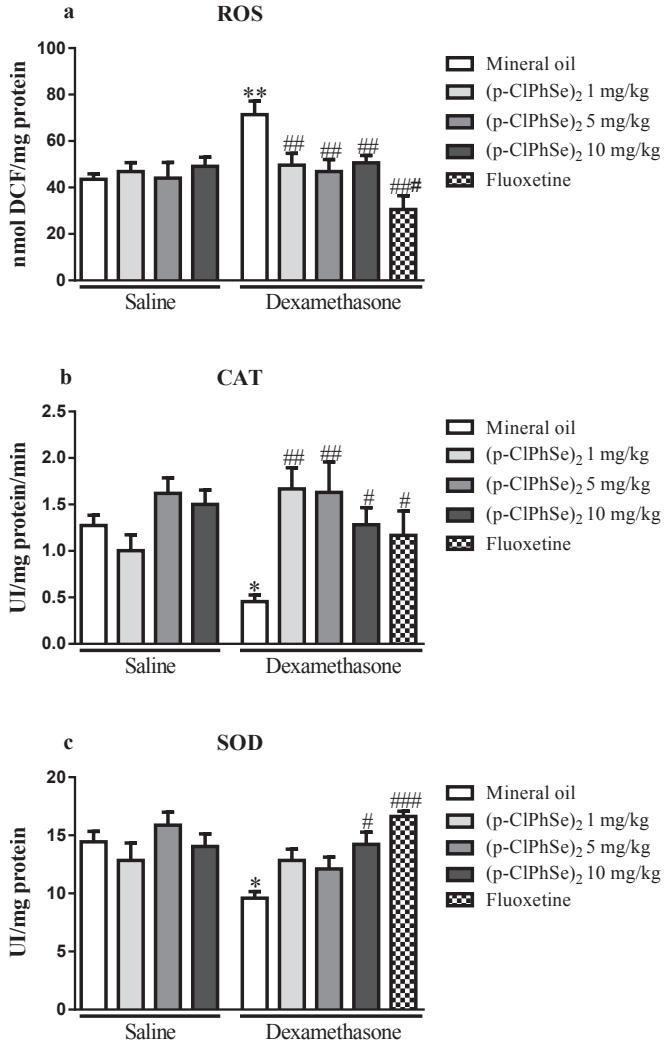

Fig. 3. Effects of (p-ClPhSe)$_2$ (1–10 mg/kg) and fluoxetine (20 mg/kg) on ROS levels (a), CAT activity (b) and SOD activity (c) in the PFC of mice subchronically exposed to DEX. The results represent the means ± S.E.M. of 8 animals per group. Data analysis was carried out through one-way ANOVA followed by the Newman-Keuls post-test. Significance: *p < 0.05, **p < 0.01 as compared with the vehicle-treated mice. #p < 0.05, ##p < 0.01 and ###p < 0.001 as compared with the DEX-exposed mice.

were different from the homeostatic glutamatergic neurotransmission; in which after glutamate release as the result of an action potential, glutamate transporters quickly remove it from the extracellular space to keep its levels low, thereby terminating the synaptic transmission (Reiner and Levitz, 2018). Considering the results obtained with DEX in this study, we hypothesize that the reduced glutamate uptake causes an increase in the extracellular glutamate concentration, leading to an increase in the levels of the NMDA R2A subunit as well as in the EAAT1 levels, a Na^+ dependent glutamate transporter, and the corresponding increase in the Na^+, K^+-ATPase activity, leading to a compensatory decrease in the [^{3}H] glutamate release in an attempt to restore the glutamate neurotransmission homeostasis in the PFC of mice. In support to this hypothesis, researchers have demonstrated a decrease in the glutamate release induced by DEX in neurons of Sprague–Dawley rats (Wang et al., 2012) and an increased activity of Na^+, K^+-ATPase, which was stimulated by elevated glutamate concentrations in human fetal astrocytes (Gegelashvili et al., 2007).

It is well known that excessive extracellular levels of glutamate can lead to neuronal injury or death, contributing to the risk of developing depression (Lipton and Rosenberg, 1994). Therefore, dysregulation of glutamatergic neurotransmission found in this study could contribute to depressive-like phenotype induced by subchronic exposure to DEX in mice. Accordingly, Li et al. (2014) reported that neonatal exposure to DEX disturbed the glutamatergic transmission, via the NMDA receptor, resulting in a depression-like behavior of the juvenile mice. Besides, the disturbance of the glutamatergic neurotransmission induced by DEX, found in the present study, could be associated to oxidative stress because the overactivation of NMDA receptors leads to an increase in the Ca^{++} influx, which can give rise to oxidative stress (Michaelis, 1998).

Studies have suggested that an increase of oxidative stress, characterized by an imbalance between pro-oxidant molecules and antioxidant defenses, contributes to the neuropathology of depression (Yusuf et al., 2018). The increased ROS levels and reduced activities of antioxidant enzymes, found in the present study, denote an increased oxidative stress frame (Yusuf et al., 2018) in the PFC of depressive-like mice induced by DEX. Accordingly, other studies associated the increase of oxidative stress with both DEX exposure (Raciti et al., 2016) and depressive-like behavioral phenotype (Patel and Udayabanu, 2014). It is well known that a rise in ROS levels is associated with excess of glutamate in the synaptic cleft, followed by prolonged overactivation of ionotropic glutamate receptors, contributing to neuronal excitotoxicity (Bondy and Lee, 1993; Lau and Tymianski, 2010). Our results showed that high ROS levels in the PFC of mice with depressive-like phenotype induced by DEX were accompanied by an increase in the levels of the NMDA R2A subunit, which could contribute to neurotoxicity induced by DEX.

Antioxidants and/or modulators of the glutamatergic system, especially via NMDA receptor, have been studied as promising candidates for the development of new antidepressants (Huang et al., 2017; Kaufmann et al., 2016). The results of the present study indicate that (p-ClPhSe)$_2$, as well as the antidepressant fluoxetine reversed the depressive-like phenotype induced by DEX in mice. In addition, both treatments modulated oxidative stress and dysregulation of glutamatergic neurotransmission in the PFC of mice exposed to DEX, which could contribute to the antidepressant-like action, of (p-ClPhSe)$_2$ and

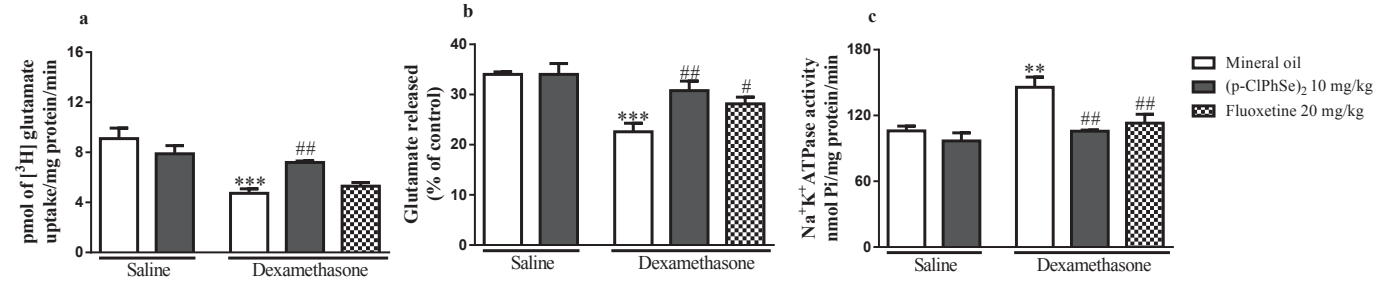

Fig. 4. Effects of (p-ClPhSe)$_2$ (10 mg/kg) and fluoxetine (20 mg/kg) on glutamate uptake (a), glutamate release (b) and Na^+K^+ATPase activity (c) in the PFC of mice subchronically exposed to DEX. The results represent the means ± S.E.M. of 5 animals per group. Data analysis was carried out through one-way ANOVA followed by the Newman-Keuls post-test. Significance: **p < 0.01, ***p < 0.001 as compared with the vehicle-treated mice. #p < 0.05, ##p < 0.01 as compared with the DEX-exposed mice.

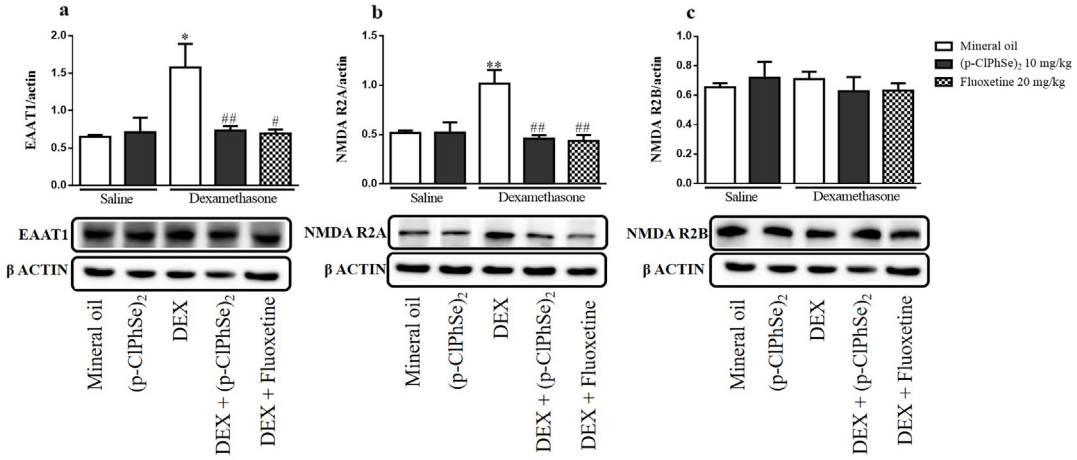

Fig. 5. Effects of (p-ClPhSe)$_2$ (10 mg/kg) and fluoxetine (20 mg/kg) on the protein contents of EAAT1 (a), NMDA R2A (b) and NMDA R2B (c) in the PFC of mice subchronically exposed to DEX. Representative qualitative Western blotting analyses are at the bottom of figure. The results represent the means ± S.E.M. of 5 animals per group. Data analysis was carried out through one-way ANOVA followed by the Newman-Keuls post-test. Significance: *p < 0.05, **p < 0.01 as compared with the vehicle-treated mice. #p < 0.05, ##p < 0.01 as compared with the DEX-exposed mice.

fluoxetine.

In addition, (p-ClPhSe)$_2$ has been reported to have central pharmacological actions that include: antidepressant-like; improvement of spatial memory in aged rats (Bortolatto et al., 2012); modulation of 5-HT1A and 5-HT3 receptors; antioxidant (Bortolatto et al., 2012); anorectic-like, modulation of hypothalamic neuropeptides involved in the appetite control (Bortolatto et al., 2017) and of 5-HT uptake (Bortolatto et al., 2015); and modulation of hippocampal [^{3}H]glutamate uptake in corticosterone-exposed mice (Zborowski et al., 2016).

Regarding the effects of fluoxetine, our present findings are in accordance with previous studies (Moretti et al., 2012; Vizi et al., 2013) which demonstrated the beneficial effects of fluoxetine on oxidative stress and glutamatergic signaling as well as the antidepressant-like action in a model of depression induced by DEX in rats (Djordjevic et al., 2012). However, the purpose of our study was not to compare the antidepressant-like effects of (p-ClPhSe)$_2$ with those of fluoxetine, a classical antidepressant. Fluoxetine was used only as positive control in this experimental protocol because the pharmacokinetic and toxicokinetic of (p-ClPhSe)$_2$ are unknown and the dose of fluoxetine used in this study was two times higher than that of (p-ClPhSe)$_2$. Moreover, the chemical structures of (p-ClPhSe)$_2$ ($C_{12}H_{10}Se_2$) and fluoxetine ($C_{17}H_{18}F_3NO$) are not similar.

In conclusion, this study shows the effectiveness of (p-ClPhSe)$_2$ treatment in reversing depressive-like behavioral phenotype of mice subchronically exposed to DEX. In addition, the present study reveals that (p-ClPhSe)$_2$ modulated oxidative stress and glutamate neurotransmission in the PFC of mice subchronically exposed to DEX.

Conflicts of interest

The authors declare that there is no conflict of interest in the present study.

Funding information/acknowledgements

We gratefully acknowledge Universidade Federal de Santa Maria, Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS, grant number 17/2551- 0000), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES/PROEX #23038.005848/2018-31), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, grant number 407118/2018-7) for the financial support.

References

Aebi, H., 1984. Catalase in vitro. Methods Enzymol. 105, 121–126. http://www.ncbi.nlm.nih.gov/pubmed/6727660.

Bondy, S.C., Lee, D.K., 1993. Oxidative stress induced by glutamate receptor agonists. Brain Res. 610 (2), 229–233. http://www.ncbi.nlm.nih.gov/pubmed/8319085.

Bortolatto, C.F., Heck, S.O., Gai, B.M., Zborowski, V.A., Neto, J.S., Nogueira, C.W., 2015. Effects of diphenyl and p-chloro-diphenyl diselenides on feeding behavior of rats. Psychopharmacology 232 (13), 2239–2249. https://doi.org/10.1007/s00213-014-3856-z. http://www.ncbi.nlm.nih.gov/pubmed/25563236.

Bortolatto, C.F., Nogueira, C.W., Porteiro, B., Imbernon, M., Nogueiras, R., 2017. Hypothalamic pathways regulate the anorectic action of p-chloro-diphenyl diselenide in rats. Eur. J. Pharmacol. 815, 241–250. https://doi.org/10.1016/j.ejphar.2017.09.032. http://www.ncbi.nlm.nih.gov/pubmed/28943102.

Bortolatto, C.F., Wilhelm, E.A., Chagas, P.M., Nogueira, C.W., 2012. p-Chloro-diphenyl diselenide, an organoselenium compound, with antidepressant-like and memory enhancer actions in aging male rats. Biogerontology 13 (3), 237–249. https://doi.org/10.1007/s10522-011-9369-9. http://www.ncbi.nlm.nih.gov/pubmed/22143824.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. http://www.ncbi.nlm.nih.gov/pubmed/942051.

Carle, G., Abgrall-Barbry, G., 2016. [Corticotherapy and suicidal behavior: a case report]. Encephale 42 (3), 272–276. https://doi.org/10.1016/j.encep.2016.01.005. http://www.ncbi.nlm.nih.gov/pubmed/26923998.

Chang, Y.P., 2014. Evidence for adverse effect of perinatal glucocorticoid use on the developing brain. Korean J Pediatr 57 (3), 101–109. https://doi.org/10.3345/kjp.2014.57.3.101. http://www.ncbi.nlm.nih.gov/pubmed/24778691.

Dean, J., Keshavan, M., 2017. The neurobiology of depression: an integrated view. Asian J Psychiatr 27, 101–111. https://doi.org/10.1016/j.ajp.2017.01.025. http://www.ncbi.nlm.nih.gov/pubmed/28558878.

Djordjevic, A., Djordjevic, J., Elakovic, I., Adzic, M., Matic, G., Radojcic, M.B., 2012. Fluoxetine affects hippocampal plasticity, apoptosis and depressive-like behavior of chronically isolated rats. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 36 (1), 92–100. https://doi.org/10.1016/j.pnpbp.2011.10.006. http://www.ncbi.nlm.nih.gov/pubmed/22019604.

Donia, M., Mangano, K., Quattrocchi, C., Fagone, P., Signorelli, S., Magro, G., ... Nicoletti, F., 2010. Specific and strain-independent effects of dexamethasone in the prevention and treatment of experimental autoimmune encephalomyelitis in rodents. Scand. J. Immunol. 72 (5), 396–407. https://doi.org/10.1111/j.1365-3083.2010.02451.x. http://www.ncbi.nlm.nih.gov/pubmed/21039734.

Duric, V., Banasr, M., Stockmeier, C.A., Simen, A.A., Newton, S.S., Overholser, J.C., ... Duman, R.S., 2013. Altered expression of synapse and glutamate related genes in post-mortem hippocampus of depressed subjects. Int. J. Neuropsychopharmacol. 16 (1), 69–82. https://doi.org/10.1017/S1461145712000016. http://www.ncbi.nlm.nih.gov/pubmed/22339950.

Fietta, P., Fietta, P., Delsante, G., 2009. Central nervous system effects of natural and synthetic glucocorticoids. Psychiatr. Clin. Neurosci. 63 (5), 613–622. https://doi.org/10.1111/j.1440-1819.2009.02005.x. http://www.ncbi.nlm.nih.gov/pubmed/19788629.

Fiske, C.H., Subbarow, Y., 1925. The calorimetric determination of phosphorus. J. Biol. Chem. 66, 375–381.

Gegelashvili, M., Rodriguez-Kern, A., Sung, L., Shimamoto, K., Gegelashvili, G., 2007. Glutamate transporter GLAST/EAAT1 directs cell surface expression of FXYD2/gamma subunit of Na, K-ATPase in human fetal astrocytes. Neurochem. Int. 50 (7–8), 916–920. https://doi.org/10.1016/j.neuint.2006.12.015. http://www.ncbi.nlm.nih.

gov/pubmed/17316900.
Gray, E.G., Whittaker, V.P., 1962. The isolation of nerve endings from brain: an electron-microscopic study of cell fragments derived by homogenization and centrifugation. J. Anat. 96, 79–88. http://www.ncbi.nlm.nih.gov/pubmed/13901297.
Grodzinsky, A.J., Wang, Y., Kakar, S., Vrahas, M.S., Evans, C.H., 2017. Intra-articular dexamethasone to inhibit the development of post-traumatic osteoarthritis. J. Orthop. Res. 35 (3), 406–411. https://doi.org/10.1002/jor.23295. http://www.ncbi.nlm.nih.gov/pubmed/27176565.
Hashimoto, K., Malchow, B., Falkai, P., Schmitt, A., 2013. Glutamate modulators as potential therapeutic drugs in schizophrenia and affective disorders. Eur. Arch. Psychiatry Clin. Neurosci. 263 (5), 367–377. https://doi.org/10.1007/s00406-013-0399-y. http://www.ncbi.nlm.nih.gov/pubmed/23455590.
Huang, Y.J., Lane, H.Y., Lin, C.H., 2017. New treatment strategies of depression: based on mechanisms related to neuroplasticity. Neural Plast. 4605971. https://doi.org/10.1155/2017/4605971. http://www.ncbi.nlm.nih.gov/pubmed/28491480.
Kanagawa, T., Tomimatsu, T., Hayashi, S., Shioji, M., Fukuda, H., Shimoya, K., Murata, Y., 2006. The effects of repeated corticosteroid administration on the neurogenesis in the neonatal rat. Am. J. Obstet. Gynecol. 194 (1), 231–238. https://doi.org/10.1016/j.ajog.2005.06.015. http://www.ncbi.nlm.nih.gov/pubmed/16389037.
Kaufmann, F.N., Gazal, M., Bastos, C.R., Kaster, M.P., Ghisleni, G., 2016. Curcumin in depressive disorders: an overview of potential mechanisms, preclinical and clinical findings. Eur. J. Pharmacol. 784, 192–198. https://doi.org/10.1016/j.ejphar.2016.05.026. http://www.ncbi.nlm.nih.gov/pubmed/27235294.
Lau, A., Tymianski, M., 2010. Glutamate receptors, neurotoxicity and neurodegeneration. Pflügers Archiv 460 (2), 525–542. https://doi.org/10.1007/s00424-010-0809-1. http://www.ncbi.nlm.nih.gov/pubmed/20229265.
Li, S.X., Fujita, Y., Zhang, J.C., Ren, Q., Ishima, T., Wu, J., Hashimoto, K., 2014. Role of the NMDA receptor in cognitive deficits, anxiety and depressive-like behavior in juvenile and adult mice after neonatal dexamethasone exposure. Neurobiol. Dis. 62, 124–134. https://doi.org/10.1016/j.nbd.2013.09.004. http://www.ncbi.nlm.nih.gov/pubmed/24051277.
Lipton, S.A., Rosenberg, P.A., 1994. Excitatory amino acids as a final common pathway for neurologic disorders. N. Engl. J. Med. 330 (9), 613–622. https://doi.org/10.1056/NEJM199403033300907. http://www.ncbi.nlm.nih.gov/pubmed/7905600.
Liu, W., Ge, T., Leng, Y., Pan, Z., Fan, J., Yang, W., Cui, R., 2017. The role of neural plasticity in depression: from Hippocampus to prefrontal cortex. Neural Plast. 2017, 6871089. https://doi.org/10.1155/2017/6871089. http://www.ncbi.nlm.nih.gov/pubmed/28246558.
Loetchutinat, C., Kothan, S., Dechsupa, S., Meesungnoen, J., Jay-Gerin, J., Mankhetkorn, S., 2005. Spectrofluorometric determination of intracellular levels of reactive oxygen speciesin drug-sensitive and drug-resistant cancer cells using the 2',7'-dichlorofluorescein diacetate assay. Radiat. Phys. Chem. 72, 323–331.
Mattson, M.P., 2008. Glutamate and neurotrophic factors in neuronal plasticity and disease. Ann. N. Y. Acad. Sci. 1144, 97–112. https://doi.org/10.1196/annals.1418.005. http://www.ncbi.nlm.nih.gov/pubmed/19076369.
Michaelis, E.K., 1998. Molecular biology of glutamate receptors in the central nervous system and their role in excitotoxicity, oxidative stress and aging. Prog. Neurobiol. 54 (4), 369–415. http://www.ncbi.nlm.nih.gov/pubmed/9522394.
Migues, P.V., Leal, R.B., Mantovani, M., Nicolau, M., Gabilan, N.H., 1999. Synaptosomal glutamate release induced by the fraction Bc2 from the venom of the sea anemone Bunodosoma caissarum. Neuroreport 10 (1), 67–70. http://www.ncbi.nlm.nih.gov/pubmed/10094135.
Misra, H.P., Fridovich, I., 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247 (10), 3170–3175. http://www.ncbi.nlm.nih.gov/pubmed/4623845.
Moretti, M., Colla, A., de Oliveira Balen, G., dos Santos, D.B., Budni, J., de Freitas, A.E., ... Severo Rodrigues, A.L., 2012. Ascorbic acid treatment, similarly to fluoxetine, reverses depressive-like behavior and brain oxidative damage induced by chronic unpredictable stress. J. Psychiatr. Res. 46 (3), 331–340. https://doi.org/10.1016/j.jpsychires.2011.11.009. http://www.ncbi.nlm.nih.gov/pubmed/22154133.
Nogueira, C.W., Rocha, J.B., 2011. Toxicology and pharmacology of selenium: emphasis on synthetic organoselenium compounds. Arch. Toxicol. 85 (11), 1313–1359. https://doi.org/10.1007/s00204-011-0720-3. http://www.ncbi.nlm.nih.gov/pubmed/21720966.
Oliveira, C.E., Sari, M.H., Zborowski, V.A., Araujo, P.C., Nogueira, C.W., Zeni, G., 2017. p,p'-Methoxyl-diphenyl diselenide elicits an antidepressant-like effect in mice without discontinuation anxiety phenotype. Pharmacol. Biochem. Behav. 154, 31–38. https://doi.org/10.1016/j.pbb.2017.02.002. http://www.ncbi.nlm.nih.gov/pubmed/28174136.
Patel, S.S., Udayabanu, M., 2014. Urtica dioica extract attenuates depressive like behavior and associative memory dysfunction in dexamethasone induced diabetic mice. Metab. Brain Dis. 29 (1), 121–130. https://doi.org/10.1007/s11011-014-9480-0. http://www.ncbi.nlm.nih.gov/pubmed/24435938.
Paulmier, C., 1986. Selenoorganic functional groups. In: Paulmier, C. (Ed.), Selenium Reagents and Intermediates in Organic Synthesis. Pergamon Press, Oxford, pp. 25–51.
Porsolt, R.D., Bertin, A., Blavet, N., Deniel, M., Jalfre, M., 1979. Immobility induced by forced swimming in rats: effects of agents which modify central catecholamine and serotonin activity. Eur. J. Pharmacol. 57 (2–3), 201–210. http://www.ncbi.nlm.nih.gov/pubmed/488159.
Quines, C.B., Rosa, S.G., Velasquez, D., Da Rocha, J.T., Neto, J.S., Nogueira, C.W., 2016. Diphenyl diselenide elicits antidepressant-like activity in rats exposed to monosodium glutamate: a contribution of serotonin uptake and Na(+), K(+)-ATPase activity. Behav. Brain Res. 301, 161–167. https://doi.org/10.1016/j.bbr.2015.12.038. http://www.ncbi.nlm.nih.gov/pubmed/26738966.
Raciti, M., Ong, J., Weis, L., Edoff, K., Battagli, C., Falk, A., Ceccatelli, S., 2016. Glucocorticoids alter neuronal differentiation of human neuroepithelial-like cells by inducing long-lasting changes in the reactive oxygen species balance. Neuropharmacology 107, 422–431. https://doi.org/10.1016/j.neuropharm.2016.03.022. http://www.ncbi.nlm.nih.gov/pubmed/26992751.
Reiner, A., Levitz, J., 2018. Glutamatergic signaling in the central nervous system: ionotropic and metabotropic receptors in concert. Neuron 98 (6), 1080–1098. https://doi.org/10.1016/j.neuron.2018.05.018. http://www.ncbi.nlm.nih.gov/pubmed/29953871.
Seibenhener, M.L., Wooten, M.C., 2015. Use of the Open Field Maze to measure locomotor and anxiety-like behavior in mice. J. Vis. Exp. 96, e52434. https://doi.org/10.3791/52434. http://www.ncbi.nlm.nih.gov/pubmed/25742564.
Sigwalt, A.R., Budde, H., Helmich, I., Glaser, V., Ghisoni, K., Lanza, S., . . Latini, A., 2011. Molecular aspects involved in swimming exercise training reducing anhedonia in a rat model of depression. Neuroscience 192, 661–674. https://doi.org/10.1016/j.neuroscience.2011.05.075. http://www.ncbi.nlm.nih.gov/pubmed/21712072.
Siwek, M., Sowa-Kucma, M., Dudek, D., Styczen, K., Szewczyk, B., Kotarska, K., ... Nowak, G., 2013. Oxidative stress markers in affective disorders. Pharmacol. Rep. 65 (6), 1558–1571. http://www.ncbi.nlm.nih.gov/pubmed/24553004.
Skupio, U., Tertil, M., Sikora, M., Golda, S., Wawrzczak-Bargiela, A., Przewlocki, R., 2015. Behavioral and molecular alterations in mice resulting from chronic treatment with dexamethasone: relevance to depression. Neuroscience 286, 141–150. https://doi.org/10.1016/j.neuroscience.2014.11.035. http://www.ncbi.nlm.nih.gov/pubmed/25433240.
Steru, L., Chermat, R., Thierry, B., Simon, P., 1985. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology 85 (3), 367–370. http://www.ncbi.nlm.nih.gov/pubmed/3923523.
Thomazi, A.P., Godinho, G.F., Rodrigues, J.M., Schwalm, F.D., Frizzo, M.E., Moriguchi, E., ... Wofchuk, S.T., 2004. Ontogenetic profile of glutamate uptake in brain structures slices from rats: sensitivity to guanosine. Mech. Ageing Dev. 125 (7), 475–481. https://doi.org/10.1016/j.mad.2004.04.005. http://www.ncbi.nlm.nih.gov/pubmed/15246742.
Treadway, M.T., Waskom, M.L., Dillon, D.G., Holmes, A.J., Park, M.T.M., Chakravarty, M.M., ... Pizzagalli, D.A., 2015. Illness progression, recent stress, and morphometry of hippocampal subfields and medial prefrontal cortex in major depression. Biol. Psychiatry 77 (3), 285–294. https://doi.org/10.1016/j.biopsych.2014.06.018. http://www.ncbi.nlm.nih.gov/pubmed/25109665.
Vizi, E.S., Kisfali, M., Lorincz, T., 2013. Role of nonsynaptic GluN2B-containing NMDA receptors in excitotoxicity: evidence that fluoxetine selectively inhibits these receptors and may have neuroprotective effects. Brain Res. Bull. 93, 32–38. https://doi.org/10.1016/j.brainresbull.2012.10.005. http://www.ncbi.nlm.nih.gov/pubmed/23089362.
Wagner, G., Schultes, M.T., Titscher, V., Teufer, B., Klerings, I., Gartlehner, G., 2018. Efficacy and safety of levomilnacipran, vilazodone and vortioxetine compared with other second-generation antidepressants for major depressive disorder in adults: a systematic review and network meta-analysis. J. Affect. Disord. 228, 1–12. https://doi.org/10.1016/j.jad.2017.11.056. http://www.ncbi.nlm.nih.gov/pubmed/29197738.
Wang, J., Shen, R.Y., Haj-Dahmane, S., 2012. Endocannabinoids mediate the glucocorticoid-induced inhibition of excitatory synaptic transmission to dorsal raphe serotonin neurons. J. Physiol. 590 (22), 5795–5808. https://doi.org/10.1113/jphysiol.2012.238659. http://www.ncbi.nlm.nih.gov/pubmed/22946098.
Warris, L.T., van den Heuvel-Eibrink, M.M., Aarsen, F.K., Pluijm, S.M., Bierings, M.B., van den Bos, C., ... van den Akker, E.L., 2016. Hydrocortisone as an intervention for dexamethasone-induced adverse effects in pediatric patients with acute lymphoblastic leukemia: results of a double-blind, randomized controlled trial. J. Clin. Oncol. 34 (19), 2287–2293. https://doi.org/10.1200/JCO.2015.66.0761. http://www.ncbi.nlm.nih.gov/pubmed/27161966.
Warris, L.T., van den Heuvel-Eibrink, M.M., den Hoed, M.A., Aarsen, F.K., Pieters, R., van den Akker, E.L., 2014. Does dexamethasone induce more neuropsychological side effects than prednisone in pediatric acute lymphoblastic leukemia? A systematic review. Pediatr. Blood Canc. 61 (7), 1313–1318. https://doi.org/10.1002/pbc.24988. http://www.ncbi.nlm.nih.gov/pubmed/24532490.
WHO, 2017. Depression and Other Common Mental Disorders: Global Health Estimates. http://apps.who.int/iris/bitstream/handle/10665/254610/WHO-MSD-MER-2017.
Wyse, A.T., Streck, E.L., Barros, S.V., Brusque, A.M., Zugno, A.I., Wajner, M., 2000. Methylmalonate administration decreases Na+,K+-ATPase activity in cerebral cortex of rats. Neuroreport 11 (10), 2331–2334. http://www.ncbi.nlm.nih.gov/pubmed/10923695.
Yusuf, M., Khan, M., Robaian, M.A., Khan, R.A., 2018. Biomechanistic insights into the roles of oxidative stress in generating complex neurological disorders. Biol. Chem. 399 (4), 305–319. https://doi.org/10.1515/hsz-2017-0250. http://www.ncbi.nlm.nih.gov/pubmed/29261511.
Zborowski, V.A., Sari, M.H., Heck, S.O., Stangherlin, E.C., Neto, J.S., Nogueira, C.W., Zeni, G., 2016. p-Chloro-diphenyl diselenide reverses memory impairment-related to stress caused by corticosterone and modulates hippocampal [(3)H]glutamate uptake in mice. Physiol. Behav. 164 (Pt A), 25–33. https://doi.org/10.1016/j.physbeh.2016.05.029. http://www.ncbi.nlm.nih.gov/pubmed/27211333.
Zhu, L., Nang, C., Luo, F., Pan, H., Zhang, K., Liu, J., ... Yan, T., 2016. Esculetin attenuates lipopolysaccharide (LPS)-induced neuroinflammatory processes and depressive-like behavior in mice. Physiol. Behav. 163, 184–192. https://doi.org/10.1016/j.physbeh.2016.04.051. http://www.ncbi.nlm.nih.gov/pubmed/27133730.
Zidan, A., Hedya, S.E., Elfeky, D.M., Abdin, A.A., 2018. The possible anti-apoptotic and antioxidant effects of acetyl l-carnitine as an add-on therapy on a relapsing-remitting model of experimental autoimmune encephalomyelitis in rats. Biomed. Pharmacother. 103, 1302–1311. https://doi.org/10.1016/j.biopha.2018.04.173. http://www.ncbi.nlm.nih.gov/pubmed/29864912.