

Research paper

Chronic clomipramine treatment reverses depressogenic-like effects of a chronic treatment with dexamethasone in rats

Abderrahim Laaziz^{a,*}, Hicham El Mostafi^a, Aboubaker Elhessni^a, Tarik Touil^{a,b}, Hanane Doumar^a, Abdelhalem Mesfioui^a

^a *Biology and Health Laboratory, Faculty of Sciences, Ibn Tofail University, Kenitra, Morocco*

^b *Higher Institute of Nursing and Health Professions of Rabat, Morocco*



ARTICLE INFO

Keywords:

Dexamethasone
Clomipramine
Cognition impairment
Depression
Animal model
Oxidative stress

ABSTRACT

Corticosteroids are widely used in medicine, for their anti-inflammatory and immunosuppressive actions, but can lead to troubling psychiatric side-effects. In fact, corticosteroids can induce many symptoms and syndromes, for example, mood disorders, anxiety and panic disorder, suicidal thinking and behavior. Furthermore, chronic stress and the administration of exogenous glucocorticoids are reported to induce affective changes in humans and rodents that relate to depressive state. Animal models are highly useful tools for studying the depression etiology. Face validity, construct validity, and predictive validity are the main criteria to evaluate animal depression models. The present study aimed to investigate the behavioral, cognitive, and biochemical effects of a chronic administration of DEX on Wistar rats. Wistar rats were administered daily with DEX (1.5 mg/kg, i.p., 21 days) or saline, the clomipramine treatment (2 mg/kg, i.p.) was realized just after the DEX injections for 21 days. DEX induced changes were evaluated by: forced swimming, novelty suppressed feeding, saccharin preference, open field, Morris water maze, and oxidative stress state in the brain. Results showed that chronic DEX administration conduct to a range of depression-related behavioral traits, including anhedonia, despair, weight loss, anxiety-like behavior, and cognitive impairments, which fill the face validity criterion. The DEX induced behavioral changes may result from the massive production of oxidative stress agents. This sustains the etiological hypothesis claiming that hyper-circulating glucocorticoid resulting from HPA dysfunction induces damage in certain neural structures related to depressive disorder, essentially the hippocampus. The antidepressant treatment has restored the behavioral state of rats which fills the predictive validity criterion.

Introduction

Depression, for interested in human health concern, represents a critical mental psychopathology, determined in the DSM V by five from nine symptoms in at least 2 weeks: depressed mood, diminished interest or pleasure, clear change in weight or appetite, perturbation in sleep, change in activity, low energy, feelings of worthlessness or guilt, decreased concentration and suicide tendency (Margaroli et al., 2021). In major population, at least 18 % has experienced one major depressive episode in their lifetime (Ramaker, and Dulawa, 2017). In 2008, the WHO ranked major depressive disorder as the third leading cause of morbidity in the world. The projection by the year 2030, this disease will rank first. Many factors are supposed to explain the etiology of depression, comprising genetic, biological, psychosocial, and environmental factors (Bains, and Abdijadid, 2021). Studies suggest that the

environmental factors account for about 75 % of the variance (Henn et al., 2004). Stress is a strength environmental trigger for the development of clinical depression (Sterner and Kalynchuk, 2010). There is a close link between chronic stress, glucocorticoids and depression development; some reports underline the relationship between an elevated glucocorticoid level in the blood and a depressed state (Wróbel et al., 2014). Furthermore, Studies over the last years have shown that hyperactivity of the hypothalamic-pituitary-adrenal axis (HPA) is one of the most substantial findings in major depression psychiatry, the high activity of the HPA axis is considered to be related, at least in part, to disrupted feedback inhibition by endogenous glucocorticoids, which is mediated by glucocorticoid receptors. Data claiming the notion that glucocorticoid-mediated feedback inhibition is decreased in major depression derive from a variety of studies showing that the HPA axis is not suppressed by pharmacological stimulation of the glucocorticoid

* Correspondence to: Biology and Health Laboratory, Department of Biology, Faculty of Sciences, Ibn Tofail University, Kenitra, Morocco.
E-mail address: ab.laaziz1@gmail.com (A. Laaziz).

<https://doi.org/10.1016/j.ibneur.2022.07.007>

Received 13 June 2022; Received in revised form 27 July 2022; Accepted 30 July 2022

Available online 1 August 2022

2667-2421/© 2022 The Author(s). Published by Elsevier Ltd on behalf of International Brain Research Organization. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

receptors with an administration of the synthetic glucocorticoid dexamethasone (DEX) (Pariante et al., 2008).

Corticosteroids are widely used in medicine, for their anti-inflammatory and immunosuppressive actions (Sigwalt et al., 2011), but can lead to psychiatric side effects. In fact, psychiatric and cognitive symptoms evoking major depression have been shown in patients using prolonged glucocorticoid treatment as well as in patients suffering from Cushing's disease (Brown et al., 1998; Kelly et al., 1983). Chronic stress and the administration of exogenous glucocorticoids are reported to induce affective perturbations in humans and rodents that recapitulate depressive state (Skupio et al., 2015). Indeed, a large body of preclinical studies indicates that exogenous corticosterone administration produces robust changes in a variety of behaviors that could be considered symptomatic of depression (increased immobility in forced swimming test (FST), reduced grooming, reduced weight gain, reduced sucrose preference, increased behavioral defense, decreased time in open arm in elevated plus maze (EPMT), increased escape behavior, elevated latency in novelty suppressed feeding test (NSFT), diminished reversal learning and increased latency to find platform in Morris water maze (MWM)) (Stern and Kalynchuk, 2010).

Studies on the effects of chronic stress on animals have shown elevated oxidative stress status (López-López et al., 2016). Immobilization restraint stress can induce an increase in lipid peroxidation (LPO) markers and nitric oxide (NO) activity (Pérez-Nievas et al., 2007). In addition, a chronic stress paradigm for 21 days was able to elevate the levels of inducible nitric oxide synthase (iNOS), which can lead to a large production of NO (Harvey et al., 2004). Chronic stress in rats showed a positive correlation between corticosterone rate and LPO (Abidin et al., 2004). Some studies were interested specifically at the impacts of glucocorticoids excess. Glucocorticoids treatment, in hippocampal cell cultures, resulted in elevated levels of Reactive Oxygen Species (ROS) production (McIntosh and Sapolsky, 1996). Cortical neuronal cells treated with corticosterone for 1–3 days exhibited high oxidative damage (Lee et al., 2009). In vivo, corticosterone increases iNOS levels (Pinnock et al., 2007), and high doses corticosterone administered orally in rats provoke more oxidative damage (Zafir and Banu, 2009). Antioxidant agents are usually measured in the studies of the oxidative stress. In rats chronically stressed or administered with chronic corticosterone showed decreased levels of superoxide dismutase (SOD) in the hippocampus, cortex (MacIntosh et al., 1998), and showed also decreases in catalase (CAT) activity (Cvijić et al., 1995).

Depression treatment is based on the use of a variety of antidepressants, such as selective serotonin reuptake inhibitors, selective norepinephrine reuptake inhibitors, tricyclic antidepressants and monoamine oxidase inhibitors. These drugs main effect is to increase monoaminergic neurotransmission either by blocking the serotonergic and/or the noradrenergic transporter or by blocking their degradation. Classical antidepressants require a minimum of 2–4 weeks of continuous treatment to elicit therapeutic benefit in depressed patients (de Souza Balk et al., 2010).

Animal models are highly useful tools for studying the depression etiology, as well as advancements in the development of therapeutic targets. Albeit animal models enhance greatly our understanding about psychiatric disorders, they are boarded by limitations (Becker et al., 2021). Three main criteria are often needed to be fulfilled to evaluate depression models: (a) face validity (reasonable degree of symptomatic homology), (b) construct validity (similar causative factors), and (c) predictive validity (depressive symptoms should be reversed by available antidepressants) (Krishnan and Nestler, 2011). Corticosterone is known to induce rodent depression like behavior, therefore, and often used for preparing depression model for the drug screening. But cortisol or glucocorticoid analogs should theoretically also induce depressive like behavior manifestations, since they act on glucocorticoid receptor (GR). There are many reports about DEX and clomipramine, but few reports about the effect of clomipramine on DEX-induced depression-like behavioral models. This study aimed to investigate whether chronic

administration of DEX could induce the characteristic behavioral, cognitive, and biochemical changes of depression in Wistar rats and meet key validity criteria for a depression model. Biochemical changes have focused on hippocampus and prefrontal cortex, two key regions for major depression and stress-related disorders. DEX is a synthetic glucocorticoid with high glucocorticoid activity, and no mineralocorticoid activity (Kenna et al., 2011), providing an opportunity to study the direct impact of chronic glucocorticoid receptor activation on development of depression. Chronic administration of DEX in many studies induced behavioral changes that are characteristic of depressive-like disorders such as despair, anhedonia, and weight loss (Laaziz et al., 2022; Gaspar et al., 2021; Sigwalt et al., 2011); anxiety-like state (Laaziz et al., 2022; Gaspar et al., 2021; Sigwalt et al., 2011); structural changes such as remodeling of microglia and neuronal morphology (Gaspar et al., 2021). Skupio et al. (2015) have shown that 21 days of DEX treatment in mice induces depressive-like symptoms, including behavioral and biochemical changes. Also, the acute use of DEX at prenatal stage induced long-lasting behavioral changes (Ferreira et al., 2021).

Material and methods

Animals and drugs

The study was conducted on 70 male Wistar rats weighting initially 100 ± 4.48 g and aged 52 ± 3 postnatal days. A natural light/dark cycle and temperature of 23 ± 1 °C were maintained. The animals were housed 5 per cage. Food and water were provided ad libitum. Rats were randomly assigned to two experimental groups. One group was administered daily with DEX (1.5 mg/kg, i.p., 21 days), while the other group was administered only with vehicle. After the 21 days of injection two cohorts (1, 2) have received chronic treatment with an antidepressant drug Clomipramine (2 mg/kg/day, i.p.) or saline solution for 21 days before performing behavioral tests. The other cohorts (3, 4, 5) underwent, one day after the final injection of DEX, the behavioral and cognitive tests. After MWM the cohort (5) has been sacrificed for oxidative stress analysis in hippocampus and prefrontal cortex (PFC). We have used different cohorts to avoid possible disturbance that can result when the tests are carried out on the same animals. The OFT, MWM and oxidative stress was used in a separate cohort without clomipramine protocol. The experimental procedures are summarized in Fig. 1. The DEX concentration was based on the work of (Laaziz et al., 2022; Sigwalt et al., 2011). DEX injections were given daily between 9:00 am and 10 am. This work has been fully realized in Biology and Health Laboratory located at Ibn Tofail University (Kenitra, Morocco) and all experimental procedures were performed according to the NIH Guide for the Care and Use of Laboratory Animals.

Evaluation of despair and anhedonia

FST

The levels of despair-based depressive-like behaviors in rat were assessed in the forced swimming procedure. Testing was similar to that described by Porsolt (1977). Rats were individually placed in a glass cylinder (height, 50 cm; diameter, 30 cm) devoid of exits and containing 27 cm of water. The water was warmed to at 26 °C \pm 1 °C, and it was changed for each rat. Animals were gently placed into the water for 6 min and then removed from the cylinder and allowed to dry in a small container with lignin. Duration of immobility was recorded visually uniquely for the 4 last minutes of the test and it is defined as the absence of active, escape-oriented behaviors (only those movements necessary to keep its head above the water) such as swimming, jumping, rearing, sniffing or diving (Abelaira et al., 2013).

Sucrose preference test (SPT)

Additionally, the animals were evaluated in the SPT. During this test, rats were individually housed and given a free choice between two

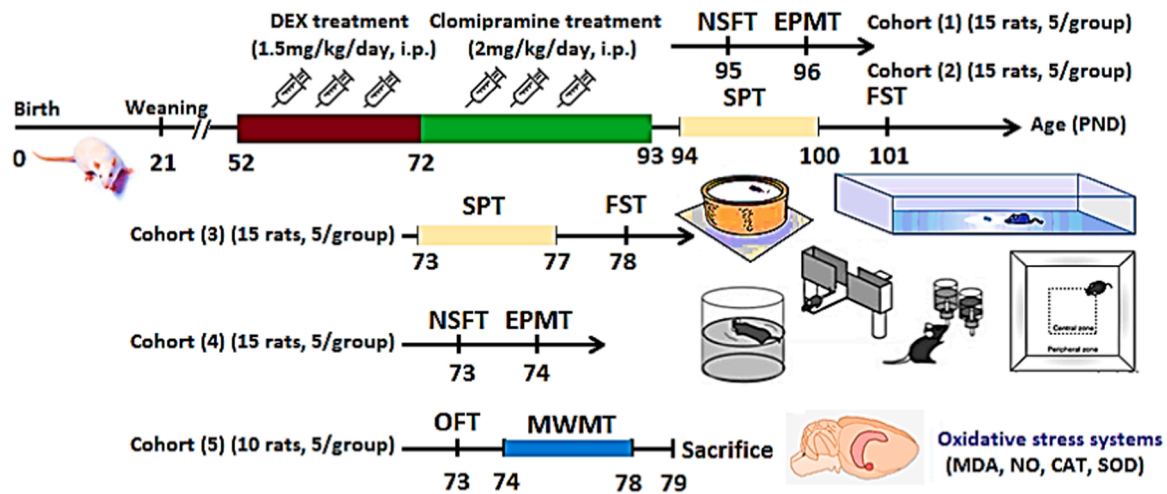


Fig. 1. Experimental design: In this study, we aimed to investigate the behavioral, cognitive effects, and oxidative stress state of prolonged activation of glucocorticoid receptors in rats aged 52 ± 3 postnatal days (PND) by the administration of the specific agonist Dexamethasone (DEX). Wistar rats were administered daily with DEX (1.5 mg/kg, i.p., 21 days) or vehicle. Two cohorts (1, 2) have received treatment with an antidepressant drug Clomipramine (2 mg/kg/day, i.p.) or saline solution before performing behavioral tests. The behavior and cognitive states of rats were evaluated in these paradigms: forced swimming test (FST), saccharin preference test (SPT), novelty suppressed feeding test (NSFT), elevated plus maze test (EPMT), open field test (OFT), spatial learning and memory in Morris water maze test (MWMT). The oxidative stress state in the brain was assessed by Malondialdehyde assay (MDA); nitrite assay (NO); catalase activity (CAT); superoxide dismutase activity (SOD).

bottles for one week: one with 2 % saccharin solution and the other with tap water. To avoid the possible effects of side preference in drinking behavior, the position of the bottles was changed after 12 h per day. No previous food or water deprivation was applied before the test. The consumption of water and saccharin solution was estimated simultaneously in the control and experimental groups by weighing the bottles. The preference for saccharin was calculated uniquely for the 4 last days of the test as a percentage of saccharin solution compared to the total amount of liquid consumed. For the first two days of the test the rats were left in cages (5 rats/group/cage) with the two bottles for the whole duration of the test, but from the third day the rats were separated. A single cage contains two rats separated by a metallic grid which still allows visual, olfactory and even tactile contact to minimize the effect of social isolation.

Anxiety-like behaviors

Open field test (OFT)

The procedure used in this study is similar to that previously described (Hazra et al., 2015). The device was a square wooden made (100 cm \times 100 cm) enclosed with 40 cm high walls. It was divided into 25 squares (16 peripherals, 9 centrals). During behavioral testing, the apparatus was placed under illumination (100 w, 2 m above). During the 10 min, each animal was gently placed in the center then video-recorded. The apparatus was cleaned with 70 % ethanol, after each trial. The OFT is primarily used to assess whether the DEX model affects the locomotor activity, an important parameter to exclude the possible confounding effects in locomotor performances that could alter results of other tests. The locomotor activity was estimated from the number of total squares visits during 10 min testing.

NSFT

Animals in The NSFT were pushed to face a conflict between the drive to eat and the fear of novel and open spaces. The latency to begin eating was measured. The ability to solve conflicts is inversely related to anxiety and depression (Francis-Oliveira et al., 2013). Animals were food-deprived for 24 h prior to the test. Then, each rat was placed in the corner of OFT arena containing an amount of rodent chow in the center on a circular white paper. An amount of sawdust covered the floor, and was mixed after each individual trial to eliminate olfactory stimuli. Rats

were allowed 10 min to stay tested if they failed to go eat. The amount of food that the rats usually ate was similar between the groups, and this was assessed throughout the whole study.

EPMT

This test is an ethological model of anxiety in rodents provoked by the novelty and repulsion as a result of elevation and illumination of the maze (Clénet et al., 2006). This test is based on the creation of a conflict between the exploratory drive of the rat and its innate fear of open and exposed areas; it has been validated for the detection of emotional responses to anxiogenic and anxiolytic substances (Pellow et al., 1985). The EPMT consists of a wooden plus-shaped platform elevated 70 cm above the floor. Two of the opposing arms (50 cm \times 10 cm) are closed by 40 cm high side and end walls, having an open roof. In order to avoid fall, the other two arms (open arms) were surrounded by 0.5 cm high edge, the four arms had at their intersection a central platform (10 cm \times 10 cm). A 100-W lamp was placed exactly over the central area of the maze facing an open arm. To eliminate any lingering olfactory cues, the apparatus was cleaned between each examination using 70 % ethyl alcohol.

Spatial learning and memory

The test was carried out as described by Wenk (2003) using a circular pool (160 cm diameter, 40 cm height) arbitrarily divided into four quadrants (Four points around the circumference of the pool were arbitrarily designated North, South, East, or West). Water opacity was obtained by adding milk powder. A transparent Plexiglas platform, 10 cm in diameter, was immersed 2 cm under the water surface at the center of one quadrant during training sessions. The rats do not like to be in the water and try to reach the platform to exit the water. In brief, the learning test was carried out as follows; the first day was the pretraining day, rats were put in the water three times for 20 s only to adapt to water. Then the rats were trained to learn the fixed location of the invisible platform during 5 days. Each training trial involved placing the rat into the pool facing the wall at one of the four positions. A different starting point was randomly used on each trial. Training consisted of four swims per day, with an intertrial interval of 10–15 min. Each animal was allowed a maximum of 120 s to find the platform and was left

for 10 s on the platform. The aim of this test is that the rats learn where the invisible platform is placed and find it in the shortest time possible. The time needed to find the hidden platform was recorded manually and used as a measure of learning of the task. If a rat failed to locate the platform within 120 s it was then manually guided to the platform by the experimenter. One hour after the last training trial, the platform was removed from the pool, the rats were allowed to swim for 120 s in the pool and the time spent in the quadrant where the platform was during training was recorded (Monfort et al., 2007).

Oxidative stress in the brain

Oxidative stress evaluation was realized after the behavioral tests. For this goal, rats were sacrificed by decapitation, and the brains were removed and cooled on dry ice. The hippocampus and PFC were dissected and isolated. The two structures has been homogenized in ice-cold 20-mM Trise-HCl buffer (pH 7.4) for measurement of LPO level, nitrite content, CAT, and SOD activities.

NO assay

To analyze nitrite levels, a supernatant was used from an aliquot of crude homogenate (10 %) for each structure centrifuged at 4 °C (800g for 10 min). protocol measurement was as described elsewhere (Green et al., 1981). In brief, each sample was incubated at room temperature for 20 min with Griess reagent (0.1%N-[1-naphtyl] ethylene diamine dihydrochloride; 1% sulfanilamide in 5% phosphoric acid; 1:1). Then, the measurement of absorbance was realized at 550 nm and compared to sodium nitrite standard solution. NO levels were expressed as $\mu\text{mol/g}$ of homogenate.

LPO assay

LPO evaluation was based on measuring thiobarbituric acid-reacting substances (TBARS) in homogenates. Protocol measurement was as previously described (Esterbauer, 1993). In brief, a supernatant was used from an aliquot of crude homogenate of each structure centrifuged at 4 °C (1000g for 10 min), and mixed with 1 ml 10 % trichloroacetic acid and 1 ml 0.67 % thiobarbituric acid. The mixture was then heated in boiling water bath for 15 min, before adding butanol (2:1, v/v) to this solution. The mixture was centrifuged (800g for 5 min), and TBARS were evaluated from absorbance at 535 nm. Results were expressed as nmol of malondialdehyde (MDA)/g wet tissue.

CAT activity

To evaluate CAT activity a method as previously described (Aebi, 1984) was used. Protocol was based on H_2O_2 transformation to H_2O . Briefly, in a 3 ml quartz tank, 1.95 ml of 50-mM phosphate buffer, 1 ml of H_2O_2 (0.019 M) and 0.05 ml of the sample were mixed. Then, at 240 nm the absorbance was measured at time 0 and after every 30 s for 2 min results were expressed as $\mu\text{mol/min/mg}$ of protein.

SOD activity

SOD activity evaluation was realized by the spectrophotometric methods by determining the inhibition of photoreduction of nitro blue tetrazolium (NBT). Protocol measurement was as previously described (Beauchamp and Fridovich, 1971). In brief, each system included a mixture of 2.4×10^{-6} M riboflavin, 0.01 M methionine, 1.67×10^{-4} M NBT and 0.05 potassium phosphate buffer at pH 7.4 and 25 °C. Mixture took blue coloration in an aerobic condition. Optical density was then measured at 560 nm. Results were expressed as $\mu\text{mol/min/mg}$ of protein.

Statistical methods

Behavioral tests were video-recorded and then visually analyzed. The study data were analyzed by Graph pad prism version 8, and they were expressed as mean \pm standard error of the mean (SEM). The

analysis of normal distribution by Shapiro-Wilk test and homogeneity of variance by an F-test or Brown-Forsythe test have been verified before performing parametric tests. Statistical comparison between groups was given by one-way ANOVA; two-way repeated measures ANOVA, and by unpaired Student's t-test. $P < 0.05$ was considered as statistically significant difference.

Results

Body weight evolution

Fig. 2H shows the evolution of mean body weights for the rats in each group throughout the DEX treatment followed by the antidepressant treatment. Body weight was significantly reduced in DEX-treated rats after the first week of treatment in the DEX injection period. A two-way ANOVA for repeated measures analysis, in the DEX treatment phase, indicated significant effects of treatment ($F(1, 26) = 9.045$; $p < 0.01$), time ($F(1.794, 46.63) = 38.19$; $p < 0.001$), and an interaction between treatment and time ($F(4, 104) = 18.30$; $p < 0.001$). In the Clomipramine treatment phase, the body weight of the three groups increased with time but with different rates. In Clm-treated group the weight gain is more pronounced compared to Dex+Sal group, from the first week of treatment by the antidepressant. A two-way ANOVA for repeated measures analysis, in the DEX treatment phase, indicated significant effects of treatment ($F(2, 9) = 52.24$; $p < 0.001$), time ($F(2.274, 20.46) = 269.4$; $p < 0.001$), and an interaction between treatment and time ($F(6, 27) = 28.75$; $p < 0.001$).

Depressive-like behavior

To assess if prolonged DEX administration (1.5 mg/kg, i.p.) can induce depressive-like behavior in rats, an FST was used. Fig. 2A revealed that one-way ANOVA exhibited significant effects of treatment ($F = 46.52$; $p < 0.001$). Post-hoc Tukey's multiple comparisons test revealed that DEX treatment conducted to more immobility time as compared with the non-treated group ($p < 0.001$), which indicates that DEX injections have induced depressive-like behavior; Treatment by antidepressant has diminished the immobility time compared to DEX+Sal group ($p < 0.001$); there is no difference in immobility state between the DEX treated group and the same group after saline treatment ($p > 0.05$), which indicates that DEX treatment induced a long-lasting changes in rats.

On other hand, anhedonia represents one of the main core symptoms related to depressive-like behavior. An SPT was used, as a way to evaluate anhedonia in rats. Fig. 2B revealed that one-way ANOVA exhibited significant effects of treatment ($F = 157.3$; $p < 0.001$). Post-hoc Tukey's multiple comparisons test revealed that DEX treatment conducted to reduced saccharine intake compared with the non-treated group ($p < 0.001$); Treatment by antidepressant has enhanced the hedonic state compared to DEX+Sal group ($p < 0.001$); there is no difference in anhedonia state between the DEX treated group and the same group after saline treatment ($p > 0.05$), which indicates that DEX treatment induced a chronic changes in rats.

Anxiety-like behavior

Evaluation of anxiety-like behaviors has been realized by two test indicators: latency time of NSFT and time passed in open arms of EPMT. In Fig. 2C, the one-way ANOVA exhibited significant effects of treatment ($F = 37.01$; $p < 0.001$). Post-hoc Tukey's multiple comparisons test revealed that DEX treatment conducted to increased latency time compared with the non-treated group ($p < 0.001$); Treatment by antidepressant has reduced the latency time compared to DEX+Sal group ($p < 0.001$); there is no difference in latency time state between the DEX treated group and the same group after saline treatment ($p > 0.05$), which indicates that DEX treatment induced a chronic changes in rats.

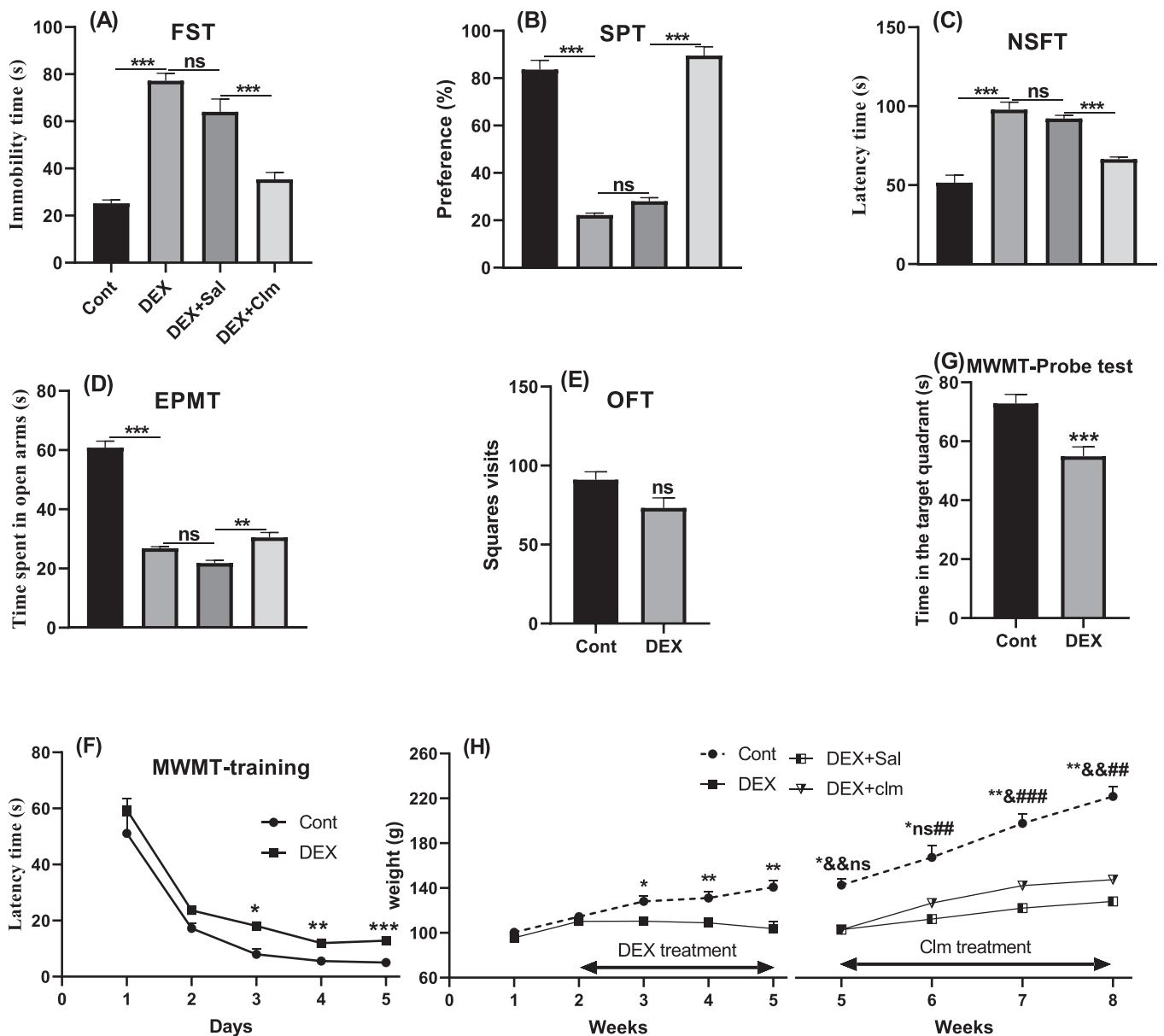


Fig. 2. Effect of chronic dexamethasone treatment on behavior and cognition. Rats after dexamethasone treatment have undergone behavioral testing to evaluate anxiety-like, depressive-like behaviors, and spatial memory. Data represent means \pm SEMs (FST; SPT; NSFT; EPMT; OFT; MWMT n = 5/group). CONT, control group; DEX, dexamethasone group; DEX+Sal, dexamethasone group treated by saline solution, DEX+Cim, dexamethasone group treated by clomipramine. ns non-significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. In body weight evolution* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ significant difference between Cont and DEX+Sal; & $P < 0.05$, && $P < 0.01$ significant difference between Cont and DEX+Cim; ### $P < 0.01$ ### $P < 0.001$ significant difference between DEX+Sal and DEX+Cim.

To exclude the possible confounding effects in appetite drive, results (not represented) showed that there was no difference in food intake between groups ($p > 0.05$). In Fig. 2D, the one-way ANOVA showed a significant effects of treatment ($F = 146.6$; $p < 0.001$). Post-hoc Tukey’s multiple comparisons test revealed that DEX treatment conducted to decreased time in open arms compared with the non-treated group ($p < 0.001$); Treatment by antidepressant has enhanced the open arms time compared to DEX+Sal group ($p < 0,01$); there is no difference in time spent in open arms between the DEX treated group and the same group after saline treatment ($p > 0.05$), which indicates that DEX treatment induced a chronic changes in rats. To investigate DEX treatment effect on horizontal (ambulatory distance) locomotor activity, in OF test the total squares visits was calculated. Fig. 2E shows that chronic DEX administration did not influence locomotor activity. A Student’s t-test showed no significant differences in total squares visits between

groups ($t = 2.199$; $p > 0.05$).

Spatial learning and memory

In MWMT, the animals training for 5 days to reach the hidden platform using extra-labyrinth wall signs, the latency (Fig. 2F) to find the platform decreases significantly in both treated and control groups. Yet, this parameter tended to be higher in treated group, as compared to control counterparts from the 3th day of training, which suggests a learning impairment induced by DEX administration. A two-way ANOVA for repeated measures analysis indicated significant effects of treatment ($F(1, 8) = 30.85$; $p < 0.001$), time ($F(1.265, 10.12) = 109.9$; $p < 0.001$). When the platform was removed to test the strength of the learning during the probe test (1 h after the 5th session) (Fig. 2G), non-treated rats showed a significant preference (expressed as time spent in

the target quadrant) for the target quadrant, with a highly significant difference between groups ($t = 4$; $p < 0.001$). This data clearly suggest that rats under DEX treatment showed altered learning and spatial memory performance.

Oxidative stress in the brain

MDA levels

The result of the Student's t-test of the MDA levels (Fig. 3A) demonstrated a significant increase in the PFC following DEX administration compared to the control group ($t = 10.07$; $p < 0.001$). In regards to the hippocampus region (Fig. 3a), the DEX administration led to an increase in the LPO in comparison to control rats ($t = 10.58$; $p < 0.001$).

NO levels

The result of the Student's t-test of the NO levels (Fig. 3B) demonstrated a significant increase in the PFC following DEX administration compared to the control group ($t = 14.31$; $p < 0.001$). In the hippocampus region (Fig. 3a), the DEX administration led to an increase in the NO in comparison to control rats ($t = 4.211$; $p < 0.01$).

SOD activity

The result of the Student's t-test of the SOD activity (Fig. 3C) demonstrated a significant decrease in the PFC following DEX administration compared to the control group ($t = 5.431$; $p < 0.001$). In the hippocampus region (Fig. 3c), the DEX administration led to a decrease in the SOD activity in comparison to control rats ($t = 10.98$; $p < 0.001$).

CAT activity

The result of the Student's t-test of the SOD activity (Fig. 3D)

demonstrated a significant decrease in the PFC following DEX administration compared to the control group ($t = 3.418$; $p < 0.01$). In the hippocampus region (Fig. 3d), the DEX administration led to a decrease in the SOD activity in comparison to control rats ($t = 10.79$; $p < 0.001$).

Discussion

Corticosteroids are widely used in medicine but can lead to troubling psychiatric side effects. The present study aimed to investigate whether a chronic administration of DEX can induce, the characteristic behavioral, cognitive, and biochemical effects of depression on Wistar rats, and fulfill the main validity criteria of a depression model.

Many finding in rats and mice (laaziz et al., 2022; Skupio et al., 2015; Ruksee et al., 2014; Sigwalt et al., 2011) sustained the hypothesis that prolonged systemic glucocorticoids administration increases despair-based depressive-like behaviors. In regards to saccharin preference, works (laaziz et al., 2022; Skupio et al., 2015) concluded that chronic administration of DEX causes anhedonia in rats. In this study, two typical tests were performed for measuring despair and anhedonia in rats: FST and SPT. The FST results showed that chronic DEX administration induced a depressive-like state in animals. DEX-treated rats exhibited significantly increased time of immobility. For saccharine preference our results showed a significant difference, treated rats exhibited decreased amount of saccharine consumption compared to vehicle-treated group. Additionally, related to body weight evolution, rats treated chronically with DEX showed a clear decrease in body weight gain, which is much observed in depression in both humans and animals (Skupio et al., 2015; Sigwalt et al., 2011; Feng et al., 2009; Johnson et al., 2006).

Anxiety-like effects often co-occur with depressive-like symptoms. In

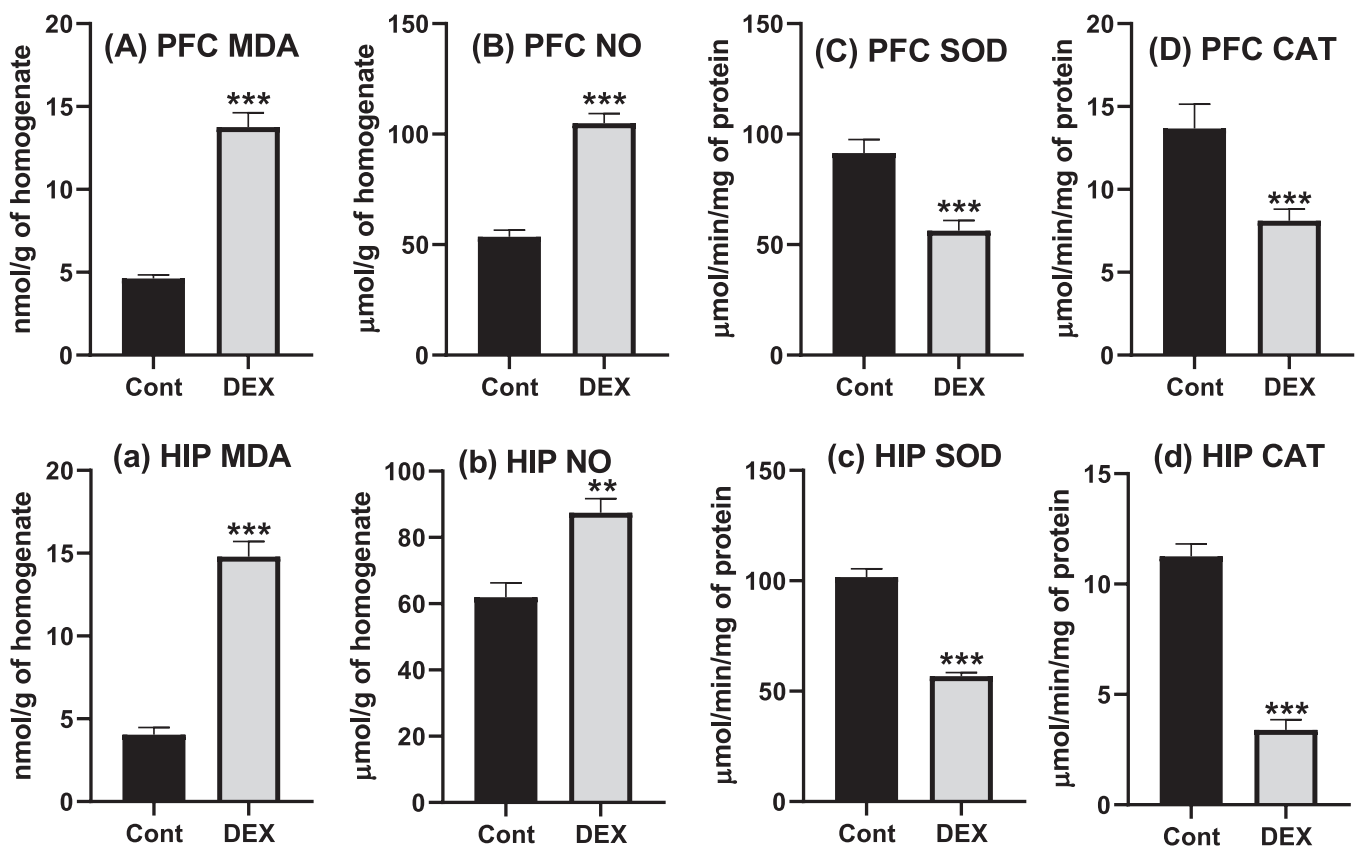


Fig. 3. : Oxidative stress status. Oxidative stress has been realized in two different regions: prefrontal cortex (PFC) and hippocampus (HIP). Data represent means \pm SEMs. CONT, control group; DEX, dexamethasone group. MDA: Malondialdehyde (nmol/g of homogenate) NO: nitrite content ($\mu\text{mol/g}$ of homogenate); CAT: catalase activity ($\mu\text{mol/min/mg}$ of protein); SOD: superoxide dismutase activity ($\mu\text{mol/min/mg}$ of protein). ns non-significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

fact, in many studies, exogenous glucocorticoids, chronically administered, have also been reported to produce anxiety-like behaviors (Laaziz et al., 2022; Delanogare et al., 2020; Skupio et al., 2015). These effects were reported in a variety of tests such as the elevated plus-maze (Pégo et al., 2008), the light/dark box (Murray et al., 2008), the novelty suppressed feeding, and the open field tests (David et al., 2009). In the present study, we used two anxiety-like test indicators. Treated rats with DEX exhibited in the NSFT greater latency to begin eating and in the EPMT reduced time in open arms.

Locomotor activity measurement is an essential parameter to evaluate, because it can influence the performance in the other tests. So, we decided to explore whether chronic DEX administration impaired locomotor performance using the OF arena. In this study, there was no significant difference between the DEX-treated group and the vehicle group in locomotor activity expressed as the number of squares visits. Our results are in line with others findings, (Laaziz et al., 2022; Skupio et al., 2015) showed that DEX injection, in 21 days, didn't impair locomotor activity.

Cumulative data indicate that exposure to stress or stress hormones decrease hippocampal-dependent forms of memory in humans (Lupien and McEwen, 1997; Li et al., 2010). In normal subjects, elevated doses of cortisol decreases verbal declarative memory (Newcomer et al., 1999). Moreover, patients with persistent hypercortisolemia or with Cushing's disease exhibit alterations in certain forms of memory (Sapolsky, 2000). Rodents exposed to stress or chronically administered with CORT show, like human studies, impairments in different tasks thought involving spatial abilities (Sapolsky, 2000; Lupien and McEwen, 1997). Rats chronically administered with CORT show defected acquisition in radial arm maze task (Dachir et al., 1993), in the Barnes maze (Coburn-Litvak et al., 2003), and in the MWMT (Sousa et al., 2000). Studies using DEX for twenty one days of exposure induced impairment of memory and learning, in senescent mice (Yao et al., 2007) and in adolescent Wistar rats (Laaziz et al., 2022). In our study we used the MWMT to investigate some memory related behaviors. Our results are in line with the abovementioned data and suggest that DEX treatment induced cognitive impairments in rats.

Treatment of depression has been based for long time on Tricyclic antidepressants (TCAs). The TCAs primary mode of action remains in blocking the reuptake of centrally active neurotransmitters (Potter et al., 1998). Clomipramine is one of TCAs and is widely used for depression and obsessive compulsive disorder treatment, essentially by blocking the serotonin and norepinephrine reuptake (Kelly and Myers, 1990). The chronic administration of clomipramine, at doses relatively low, promotes benefits on depression (Bhagya et al., 2008; Srikumar et al., 2006) and reduces the behavioral deficits induced by stress in rats (D'Aquila et al., 2000). In this study chronic clomipramine treatment has restored the behavioral state of rats in FST, SPT, NSFT, and EPMT which fills the predictive validity criterion. In both, rodent models and depressed patients, Anhedonia implies a defective reward system (Willner et al., 1992) that can be effectively treated by the TCAs (Yu et al., 2007; Monleon et al., 1995). Liu et al. (2009) showed that a chronic antidepressant treatment with clomipramine improve the physical states of depression model rats by decreasing the immobility time in FST. Antidepressant drug can also have anxiolytic effects, Poltronieri et al. (2003) showed that clomipramine chronically administered impaired the one-way escape, an antipanic-like effect, in elevated T maze. Recently some clarifications of mechanism underlying the neuroprotective effects of antidepressants comprising tricyclic ones have been proposed. The main actor is The FK506-binding protein 51 (FKBP51), which is an important inhibitor of the glucocorticoid receptor (GR) signaling. High FKBP51 levels are associated to stress-related disorders, which are linked to GR resistance. SUMO conjugation to FKBP51 is necessary for FKBP51's inhibitory action on GR. The GR/FKBP51 pathway is target of antidepressant action. Tricyclic antidepressants- particularly clomipramine- inhibited FKBP51 SUMOylation. The inhibition of FKBP51 SUMOylation decreased its binding to Hsp90 and GR facilitating FKBP52

recruitment, and enhancing GR activity. These results describe the action of antidepressants as repressors of FKBP51 SUMOylation as a molecular switch for restoring GR sensitivity, thereby providing new potential routes of antidepressant intervention (Budziński et al., 2022).

The brain has been reported to use 20 % of metabolized oxygen but has a very limited antioxidant capacity, which makes it very vulnerable to ROS production (Wang et al., 2012). It has been shown that chronic stress can lead to an elevation of metabolic rate and ROS production (Lv et al., 2019). ROS production can be the cause of several brain tissues damage, resulting from oxidative stress (Zhong et al., 2019). In normal conditions, there is a balance between the oxidant and antioxidative systems. However, damage reduces the antioxidant defense capacity and alters this balance (Biala et al., 2018). Psychiatric disorders like anxiety and depression observed with the chronic stress model could be considered as result of oxidative damage (Duda et al., 2016). Chronic stress has been reported to facilitate oxidative stress leading to free radicals' production in the brain, so the antioxidant systems and glutathione rates are reduced, LPO increased, and SOD/CAT activity altered (Cameron et al., 2018). Patients with depressive disorder showed high levels of oxidative stress with decreased antioxidant enzyme defenses (Xu et al., 2014). Corticosterone administration can lead to an increase in the iNOS levels (Pinnock et al., 2007), and rats orally supplemented with high doses of corticosterone showed more oxidative damage (Zafir and Banu, 2009). Rats chronically stressed or received chronic corticosterone injections showed decreased levels of SOD in the hippocampus and cortex (MacIntosh et al., 1998), and showed also decreases in CAT activity (Cvijić et al., 1995). Athira et al. (2018) showed that chronic corticosterone administration can significantly increase hippocampal MDA and iNOS levels while lowering glutathione, as compared to control. In our study, chronic DEX treatment was able to alter the oxidative stress balance by inducing a decrease in SOD and CAT activity and by elevations of MDA and NO levels in the hippocampus and PFC.

Based in These results and in other findings (Budziński et al., 2022; Guidotti et al., 2013) we can suggest that DEX treatment induced anxiety and depression-like behaviors in rats, via the massive production of oxidative stress agents in the brain. Oxidative stress can induce cell death in hippocampus, since the hippocampus plays a central role in inhibiting the activity of the HPA axis, hippocampal damage could produce a repetitive cycle of increasing HPA axis dysregulation and ongoing hippocampal injury. Add to that, as primary cause of the above cited variations, the altered GR function in the hypothalamus and in the hippocampus, which may result in an inability of GCs themselves to exert their effects on these targets leads to the so-called 'glucocorticoid resistance', characterized by increased CRH production and over-activity of the HPA axis, supporting the hypothesis that reduced expression and function of GRs may be relevant for the pathogenesis of stress-related psychiatric disorders. The resulted HPA hyperactivity is a main feature of depressive disorder. TCAs like clomipramine can modulate some GR related proteins (e.g. FKBP51) to enhance their sensibility to glucocorticoid, and regain their ability to exercise the negative feedback via hippocampus and prefrontal cortex and consequently diminish their secretion from HPA axis. In the other hand, it is possible that augmentation of antioxidant defenses and/or reduction of pro-oxidant systems could serve as a convergence point for multiple classes of antidepressants (e.g. clomipramine) as an important mechanism underlying the neuroprotective pharmacological effects of these drugs observed clinically in the treatment of various stress disorders (Zafir et al., 2009).

Conclusion

In conclusion, chronic DEX administration conducted to a range of depression-related behavioral traits, including anhedonia, despair, weight loss, anxiety-like behavior, and cognitive impairments, which fill the face validity criterion.

The anxiety and depression-like behaviors noticed following the

chronic dexamethasone stress simulation may result from the massive production of oxidative stress agents in the brain. This sustains the etiological hypothesis claiming that hyper-circulating glucocorticoid resulting from HPA dysfunction induces damage in certain neural structures related to depressive disorder, essentially the hippocampus.

The antidepressant treatment has restored the behavioral state of rats which fills the predictive validity criterion.

CRedit authorship contribution statement

Laaziz Abderrahim: Conceptualization, Methodology, Investigation and Writing – review & editing, **Elmostafi Hicham:** Methodology and Writing – review & editing, **Elhessni Aboubaker:** Supervision, **Touil tarik:** Writing – review & editing, **Doumar Hanane:** Writing – review & editing, **Mesfioui Abdelhalim:** Supervision.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declarations of Competing Interest

None.

References

- Abelaira, H.M., Reus, G.Z., Quevedo, J., 2013. Animal models as tools to study the pathophysiology of depression. *Rev. Bras. De. Psiquiatri.* 35, S112–S120. <https://doi.org/10.1590/1516-4446-2013-1098>.
- Abidin, I., Yargicoglu, P., Agar, A., Gümüslü, S., Aydin, S., Aydin, S., Sahin, E., 2004. The effect of chronic restraint stress on spatial learning and memory: relation to oxidant stress. *Int. J. Neurosci.* 114 (5), 683–699. <https://doi.org/10.1080/00207450490430543>.
- Aebi, H., 1984. Catalase in vitro. *Methods Enzymol.* 105, 121–126. [https://doi.org/10.1016/S0076-6879\(84\)05016-3](https://doi.org/10.1016/S0076-6879(84)05016-3).
- Athira, K.V., Madhana, R.M., Js, I.C., Lahkar, M., Sinha, S., Naidu, V.G.M., 2018. Antidepressant activity of vorinostat is associated with amelioration of oxidative stress and inflammation in a corticosterone-induced chronic stress model in mice. *Behav. Brain Res.* 344, 73–84. <https://doi.org/10.1016/j.bbr.2018.02.009>.
- Bains, N., Abdijadid, S., 2021. Major Depressive Disorder. *StatPearls Publishing (StatPearls [Internet])*.
- Beauchamp, C., Fridovich, I., 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal. Biochem.* 44 (1), 276–287. [https://doi.org/10.1016/0003-2697\(71\)90370-8](https://doi.org/10.1016/0003-2697(71)90370-8).
- Becker, M., Pinhasov, A., Ornoy, A., 2021. Animal models of depression: what can they teach us about the human disease. *Diagnostics* 11 (1), 123. <https://doi.org/10.3390/diagnostics11010123>.
- Bhagya, V., Srikumar, B.N., Raju, T.R., Rao, B.S., 2008. Neonatal clomipramine induced endogenous depression in rats is associated with learning impairment in adulthood. *Behav. Brain Res.* 187 (1), 190–194. <https://doi.org/10.1016/j.bbr.2007.08.020>.
- Biala, G., Pekala, K., Boguszewska-Czubarba, A., Michalak, A., Kruk-Slomka, M., Grot, K., Budzynska, B., 2018. Behavioral and biochemical impact of chronic unpredictable mild stress on the acquisition of nicotine conditioned place preference in rats. *Mol. Neurobiol.* 55 (4), 3270–3289. <https://doi.org/10.1007/s12035-017-0585-4>.
- Brown, E.S., Suppes, T., 1998. Mood symptoms during corticosteroid therapy: a review. *Harv. Rev. Psychiatry* 5 (5), 239–246. <https://doi.org/10.3109/10673229809000307>.
- Budziński, M.L., Sokn, C., Gobbini, R., Ugo, B., Antunica-Noguerol, M., Senin, S., Liberman, A.C., 2022. Tricyclic antidepressants target FKBP51 SUMOylation to restore glucocorticoid receptor activity. *Mol. Psychiatry* 27 (5), 2533–2545. <https://doi.org/10.1038/s41380-022-01491-0>.
- Cameron, L.P., Benson, C.J., Dunlap, L.E., Olson, D.E., 2018. Effects of N, N-dimethyltryptamine on rat behaviors relevant to anxiety and depression. *ACS Chem. Neurosci.* 9 (7), 1582–1590. <https://doi.org/10.1021/acschemneuro.8b00134>.
- Clénet, F., Bouyon, E., Hascoët, M., Bourin, M., 2006. Light/dark cycle manipulation influences mice behaviour in the elevated plus maze. *Behav. Brain Res.* 166 (1), 140–149. <https://doi.org/10.1016/j.bbr.2005.07.018>.
- Coburn-Litvak, P.S., Pothakos, K., Tata, D.A., McCloskey, D.P., Anderson, B.J., 2003. Chronic administration of CORT impairs spatial reference memory before spatial working memory in rats. *Neurobiol. Learn. Mem.* 80 (1), 11–23. <https://doi.org/10.1016/S1074->
- Cvijić, G., Radojčić, R., Djordjević, J., Davidović, V., 1995. The effect of glucocorticoids on the activity of monoamine oxidase, copper-zinc superoxide dismutase and catalase in the rat hypothalamus. *Funct. Neurol.* 10 (4–5), 175–181.
- Dachir, S., Kadar, T., Robinson, B., Levy, A., 1993. Cognitive deficits induced in young rats by long-term corticosterone administration. *Behav. Neural Biol.* 60 (2), 103–109. [https://doi.org/10.1016/0163-1047\(93\)90173-F](https://doi.org/10.1016/0163-1047(93)90173-F).
- David, D.J., Samuels, B.A., Rainer, Q., Wang, J.W., Marsteller, D., Mendez, I., Hen, R., 2009. Neurogenesis-dependent and-independent effects of fluoxetine in an animal model of anxiety/depression. *Neuron* 62 (4), 479–493. <https://doi.org/10.1016/j.neuron.2009.04.017>.
- Delanogare, E., de Souza, R.M., Rosa, G.K., Guanabara, F.G., Rafacho, A., Moreira, E.L.G., 2020. Enriched environment ameliorates dexamethasone effects on emotional reactivity and metabolic parameters in mice. *Stress* 23 (4), 466–473. <https://doi.org/10.1080/10253890.2020.1735344>.
- Duda, W., Curzytek, K., Kubera, M., Iciek, M., Kowalczyk-Pachel, D., Bilska-Wilkosz, A., Antkiewicz-Michaluk, L., 2016. The effect of chronic mild stress and imipramine on the markers of oxidative stress and antioxidant system in rat liver. *Neurotoxicol. Res.* 30 (2), 173–184. <https://doi.org/10.1007/s12640-016-9614-8>.
- Esterbauer, H., 1993. Cytotoxicity and genotoxicity of lipid-oxidation products. *Am. J. Clin. Nutr.* 57 (5), 779S–786S. <https://doi.org/10.1093/ajcn/57.5.779S>.
- Feng, Y., Rhodes, P.G., Liu, H., Bhatt, A.J., 2009. Dexamethasone induces neurodegeneration but also up-regulates vascular endothelial growth factor A in neonatal rat brains. *Neuroscience* 158, 823–832. <https://doi.org/10.1016/j.neuroscience.2008.10.024>.
- Ferreira, A.S., Galvão, S., Gaspar, R., Rodrigues-Neves, A.C., Ambrósio, A.F., Matafome, P., Baptista, F.I., 2021. Sex-specific changes in peripheral metabolism in a model of chronic anxiety induced by prenatal stress. *Eur. J. Clin. Investig.* 51 (12), e13639. <https://doi.org/10.1111/eci.13639>.
- Francis-Oliveira, J., Ponte, B., Barbosa, A.P.M., Veríssimo, L.F., Gomes, M.V., Pelosi, G. G., Moreira, E.G., 2013. Fluoxetine exposure during pregnancy and lactation: effects on acute stress response and behavior in the novelty-suppressed feeding are age and gender-dependent in rats. *Behav. Brain Res.* 252, 195–203. <https://doi.org/10.1016/j.bbr.2013.05.064>.
- Gaspar, R., Soares-Cunha, C., Domingues, A.V., et al., 2021. Resilience to stress and sex-specific remodeling of microglia and neuronal morphology in a rat model of anxiety and anhedonia. *Neurobiol. Stress* 14, 100302. <https://doi.org/10.1016/j.ynstr.2021.100302>.
- Green, L.C., Tannenbaum, S.R., Goldman, P., 1981. Nitrate synthesis in the germfree and conventional rat. *Science* 212 (4490), 56–58. <https://doi.org/10.1126/science.6451927>.
- Guidotti, G., Calabrese, F., Anacker, C., Racagni, G., Pariante, C.M., Riva, M.A., 2013. Glucocorticoid receptor and FKBP5 expression is altered following exposure to chronic stress: modulation by antidepressant treatment. *Neuropsychopharmacology* 38 (4), 616–627. <https://doi.org/10.1038/npp.2012.225>.
- Harvey, B.H., Oosthuizen, F., Brand, L., Wegener, G., Stein, D.J., 2004. Stress–restress evokes sustained iNOS activity and altered GABA levels and NMDA receptors in rat hippocampus. *Psychopharmacology* 175 (4), 494–502. <https://doi.org/10.1007/s00213-004-1836-4>.
- Hazra, S., Kumar, S., Saha, G.K., Mondal, A.C., 2015. Chronic administration of bacopa monniera alleviates depressive like behavior and increases the expression of ERK1/2 in hippocampus and pre frontal cortex of chronic unpredictable stress induced rats. *Int. Neuropsychiat Dis. J.* 3 (3), 47–58. <https://doi.org/10.9734/IJNDJ/2015/14414>.
- Henn, F., Vollmayr, B., Sartorius, A., 2004. Mechanisms of depression: the role of neurogenesis. *Drug Discov. Today.* Dis. Mech. 1 (4), 407–411. <https://doi.org/10.1016/j.ddmcc.2004.10.007>.
- Johnson, S.A., Fournier, N.M., Kalynchuk, L.E., 2006. Effect of different doses of corticosterone depression-like behavior and HPA axis responses to a novel stressor. *Behav. Brain Res.* 168, 280–288. <https://doi.org/10.1016/j.bbr.2005.11.019>.
- Kelly, W.F., Checkley, S.A., Bender, D.A., Mashiter, Keith, 1983. Cushing's syndrome and depression – a prospective study of 26 patients. *Br. J. Psychiatry* 142 (1), 16–19. <https://doi.org/10.1192/bjp.142.1.16>.
- Kelly, M.W., Myers, C.W., 1990. Clomipramine: a tricyclic antidepressant effective in obsessive compulsive disorder. *Dicp* 24 (7–8), 739–744. <https://doi.org/10.1177/106002809002400718>.
- Kenna, H.A., Poon, A.W., de los Angeles, C.P., et al., 2011. Psychiatric complications of treatment with corticosteroids: review with case report. *Psychiatry Clin. Neurosci.* 65, 549–560. <https://doi.org/10.1111/j.1440-1819.2011.02260.x>.
- Krishnan, V., Nestler, E.J., 2011. Animal models of depression: molecular perspectives. *Mol. Funct. Models Neuropsychiatry* 121–147. https://doi.org/10.1007/7854_2010_108.
- Laaziz, A., El Mostafi, H., Elhessni, A., Azeroil, F., Touil, T., Boumlah, S., Mesfioui, A., 2022. Sex differences in behavioral, cognitive and voluntary ethanol-intake effects in Dexamethasone-induced depression-like state in Wistar rat. *AIMS Neurosci.* 9 (2), 228–249. <https://doi.org/10.3934/Neuroscience.2022012>.
- Lee, K.W., Kim, J.B., Seo, J.S., Kim, T.K., Im, J.Y., Baek, I.S., Han, P.L., 2009. Behavioral stress accelerates plaque pathogenesis in the brain of Tg2576 mice via generation of metabolic oxidative stress. *J. Neurochem.* 108 (1), 165–175. <https://doi.org/10.1111/j.1471-4159.2008.05769.x>.
- Li, W.Z., Li, W.P., Yao, Y.Y., Zhang, W., Yin, Y.Y., Wu, G.C., Gong, H.L., 2010. Glucocorticoids increase impairments in learning and memory due to elevated amyloid precursor protein expression and neuronal apoptosis in 12-month old mice. *Eur. J. Pharmacol.* 628 (1–3), 108–115. <https://doi.org/10.1016/j.ejphar.2009.11.045>.
- Liu, Q., Li, B., Zhu, H.Y., Wang, Y.Q., Yu, J., Wu, G.C., 2009. Clomipramine treatment reversed the glial pathology in a chronic unpredictable stress-induced rat model of depression. *Eur. Neuropsychopharmacol.* 19 (11), 796–805. <https://doi.org/10.1016/j.euroneuro.2009.06.010>.
- López-López, A.L., Jaime, H.B., Villanueva, M.D.C.E., Padilla, M.B., Palacios, G.V., Aguilar, F.J.A., 2016. Chronic unpredictable mild stress generates oxidative stress and systemic inflammation in rats. *Physiol. Behav.* 161, 15–23. <https://doi.org/10.1016/j.physbeh.2016.03.017>.

- Lupien, S.J., McEwen, B.S., 1997. The acute effects of corticosteroids on cognition: integration of animal and human model studies. *Brain Res. Rev.* 24 (1), 1–27. [https://doi.org/10.1016/S0165-0173\(97\)00004-0](https://doi.org/10.1016/S0165-0173(97)00004-0).
- Lv, H., Wu, R., Ni, H., Lian, J., Xu, Y., Xia, Y., Chen, Y., 2019. Chronic mild stress induced anxiety-like behaviors can be attenuated by inhibition of NOX2-derived oxidative stress. *J. Psychiatr. Res.* 114, 55–66. <https://doi.org/10.1016/j.jpsychires.2019.04.008>.
- Malgaroli, M., Calderon, A., Bonanno, G.A., 2021. Networks of major depressive disorder: a systematic review. *Clin. Psychol. Rev.* 85, 102000 <https://doi.org/10.1016/j.cpr.2021.102000>.
- McIntosh, L.J., Sapolsky, R.M., 1996. Glucocorticoids increase the accumulation of reactive oxygen species and enhance adriamycin-induced toxicity in neuronal culture. *Exp. Neurol.* 141 (2), 201–206. <https://doi.org/10.1006/exnr.1996.0154>.
- McIntosh, L.J., Hong, K.E., Sapolsky, R.M., 1998. Glucocorticoids may alter antioxidant enzyme capacity in the brain: baseline studies. *Brain Res.* 791 (1–2), 209–214. [https://doi.org/10.1016/S0006-8993\(98\)00115-2](https://doi.org/10.1016/S0006-8993(98)00115-2).
- Monfort, P., Erceg, S., Piedrafita, B., Llansola, M., Felipe, V., 2007. Chronic liver failure in rats impairs glutamatergic synaptic transmission and long-term potentiation in hippocampus and learning ability. *Eur. J. Neurosci.* 25 (7), 2103–2111. <https://doi.org/10.1111/j.1460-9568.2007.05444.x>.
- Monleon, S., Parra, A., Simon, V.M., Brain, P.F., D'Aquila, P., Willner, P., 1995. Attenuation of sucrose consumption in mice by chronic mild stress and its restoration by imipramine. *Psychopharmacology* 117 (4), 453–457. <https://doi.org/10.1007/BF02246218>.
- Murray, F., Smith, D.W., Hutson, P.H., 2008. Chronic low dose corticosterone exposure decreased hippocampal cell proliferation, volume and induced anxiety and depression like behaviours in mice. *Eur. J. Pharmacol.* 583 (1), 115–127. <https://doi.org/10.1016/j.ejphar.2008.01.014>.
- Newcomer, J.W., Selke, G., Melson, A.K., Hershey, T., Craft, S., Richards, K., Alderson, A. L., 1999. Decreased memory performance in healthy humans induced by stress-level cortisol treatment. *Arch. Gen. Psychiatry* 56 (6), 527–533 <https://doi.org/10.1001/archgenpsychiatry.56.6.yoa8291>.
- Pariante, C.M., Lightman, S.L., 2008. The HPA axis in major depression: classical theories and new developments. *Trends Neurosci.* 31 (9), 464–468. <https://doi.org/10.1016/j.tins.2008.06.006>.
- Pêgo, J.M., Morgado, P., Pinto, L.G., Cerqueira, J.J., Almeida, O.F.X., Sousa, N., 2008. Dissociation of the morphological correlates of stress-induced anxiety and fear. *Eur. J. Neurosci.* 27 (6), 1503–1516. <https://doi.org/10.1111/j.1460-9568.2008.06112.x>.
- Pellow, S., Chopin, P., File, S.E., Briley, M., 1985. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J. Neurosci. Methods* 14 (3), 149–167. [https://doi.org/10.1016/0165-0270\(85\)90031-7](https://doi.org/10.1016/0165-0270(85)90031-7).
- Pérez-Nievas, B.G., García-Bueno, B., Caso, J.R., Menchén, L., Leza, J.C., 2007. Corticosterone as a marker of susceptibility to oxidative/nitrosative cerebral damage after stress exposure in rats. *Psychoneuroendocrinology* 32 (6), 703–711. <https://doi.org/10.1016/j.psyneuen.2007.04.011>.
- Pinnock, S.B., Balendra, R., Chan, M., Hunt, L.T., Turner-Stokes, T., Herbert, J., 2007. Interactions between nitric oxide and corticosterone in the regulation of progenitor cell proliferation in the dentate gyrus of the adult rat. *Neuropsychopharmacology* 32 (2), 493–504. <https://doi.org/10.1038/sj.npp.1301245>.
- Poltronieri, S.C., Zangrossi Jr, H., de Barros Viana, M., 2003. Antipanic-like effect of serotonin reuptake inhibitors in the elevated T-maze. *Behav. Brain Res.* 147 (1–2), 185–192. [https://doi.org/10.1016/S0166-4328\(03\)00151-7](https://doi.org/10.1016/S0166-4328(03)00151-7).
- Porsolt, R.D., Le Pichon, M., Jalfre, M.L., 1977. Depression: a new animal model sensitive to antidepressant treatments. *Nature* 266 (5604), 730–732. <https://doi.org/10.1038/266730a0>.
- Potter, W.Z., 1998. Tricyclics and tetracyclics. *Textb. Psychopharmacol.* 199–218.
- Ramaker, M.J., Dulawa, S.C., 2017. Identifying fast-onset antidepressants using rodent models. *Mol. Psychiatry* 22 (5), 656–665. <https://doi.org/10.1038/mp.2017.36>.
- Ruksee, N., Tongjaroenbuangam, W., Mahanam, T., Govitrapong, P., 2014. Melatonin pretreatment prevented the effect of dexamethasone negative alterations on behavior and hippocampal neurogenesis in the mouse brain. *J. Steroid Biochem. Mol. Biol.* 143, 72–80. <https://doi.org/10.1016/j.jsbmb.2014.02.011>.
- Sapolsky, R.M., 2000. The possibility of neurotoxicity in the hippocampus in major depression: a primer on neuron death. *Biol. Psychiatry* 48 (8), 755–765. [https://doi.org/10.1016/S0006-3223\(00\)00971-9](https://doi.org/10.1016/S0006-3223(00)00971-9).
- Sigwalt, A.R., Budde, H., Helmich, I., et al., 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>.
- Skupio, U., Tertilt, 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>.
- Sousa, N., Lukoyanov, N.V., Madeira, M.D., Almeida, O.F.X., Paula-Barbosa, M.M., 2000. Reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioral improvement. *Neuroscience* 97 (2), 253–266. [https://doi.org/10.1016/S0306-4522\(00\)00050-6](https://doi.org/10.1016/S0306-4522(00)00050-6).
- de Souza Balk, R., Bridi, J.C., de Lima Portella, R., Carvalho, N.R., Dobrachinski, F., Da Silva, M.H., Soares, F.A.A., 2010. Clomipramine treatment and repeated restraint stress alter parameters of oxidative stress in brain regions of male rats. *Neurochem. Res.* 35 (11), 1761–1770. <https://doi.org/10.1007/s11064-010-0240-1>.
- Srikumar, B.N., Raju, T.R., Rao, B.S., 2006. The involvement of cholinergic and noradrenergic systems in behavioral recovery following oxotremorine treatment to chronically stressed rats. *Neuroscience* 143 (3), 679–688. <https://doi.org/10.1016/j.neuroscience.2006.08.041>.
- Sterner, E.Y., Kalynchuk, L.E., 2010. Behavioral and neurobiological consequences of prolonged glucocorticoid exposure in rats: relevance to depression. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 34 (5), 777–790. <https://doi.org/10.1016/j.pnpbp.2010.03.005>.
- Wang, C., Wu, H.M., Jing, X.R., Meng, Q., Liu, B., Zhang, H., Gao, G.D., 2012. Oxidative parameters in the rat brain of chronic mild stress model for depression: relation to anhedonia-like responses. *J. Membr. Biol.* 245 (11), 675–681. <https://doi.org/10.1007/s00232-012-9436-4>.
- Wenk, G.L., 2003. Assessment of spatial memory using the radial arm and Morris water maze. In: Taylor, G.P. (Ed.), *Current Protocols in Neuroscience*. John Wiley & Sons, Inc, New York, p. 8.5A5.12. <https://doi.org/10.1002/0471142301.ns0805as26>.
- Willner, P., Muscat, R., Papp, M., 1992. Chronic mild stress-induced anhedonia: a realistic animal model of depression. *Neurosci. Biobehav. Rev.* 16 (4), 525–534. [https://doi.org/10.1016/S0149-7634\(05\)80194-0](https://doi.org/10.1016/S0149-7634(05)80194-0).
- Wróbel, A., Serefko, A., Wlaz, P., Poleszak, E., 2014. The depressogenic-like effect of acute and chronic treatment with dexamethasone and its influence on the activity of antidepressant drugs in the forced swim test in adult mice. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 54, 243–248. <https://doi.org/10.1016/j.pnpbp.2014.06.008>.
- Xu, Y., Wang, C., J Klabnik, J., O'Donnell, J. M., 2014. Novel therapeutic targets in depression and anxiety: antioxidants as a candidate treatment. *Curr. Neuropharmacol.* 12 (2), 108–119.
- Yao, Y.Y., Liu, D.M., Xu, D.F., Li, W.P., 2007. Memory and learning impairment induced by dexamethasone in senescent but not young mice. *Eur. J. Pharmacol.* 574 (1), 20–28. <https://doi.org/10.1016/j.ejphar.2007.07.021>.
- Yu, J., Liu, Q., Wang, Y.Q., Wang, J., Li, X.Y., Cao, X.D., Wu, G.C., 2007. Electroacupuncture combined with clomipramine enhances antidepressant effect in rodents. *Neurosci. Lett.* 421 (1), 5–9. <https://doi.org/10.1016/j.neulet.2007.02.052>.
- Zafir, A., Banu, N., 2009. Modulation of in vivo oxidative status by exogenous corticosterone and restraint stress in rats. *Stress* 12 (2), 167–177. <https://doi.org/10.1080/10253890802234168>.
- Zhong, J., Li, G., Xu, H., Wang, Y., Shi, M., 2019. Baicalin ameliorates chronic mild stress-induced depression-like behaviors in mice and attenuates inflammatory cytokines and oxidative stress. *Braz. J. Med. Biol. Res.* 52 <https://doi.org/10.1590/1414-431X20198434>.