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ORIGINAL ARTICLE

Combined corticosterone treatment and chronic restraint stress lead to depression associated with early cognitive deficits in mice

Gwladys Temkou Ngoupaye[1,2] · Francis Bray Yassi[3] · Doriane Amanda Nguepi Bahane[3] · Elisabeth Ngo Bum[3,4]

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Abstract Many models, such as chronic mild stress, chronic stress or chronic corticosterone injections are used to induce depression associated with cognitive deficits. However, the induction period in these different models is still long and face constraints when it is short such as in the chronic mild stress done in a minimum period of 21 days. This study aimed to characterize a model of depression with early onset cognitive deficit. 14 days combined chronic injection of corticosterone followed by 2 h restraint stress using a restrainer was used to induce depression with early cognitive deficit onset. The forced swim test, sucrose test and plasma corticosterone concentration were used to assess depression-like characteristics. The Morris water maze, novel object recognition task, as well as hippocampal acetylcholinesterase activity were used to assess cognitive deficit. The combined corticosterone injection + chronic restraint stress group presented with marked depression-like behaviour and a higher plasma corticosterone concentration compared to corticosterone injection alone and restraint stress alone. It also showed an alteration in the learning process, memory deficit as well as increased acetylcholinesterase activity compared to corticosterone injection and restraint stress alone groups. These findings suggest that the combined corticosterone administration and chronic restraint stress can be used not only as an animal model for severe depression, but also for depression with early onset cognitive deficit.

Keywords Depression · Cognitive deficit · Corticosterone · Restraint · Memory · Acetylcholinesterase

Introduction

According to the World Health Organization (WHO 2012), depression is estimated to affect around 350 million people across the world, making it the leading cause of disability worldwide in terms of the total years lost due to disability (Baune and Renger 2014). Several lines of evidence suggest that depression arises from a combination of genetic and environmental factors (Gold 2015). Amongst all the environmental risk factors, stress is the major one, and its exposure can lead to /or exacerbate many neuropsychiatric disorders including depression (Duman et al. 2016). There is a well-established connection between stress-mediated hyperactivity of the hypothalamus– pituitary–adrenal axis (HPA) and depression (Barden 2004; Mazurka et al. 2016; Di et al. 2017). This connection is primarily associated with reduced expression and function of glucocorticoid receptors (GRs) which may lead to feed-back inhibition, resulting in elevated levels of circulating glucocorticoids and protracted responses to stressors (O'Keane et al. 2012; Farrell and O'Keane 2016; Di et al. 2017). While early-onset depression is associated with greater disease severity and higher levels of recurrence (Castaneda et al. 2008; Darcet et al. 2016), it is also often accompanied by cognitive deficits (McIntyre et al. 2013), with an estimated two-thirds of depressed patients presenting with impaired cognition (Abas et al. 1990; Butters et al. 2004; Afridi et al. 2011; Rock et al. 2014). Several models exist in attempts to study cognitive deficits associated with depression or to understand the aetiology of the disease, implicating a correlation between the two (Wang and Blazer 2015; Depp et al. 2015). Interestingly, notwithstanding of the stressor type, in experimentally induced depression

✉ Gwladys Temkou Ngoupaye
gtngoupaye@gmail.com

[1] School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban 4000, South Africa

[2] Department of Animal Biology, University of Dschang, Dschang 67, Cameroon

[3] Department of Biological Science, University of Ngaoundéré, Ngaoundéré 454, Cameroon

[4] Institute of Mines and Petroleum Industries, University of Maroua, Maroua 46, Cameroon

with associated cognitive deficits (restraint, maternal separation, prenatal stress, or chronic corticosterone administration), animals exhibit cognitive deficits after an average period of 4 weeks (Mehta et al. 2017; Coburn-Litvak et al. 2003). For example, exposure to restraint stress 1 h per day for 40 days was used to induce depression with cognitive deficits (Gamaro et al. 1999). Since the duration of these protocols is still fairly long, the chronic mild stress (CMS) procedure was then proposed, consisting of a variety of unpredictable mild stressors for a 5-weeks period with the stressors set for a week and the repetition starting again after the stressing procedure of the previous week being over (Song et al. 2006). Interestingly, Mc Laughlin and colleagues in 2007 succeeded to impair the spatial memory in a shorter time by restraint stress for 6 h per day for 21 consecutive days. However, this protocol was limited because the impairment affected only the spatial memory and therefore an alternative method of chronic stress paradigms was required. A new method of depression with cognitive deficits induction was developed by chronic injection of corticosterone which ablated the need for a physical stressor (Coburn-litvak et al. 2003; Wong and Herbert 2005; Kott et al. 2016). Coburn-litvak and colleagues in 2003 first showed that a 56 days consecutive injection of corticosterone (26.8 mg/kg) to male Sprague–Dawley rats impaired spatial working memory in the Y-maze. Later on, Wong and Herbert (2005) showed that a 27 days administration of corticosterone (40 mg/kg) in Lister hooded male rats reduced hippocampal neurons survival that may impaired hippocampal-dependent memory consolidation and memory retrieval. Additionally, Kott et al. (2016) showed that administration of corticosterone (40 mg/kg) to female Sprague Dawley rats for 23 days increased the immobility time and lowered neurogenesis levels in the ventral hippocampus.

A recent study by Mehta et al. (2017) showed that male Swiss albino mice experienced cognitive deficits after 21 days of chronic unpredictable stress (CUS) using a combination of different stressors or length of stress per day with no repetition. A particular task was assigned each day to allow for unpredictability which may lead to the impaired spatial and episodic memories. Although many models have been used to reduce the onset of cognitive deficits (Gamaro et al. 1999; Coburn-Litvak et al. 2003; Wong and Herbert 2005; Song et al. 2006; McLaughlin et al. 2007; Kott et al. 2016; Mehta et al. 2017), there have been no hypothesis about the effect of a combination of two models such as restraint stress and chronic corticosterone injection. This combination can reduce the constraints faced in the 21 days chronic unpredictable stress, or the long time period observed in a period of 27 days in chronic corticosterone administration and therefore can shorten the onset of the cognitive deficit in major depressive disorder. Hence, we hypothesized that the combination of chronic restraint stress and chronic administration of corticosterone, mimicking stressful events can present a synergistic effect and can induce an early onset of cognitive deficit in major depressive disorder. Thus, this study aims first to characterize a model combining two stressors leading to a severe depression-like phenotype; and further to assess the occurrence of cognitive deficits after this short time period of induction.

Materials and methods

Experimental animals

Young adult Swiss albino mice (8–10 weeks old) from both sexes ($n = 44$), weighing 19–25 g at the beginning of the experiment, were obtained from the Animal veterinary laboratory (LANAVET) Garoua, Cameroon. The animals were housed in polyacrylic cages and were randomly distributed in each group following a setup of 5 females and 6 males for a total of 11 animals in each group. At the beginning of the experiment mice were gathered together according to their respective group before being split in subgroups according to sex. Meaning in each group there was a subgroup of female and male. Food and water was available ad libitum. Mice were treated in accordance with the guidelines of the Cameroonian bioethics committee (reg N°.FWA-IRB00001954) and in accordance with NIH- Care and Use of Laboratory Animals manual. Efforts were also made to minimize animal suffering and to reduce the number of animal used in the experiment.

Swiss Albino mice were used in the present study as they have been used extensively as a primary screen in different models of depression. They are less expensive, require less test substance, and present stable levels of immobility after only a single exposure to the forced swim test and are sensitive to drugs that produce antidepressant-like activity (Castagné et al. 2010; Ngoupaye et al. 2014).

Treatments

Corticosterone (Sigma Aldrich, St Louis, USA), emulsified in distilled water and 5% tween 80 (Sigma Aldrich, St Louis, USA) was administered subcutaneously at a dose of 40 mg/kg with an injection volume of 1 mL/kg. The other group of animals was injected with vehicle (distilled water and 5% tween 80). Corticosterone 40 mg/kg, was used, based on the previous studies showing that two weeks corticosterone (40 mg/kg) injections induce depressive -like behaviour; however, it was not enough to induce cognitive deficit (Wong and Herbert 2005; Lussier et al. 2013; Yau et al. 2016).

All the animals received either corticosterone injections or vehicle every day from 9:00 am for 14 days one hour before the restraint stress (Fig. 1).

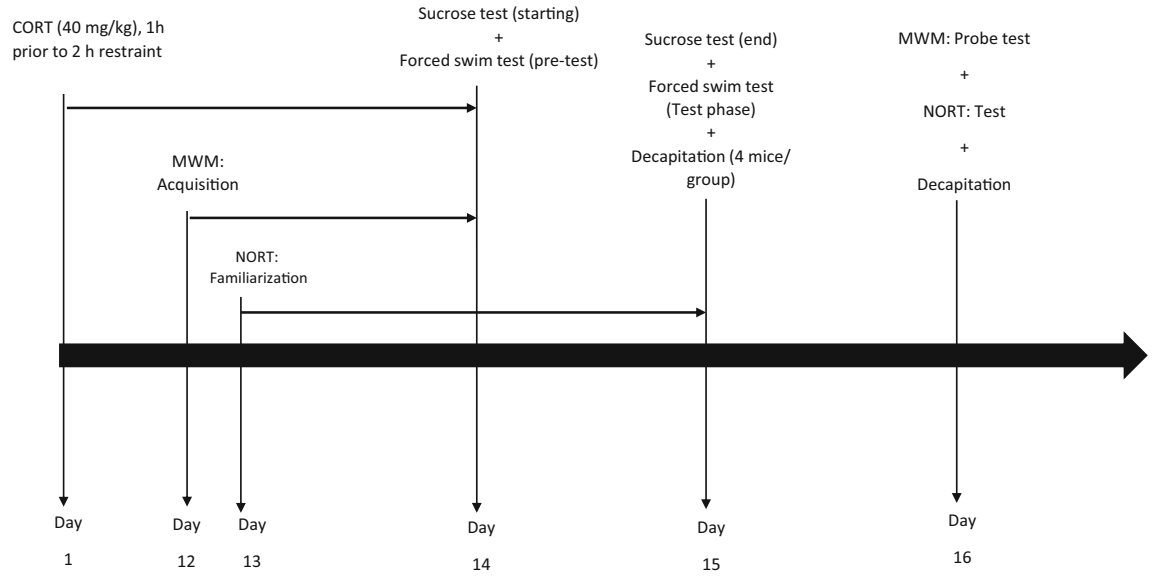

Fig. 1 Diagrammatic depiction of the experimental procedure. Restraint: Restraint stress; MWM: Morris water maze; NORT: Novel object recognition test

Restraint stress

Restraint stress of the animals was carried out one hour after the corticosterone injection, by placing mice individually in a ventilated polypropylene tube (diameter of 3 cm and length of 10 cm, and with a 1 cm hole in the wall of the cylinder for air diffusion) for 2 h every day for 14 days.

The procedure was performed with an alteration of restraint time, to avoid familiarization, according to this schedule:

Day 1 = 2 h + 0 h, Day 2 = 1 h + 1 h, Day 3 = 0 h - 2 h, Day 4 = 2 h + 0 h, Day 5 = 1 h + 1 h, Day 6 = 0 h - 2 h, Day 7 = 2 h + 0 h, with 1 h pause between restraint sessions. During the 1 h pause, animals were returned to their home cage in the housing room. The schedule was repeated for the next seven days. All the animals from the control group were handled daily for the duration of the 14 day.

The sucrose preference test (SPT)

The SPT was conducted over a 24 h period (9:00 a.m. to 9:00 a.m.) from day 14 to day 15, immediately after CORT or vehicle injection. The animals were tested for sucrose preference. For this purpose, two bottles were presented to the animals for 24 h. One bottle contained pure drinking water and the other 5% sucrose solution. The bottle positions were changed every 12 h during 24 h period in order to prevent the development of place preference. All bottles were weighed and converted to ml, prior to and after each 12-h period to measure liquid consumption. The preference for sucrose solution was calculated using the formula (ml sucrose)/(ml sucrose + ml water). Food was available ad libitum during the whole period of testing.

Novel object recognition task

The novel object recognition task (NORT) was conducted to assess the mice's ability to recognize a novel object, determined by their exploratory behavior. This task measures the ability to discriminate between novel and familiar, previously encountered objects. Familiarization of the animals with the experimental procedures occurred on the first 3 days, where the animal was individually placed in an empty open field without any objects. The mice were habituated to the open field by allowing them to explore it without the objects for 15 min every day for 3 days prior to the test. The tests (sample phase and test phase) were performed on the 4th day. The objects were two small identical wooden cubes located in an open field, a square wooden box (40 × 40 × 45 cm).

The NORT is devided into two phases; 1) the sample phase, where each animal was exposed to the object for 5 min; and 2) the test phase, where one object was replaced by a novel object and the time spent exploring the novel and the familiar objects were recorded for 5 min. The test phase takes place one hour after the sample phase.

The objects were cleaned between testing with ethanol 70%. Intact memory is evidenced by subjects spending more time investigating the novel object.

Forced swim test

The forced swim test was performed on day 14 and 15, one hour after the NORT. It is a well-characterized model used to assess despair in mice (Hirani et al. 2002; Ngoupaye et al. 2014). Briefly, animals were placed in a cylindrical Perspex tanks (25 cm height, 10 cm diameter),

filled to a depth of 15 cm with water kept at constant temperature of 22 ± 1 °C, and changed between animals were used. Testing was performed in two phases, the induction phase and the test phase. During the induction phase, animals were placed in the water for 15 min, towel dried and returned to their home cages. After 24 h, the mice were placed in the same tank for 6 min.

The movements of the mice were recorded and the duration of immobility (s), swimming and climbing, was measured during this second test phase by an experienced observer that was blind to the experimental conditions. In order to minimize interference with the animal's behaviour, the observer remained at the same location in the room during all trials (Cannizzaro et al. 2001). The behavioural variable "immobility" was defined as: making no movements for at least 2 s or making only those movements that were necessary to keep the nose above water. Mice were allowed to move their forepaws slightly or support themselves by pressing their paws against the wall of the cylinder.

Morris water maze

The Morris water maze was used to evaluate spatial learning and memory in experimental rodents. It is a circular tank (diameter 100 cm and height 45 cm), which was filled with water and maintained at 25°C. The tank was divided into four equal quadrants. A platform (diameter = 6 cm, height = 29 cm) centered in one of the four quadrants of the pool was submerged approximately 1 cm below the surface of the water. The position of the platform and visual cues (4) fixed in four sides of the surrounding walls dividing the pool into equal quadrants were kept constant throughout the training sessions. Each animal was subjected to four consecutive acquisition training sessions from 1 pm daily on each day with each session lasting 60 s and an interval of 5 min between sessions. During each training session, the mice were allowed to locate the hidden platform and to remain there for 20 s. If the animal was unable to locate the hidden platform within 60 s, it was gently guided by hand to the platform and allowed to remain there for 20 s. During each trial, the latencies of mice to locate the hidden platform were recorded and the latency was considered to be an index of acquisition and learning. On the 5th day, the platform was removed and each mouse was allowed to explore the pool for 60 s. The latency to enter the target quadrant and the total time spent in the target quadrant were noted as indices of spatial memory retrieval.

Neurochemical assays

Assessment of corticosterone level

One hour after the forced swim test on day 15, a set (4) of the animals mice were decapitated, as the corticosterone level was shown to be significantly high one hour after forced swim test-phase exposure (Connor et al. 1997). Trunk blood was collected in EDTA tubes and centrifuged at 3500 rpm for 15 min and stored at −20 °C until analysis of plasma corticosterone determinations using ELABSCIENCE enzyme immunoassay kits commercially available.

Brain tissue preparation

One hour after the novel object recognition test on day 16, all animals were decapitated, and a total of 4 animals per group were randomly chosen with regard to sex difference for further neurochemical analysis. The hippocampus was removed from the whole brain and used to prepare a homogenate for acetyl cholinesterase (ACHE) determination. The brain was exposed from its dorsal side. The whole brain was immediately removed and cleaned with chilled normal saline on ice. A 10% (*w*/*v*) homogenate was prepared with 0.1 M phosphate buffer containing 1% Triton-100X (pH 7.4). The homogenates of hippocampus were individually centrifuged (15 min at 3000 rpm). The supernatant was used for assays of ACHE.

Determination of ACHE

The cholinergic marker, ACHE was measured in hippocampal tissue according to the method of Ellman (Ellman et al. 1961). The enzymatic activity was assessed by measuring the yellow colour obtained when thiocholine, released due to the cleavage of acetylthiocholine by AChE, reacted with 5,5′-dithiobis-2-nitrobenzoic acid (DTNB). Briefly, 100 μL, 650 μL phosphate buffer (100 mM, pH 7.4), 100 μL of homogenate, and 25 μL of acetylthiocholine iodide (75 mM) as substrate were added for AChE estimation. Absorbance was measured at 412 nm for 5 min at 1 min intervals using a Spectrumlab 752S. Acetylcholinesterase activity was expressed as nmol of acetylthiocholine iodide hydrolyzed/mg protein/min. The protein concentration was assessed by a commercially BCA protein assay available kit.

Statistical analysis

Statistical analysis was performed using the software program Graph Pad Prism version 5.00 (San Diego, CA, USA). The Shapiro-Wilk normality test was used to assess the distribution of the data. When the data showed normal distribution, parametric tests were used. Repeated measure ANOVA followed by Newman-Keuls post hoc test was used to analyze data from the probe test in the Morris water maze, sucrose preference test, discrimination index from Novel object recognition tasks, forced swim test, open field test, sucrose test, corticosterone level, and ACHE level in the hippocampus. Two-way ANOVA followed by the Bonferroni test was used to analyze data from exploration time in the Novel object

recognition task as well as learning. All data are presented as mean ± S.E.M per group. When the $P < 0.05$, the difference between groups was considered statistically significant.

Results

Effect of the combination of restraint stress and corticosterone injection on the forced swim test

Figure 2 depicts the average activity of different groups recorded in the forced swim test using two-way ANOVA. There was an interaction between the different parameters, immobility, swimming and climbing between groups [$F_{(6,88)} = 37.61$; $p < 0.0001$]. Two-way ANOVA showed that there was a significant difference in time spent in the different parameters between groups [$F_{(2,88)} = 614.6$; $p < 0.0001$].

The Bonferroni post hoc test showed that the immobility time was significantly higher in RS, CORT and CORT + RS groups compared to the control ($p < 0.05$). The immobility time of CORT + RS group was significantly longer than RS and CORT groups alone ($p < 0.001$).

Differences in swimming time were also observed in the three groups. A significant decrease of swimming time was observed in the CORT + RS group compared to the CORT and control groups ($p < 0.05$). While climbing time was significantly decreased in CORT ($p < 0.05$) and CORT + RS ($p < 0.01$) groups compared to RS and the control group ($p < 0.001$).

Effect of the combination of restraint stress and corticosterone injection on sucrose consumption

Figure 3a depicts the average liquid intake assessed in the sucrose preference test after 14 days of chronic Restraint stress and administration of corticosterone (40 mg/kg). One way ANOVA showed that there was a difference amongst the group ($p < 0.0001$). Post hoc test showed that CORT + RS consumed more water [$F(3,43) = 24.39$; $p < 0.0001$] and less sucrose [$F(3,43) = 327$; $p < 0.0001$] than the control, CORT and RS alone. The sucrose consumption ratio further showed that combined corticosterone and chronic Restraint, consumed significantly less sucrose than the control, RS and CORT alone ($P < 0.001$).

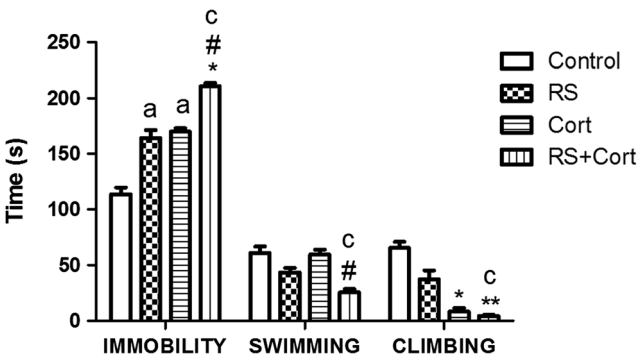

Fig. 2 Average activity of different groups recorded on the forced swim test. $N = 11$. All the data are expressed as mean ± SEM. $^{a}p < 0.05$, $^{c}p < 0.001$, when compared to control; $*p < 0.05$, $**p < 0.01$, when compared to RS; # $p < 0.05$ when compared to CORT; two-way ANOVA followed by Bonferroni test. CORT: Corticosterone injections only; RS: Restraint stress CORT + RS = corticosterone + restraint stress

Effect of the combination of restraint stress and corticosterone injection on plasma corticosterone concentration

The average concentration of plasma corticosterone after 14 days of stress and measure one hour after the forced swim test is depicted in Fig. 4. ANOVA one way showed evidence of the combined stress activity, as the corticosterone concentration was increased in all the stressed group as compared to the control [$F(3,11) = 21.13$; $p = 0.0004$]. Amongst the three groups stressed, CORT + RS showed a greater increase in corticosterone concentration ($p < 0.001$).

Effect of the combination of restraint stress and corticosterone injection in the Morris water maze test

Two way ANOVA showed that there was an interaction effect between the different groups during the three days of learning [$F(6,48) = 2.593$; $p = 0.0294$]. Mice immobilized (RS) and those exposed to CORT treatment only, showed a decrease time to reach the platform compared to the combined stress (CORT + RS) throughout the three day of learning ($p < 0.05$). The combined stress group CORT+ RS did not show any difference on the second day compared to the control. By the third day the combined stress group CORT+ RS showed a significant increase of the latency to reach the hidden platform as compared to the control ($P < 0.01$) (Fig. 5a).

Figure 5b depicts the average time spent in the target quadrant during the probe test, One way ANOVA showed that there was significant decrease of the time spent in the target quadrant between groups [$F(3,27) = 10.11$; $p = 0.0002$]. Post hoc test further showed the difference between specific groups. Combined stress group CORT+ RS showed a significant decrease in the time spent in the quadrant as compared to the RS, CORT and the control ($p < 0.001$).

Effect of the combination of restraint stress and corticosterone injection in the novel object recognition task

Mice restricted (RS) and submitted to CORT treatment spent more time exploring the familiar object than the other groups,

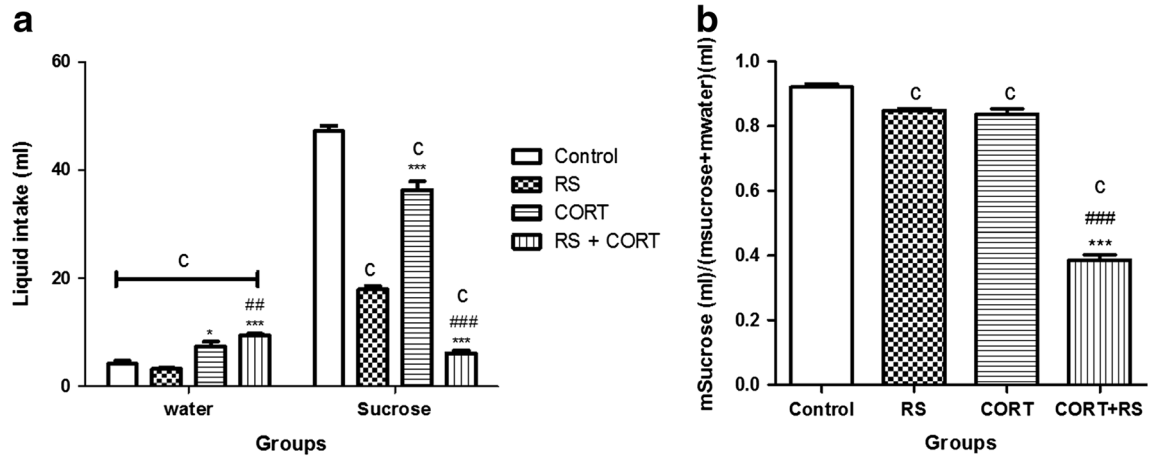

Fig. 3 Sucrose preference of the different groups after 14 days of administration of corticosterone and restraint stress in mice. N = 11. All the data are expressed as Mean ± SEM. $^{c}p < 0.001$, when compare to control; $***p < 0.001$ when compare to RS; $\#p < 0.05$ and ### $p < 0.001$ when compare to CORT. ANOVA one way followed by Newman Keuls test. CORT: Corticosterone; RS: Restraint stress CORT + RS = corticosterone + restraint stress

which spent more time exploring the novel object. Two way ANOVA showed that there was an interaction between the different group [$F(3,36) = 9.24$; $p = 0.0001$]. It also showed that a significant exploration time difference amongst the groups [$F(1,36) = 25.38$; $p = 0.0003$]. Post hoc test further showed that there was a significant difference in time spent in the familiar and novel object in Control, RS and CORT (Fig. 6a).

Figure 6b depicts the Discrimination Index. One way ANOVA showed that there was a significant difference between groups [$F(3,27) = 3.343$; $p = 0.0058$]. Post hoc analysis confirm the preference for the familiar object in mice CORT + RS ($p < 0.01$).

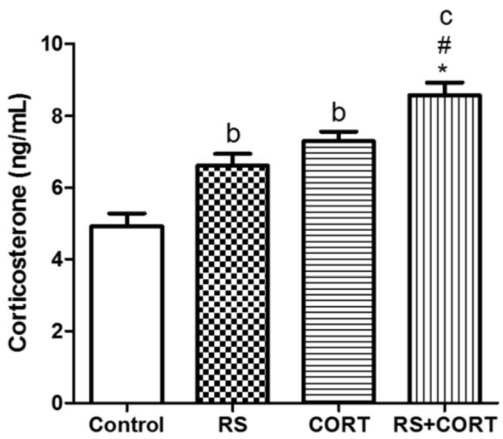

Fig. 4 Effect of the combination of restraint stress and corticosterone injection on corticosterone level in mice. $N = 4$. All the data are expressed as Mean ± SEM. $^{b}p < 0.01$, $^{c}p < 0.001$, when compare to control; $\#p < 0.05$ when compare to CORT; $*p < 0.001$ when compare to RS. ANOVA one way followed by Newman Keuls test. CORT: Corticosterone; RS: Restraint stress CORT + RS = corticosterone + restraint stress

Effect of the combination of restraint stress and corticosterone injection on AChE activity in the hippocampus

Figure 7 depicts the average of hippocampal AChE activity in the different groups. One way ANOVA further showed that there was a difference between specific groups [$F(3,15) = 15.17$; $p = 0.0002$]. Post hoc test showed that mice subjected to a combined stress showed an increase in ACHE activity as compared to the control ($P < 0.001$). CORT +RS showed a pronounced increase of ACHE activity compare to RS and CORT ($p < 0.001$).

Discussion

Our studies aimed to characterize a model combining two stress inducers leading to a more severe depression; and further assess the occurrence of cognitive deficits after this short time period of induction.

The forced swim test showed an increase in immobility time in all the stressed group compared to the control. CORT + RS showed a marked increase in immobility time compared to RS and CORT alone, showing a pronounced depressive behaviour in this group. Behavioural despair in animals assessed in forced swim test have been related to an increase in immobility time (Liu et al. 2017). Besides an increase in immobility time as a core of behavioural despair, anhedonia has been also shown as a core of depression. All the stressed animals' consumed less sucrose compared to the control, with a marked decrease in CORT + RS compared to the other stressed group. These finding are in agreement with Ali et al. (2015) who found that three weeks of corticosterone

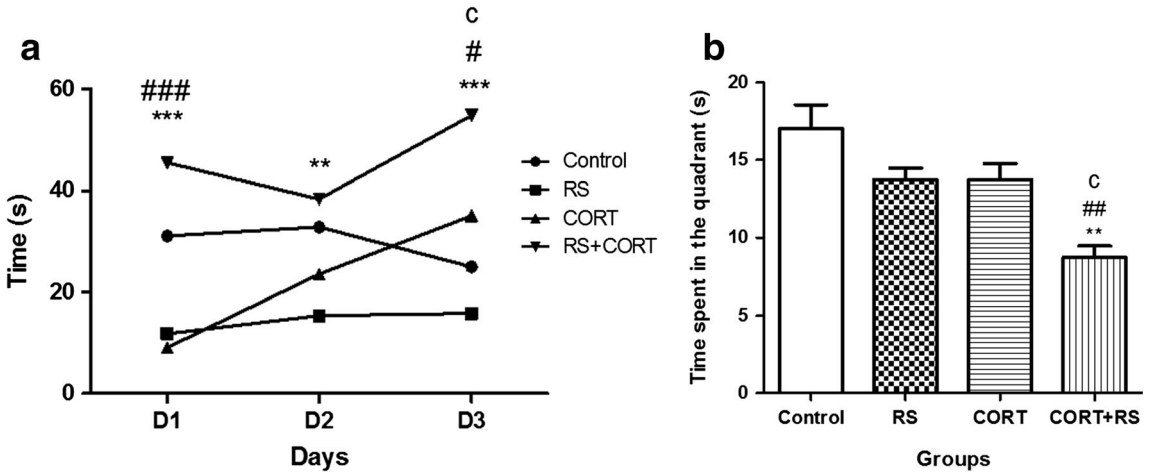

Fig. 5 Effect of the combination of restraint stress and corticosterone injection in the Morris Water Maze test. A- Represents the latency to reach the hidden platform. B- Represents the time spent in the target quadrant. $N = 7$. All the data are expressed as Mean ± SEM. $^{c}p < 0.001$, when compare to TNe; ** $p < 0.01$; ***$p < 0.001$ when compare to RS. #$p < 0.05$; ##$p < 0.01$; ###$p < 0.001$, when compare to CORT. ANOVA two way followed by Bonferonni test; ANOVA one way, followed by Student Newman - Keuls test. CORT: Corticosterone; RS: Restraint stress CORT + RS = corticosterone + restraint stress

injection resulted in a decrease in the percentage of sucrose consumption of Swiss albino mice, and attributed the reduction of sucrose intake to anhedonia, classified itself as a characteristic of major depressive disorder (Wang et al. 2017). Therefore the marked decrease in sucrose intake noted in the RS + CORT group suggest the presence of a more severe depression state.

Chronic stress in adulthood has been shown to be an increasing factor for major depressive disorder (MDD) (Risch et al. 2009). The well described effects of stress on susceptibility to develop MDD have been supported by findings showing that there are abnormalities in the hypothalamic–pituitary–adrenal (HPA) axis in patients with MDD which may impact the release of glucocorticoids. The dysregulated HPA axis is evidenced in humans by cortisol hypersecretion (Barden 2004; Travis et al. 2016), and in animals by an increased level of corticosterone (Ngoupaye et al. 2013; Harlé et al. 2017). Our results showed that there was an increased release of corticosterone in all the stressed groups, with a marked release in CORT + RS. These data confirm the depressive state, with a severe stage observed in animals that were subjected both to RS and CORT. The combination of the exposure to corticosterone injections and chronic restraint stress could have been speculated to induce post-traumatic stress disorders, due to the combination of stress happening in a short period. Many studies have shown that post-traumatic

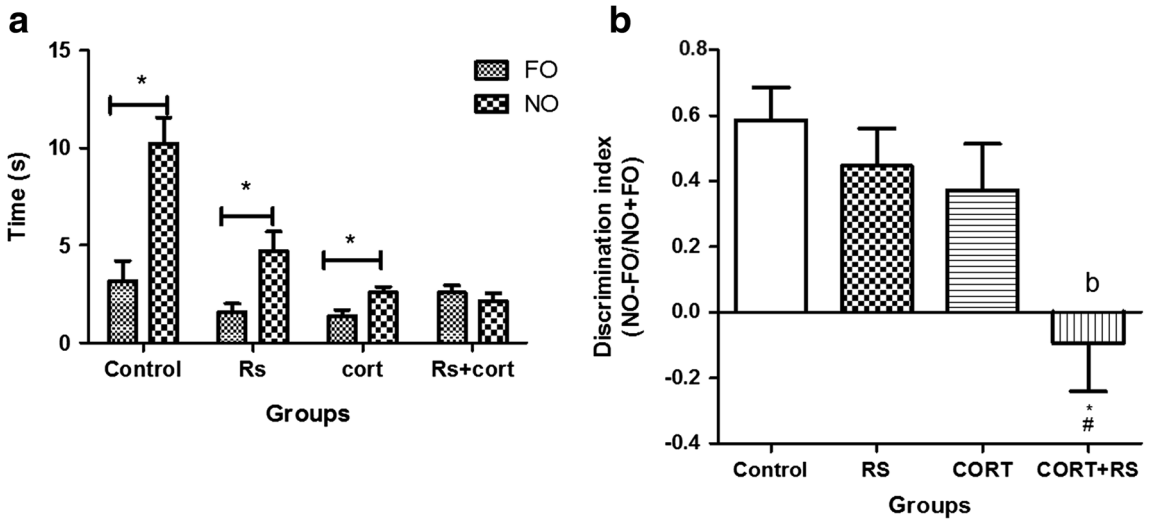

Fig. 6 Effect of the combination of restraint stress and corticosterone injection in the Novel object recognition task. A- Represents the exploratory time between the two objects. B- Represents discrimination index. N = 7. All the data are expressed as Mean ± SEM. $^{b}p < 0.05$ when compare to control; #$p < 0.05$ when compare to CORT;* $p < 0.05$; when compare to RS. ANOVA followed by Student Newman - Keuls test. CORT: Corticosterone; RS: Restraint stress CORT + RS = corticosterone + restraint stress

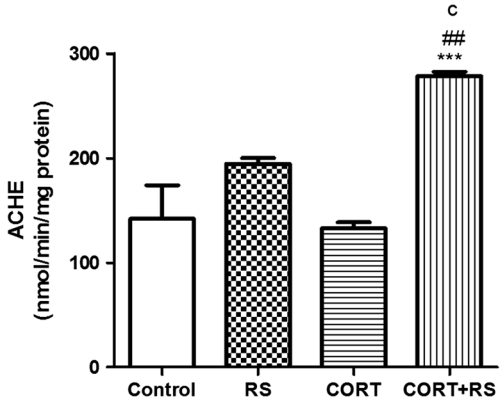

Fig. 7 Effect of the combination of restraint stress and corticosterone injection on ACHE levels in the hippocampus of mice. N = 4. All the data are expressed as mean ± SEM. $^{c}p < 0.001$ when compared to control. $^{***}p < 0.001$, when compare to RS. $^{\#\#}p < 0.01$ when compare to CORT. ANOVA followed by Student Newman-Keuls, post hoc test. CORT: Corticosterone; RS: Restraint stress CORT + RS = corticosterone + restraint stress

stress disorder is related to a lower level of cortisol rather than a high level, while major depressive disorder is related to a high level (Connor et al. 1997; Zoladz et al. 2012; Flory and Yehuda 2015). We therefore ruled out that speculation as in our study we observed an increase level of corticosterone in the combined stressed group, probably due to the severity of the depression induced.

It has been well documented that corticosterone produces its effects in the central nervous system via activation of glucocorticoid and mineralocorticoid receptors (Robinson et al. 2016). Though these receptors are ubiquitous throughout the brain, they are highly abundant in the hippocampus, where they provide crucial inhibitory feedback signals to the HPA axis, and make this structure highly responsive to changes in stress hormones (Sapolsky et al. 1984; Jacobson and Sapolsky 1991; Lucassen et al. 2014; Robinson et al. 2016). Furthermore, prolonged exposure to corticosterone can inhibit the proliferation and survival of adult-born hippocampal neurons, which have been shown to play an important role in the behavioural and neuroendocrine components of stress responses in rodents (Gould et al. 1999; Snyder et al. 2011; Robinson et al. 2016). Indeed, prolonged hippocampal exposure to glucocorticoids, has been shown to induce a reduction of hippocampal weight by negatively affecting hippocampal plasticity (Dranovsky and Hen 2014; Vinkers et al. 2014). Since the hippocampus is one of the essential structures of the brain involved in cognitive function (Rubin et al. 2014), it has been speculated that the reduced hippocampal size can be a leading cause of memory deficit seen in people with MDD (Murrough et al. 2011; Gray et al. 2013).

Many models of depression have been used to induce cognitive deficit associated with depression. In this regard, chronic stress induced either by restraint stress or exogenous glucocorticoids such as corticosterone have been used to induce memory deficits (McLaughlin et al. 2007). However, the time of memory deficit induction is long or face constraints, as seen in a minimum of 21 days of chronic unpredictable stress where by animals are exposed to a combination of daily different stressors such as Cold swim (8 °C) for 5 min, Tail pinch for 1 min, Food and water deprivation for 24 h, Swimming at room temperature (24 ± 2 °C) for 20 min, Overnight illumination, No stress, Tail pinch for 1.5 min, Cold swim (10 °C) for 5 min, Swimming at room temperature (24 ± 2 °C) for 15 min), Tail pinch for 2 min and Cold swim (6 °C) for 5 min. Each day had a particular task assigned to make the stress unpredicted; or administration of corticosterone (40 mg/kg) for 27 days (Coburn-Litvak et al. 2003; Wong and Herbert 2005; Can et al. 2012; Mehta et al. 2017) or a minimum of 40 days of 1 h per day of restraint stress (Gamaro et al. 1999). Indeed, the hypothesis behind these three types of stress being that, the prolonged exposure of the hippocampus to glucocorticoids would affect neuronal plasticity (Coburn-Litvak et al. 2003; Lucassen et al. 2015). We therefore hypothesized that a combination of two different chronic stressors can increase the severity of the depression and then shorten the memory deficit onset. Our results showed that neither two weeks of administration of CORT (40 mg/kg) nor two weeks of 2 h restriction altered the learning process during the three days training. However, the combined stressors did not learn how to reach the platform, as seen by the longest time observed to reach the hidden platform on the third day, suggesting that the prolonged exposure to the glucocorticoid have altered the synaptic plasticity supporting the learning process, impairing the spatial learning. Likewise, the probe test further supported findings in the training phase, as the combine stressor group showed a decrease time spent in the target quadrant, suggesting a spatial reference memory deficit. These findings are in agreement with recent studies which have shown that glucocorticoids impair the retrieval of hippocampus-dependent spatial or contextual memory in rodents (de Quervain et al. 2009; de Quervain et al. 2017). Indeed, the Morris water maze task is commonly used to evaluate hippocampus-dependent spatial long-term memory (Morris 1994; Vorhees and Williams 2006; Sprowles et al. 2016). Therefore, the combined stressed group spending less time in the target quadrant indicates an impairment of the spatial memory. Cognitive functions have been also assessed in the object recognition task, which further showed that animals from the combined stressors did not discriminate the novel and familiar object, as seen during the exploration time. In addition, they further spent more time in the familiar object compare to the novel as shown the discrimination index, confirming once more the learning and memory deficit. The novel object test has been used to assess the short-term as well as long-term episodic memory deficits (Abush and Akirav 2012). Since the combined stress animals chose the familiar rather than the novel object, this indicates that the episodic memory assessed in this model has been impaired.

This also shows that this model has a shorter onset of deficit than other models, and affect the spatial and episodic memories. Indeed, the episodic memory has been seen to be altered in corticosterone induced cognitive deficit after 51 days of consecutive administration (Coburn-Litvak et al. 2003), and 21 days of chronic unpredictable stress.

Acetylcholine (ACh) is the principal neurotransmitter in the central nervous system, which plays a vital role in cognitive functions (Kim et al. 2004; Reis et al. 2009; Drever et al. 2011; Deshmukh et al. 2015; Haider et al. 2016). One of the most important mechanisms responsible for correct cholinergic function is performed by enzyme acetylcholinesterase (AChE). Excessive AChE activity leads to constant Ach deficiency and cognitive impairments.

Our results showed an increase in AChE activities in the combined stressor group. This increase in AChE support the behavior changed observed in the Morris water maze and novel object recognition task, confirming the memory deficit in that group. Indeed, an increase in AChE has been associated with memory deficits (de Fátima et al. 2015), confirming the memory deficit in our model.

This was a preliminary study to assess the use of a combination of stressors to shorten the cognitive deficit onset in a mouse model of major depressive disorder. The results show that this model induces major depressive-like behaviour and cognitive deficits in a shorter time period than the 4 weeks average period use in various other models. Therefore, this model may be used as an alternative animal model for chronic depression induced memory deficit.

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Compliance with ethical standards

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References

Abas MA, Sahakian BJ, Levy R (1990) Neuropsychological deficits and CT scan changes in elderly depressives. Psychol Med 20(3):507–520. https://doi.org/10.1017/S0033291700017025

Abush H, Akirav I (2012) Short-and long-term cognitive effects of chronic cannabinoids administration in late-adolescence rats. PLoS One 7(2):e31731. https://doi.org/10.1371/journal.pone.0031731

Afridi MI, Hina M, Qureshi IS, Hussain M (2011) Cognitive disturbance comparison among drug-naive depressed cases and healthy controls. J Coll Physicians Surg Pak 21:351–355, 6, https://doi.org/07.2011/JCPSP.351355

Ali SH, Madhana RM, Athira KV, Kasala ER, Bodduluru LN, Pitta S, Mahareddy JR, Lahkar M (2015) Resveratrol ameliorates depressive-like behavior in repeated corticosterone-induced depression in mice. Steroids 30(101):37–42

Barden N (2004) Implication of the hypothalamic–pituitary–adrenal axis in the physiopathology of depression. J Psychiatry Neurosci 29(3):185–193

Baune BT, Renger L (2014) Pharmacological and non-pharmacological interventions to improve cognitive dysfunction and functional ability in clinical depression–a systematic review. Psychiatry Res 219(1):25–50. https://doi.org/10.1016/j.psychres.2014.05.013

Butters MA, Whyte EM, Nebes RD, Begley AE, Dew MA, Mulsant BH, Zmuda MD, Bhalla R, Meltzer CC, Pollock BG, Reynolds CF (2004) The nature and determinants of neuropsychological functioning in late-lifedepression. Arch Gen Psychiatry 61(6):587–595. https://doi.org/10.1001/archpsyc.61.6.587

Can A, Dao DT, Arad M, Terrillion CE, Piantadosi SC, Gould TD (2012) the mouse forced swim test. J Vis Exp (59). https://doi.org/10.3791/3638

Cannizzaro C, Martire M, Cannizzaro E, Provenzano G, Gagliano M, Carollo A, Mineo A, Steardo L (2001) Long-lasting handling affects behavioural reactivity in adult rats of both sexes prenatally exposed to diazepam. Brain Res 904(2):225–233. https://doi.org/10.1016/S0006-8993(01)02462-3

Castagné V, Moser P, Roux S, Porsolt RD (2010) Rodent models of depression: forced swim and tail suspension behavioral despair tests in rats and mice. Curr Protoc Neurosci 5–8. https://doi.org/10.1002/0471141755.ph0508s49

Castaneda AE, Tuulio-Henriksson A, Marttunen M, Suvisaari J, Lönnqvist J (2008) A review on cognitive impairments in depressive and anxiety disorders with a focus on young adults. J Affect Disord 106(1):1–27. https://doi.org/10.1016/j.jad.2007.06.006

Coburn-Litvak PS, Pothakos K, Tata DA, McCloskey DP, Anderson BJ (2003) Chronic administration of corticosterone impairs spatial reference memory before spatial working memory in rats. Neurobiol Learn Mem 80(1):11–23. https://doi.org/10.1016/S1074-7427(03)00019-4

Darcet F, Gardier AM, Gaillard R, David DJ, Guilloux JP (2016) Cognitive dysfunction in major depressive disorder. A translational review in animal models of the disease. Pharmaceuticals 9(1):9. https://doi.org/10.3390/ph9010009

Depp CA, Ceglowski J, Wang VC, Yaghouti F, Mausbach BT, Thompson WK, Granholm EL (2015) Augmenting psychoeducation with a mobile intervention for bipolar disorder: a randomized controlled trial. J Affect Disord 174:23–30. https://doi.org/10.1016/j.jad.2014.10.053

de Quervain DJF, Aerni A, Schelling G, Roozendaal B (2009) Glucocorticoids and the regulation of memory in health and disease. Front Neuroendocrinol 30(3):358–370. https://doi.org/10.1016/j.yfrne.2009.03.002

Deshmukh R, Kaundal M, Bansal V (2015) Caffeic acid attenuates oxidative stress, learning and memory deficit in intra-cerebroventricular streptozotocin induced experimental dementia in rats. Biomed Pharmacother 81:56–62

Di T, Zhang S, Hong J, Zhang T, Chen L (2017) Hyperactivity of hypothalamic-pituitary-adrenal Axis due to dysfunction of the hypothalamic glucocorticoid receptor in Sigma-1 receptor knockout mice. Front Mol Neurosci 10:287. https://doi.org/10.3389/fnmol.2017.00287

Dranovsky A, Hen R (2014) Hippocampal neurogenesis: regulation by stress and antidepressants. Biol Psychiatry 59(12):1136–1143

Drever BD, Riedel G, Platt B (2011) The cholinergic system and hippocampal plasticity. Behav Brain Res 221(2):505–514. https://doi.org/10.1016/j.bbr.2010.11.037

Duman RS, Aghajanian GK, Sanacora G, Krystal JH (2016) Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. Nat Med 22(3):238–249. https://doi.org/10.1038/nm.4050

Ellman GL, Courtney KD, Andres V, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7(2):88IN191–88I9095

Farrell C, O'Keane V (2016) Epigenetics and the glucocorticoid receptor: a review of the implications in depression. Psychiatry Res 242:349–356. https://doi.org/10.1016/j.psychres.2016.06.022

de Fátima O-SI, Pereira SR, Fernandes PA, Ribeiro AF, Pires RG, Ribeiro AM (2015) Mild thiamine deficiency and chronic ethanol consumption modulate acetylcholinesterase activity change and spatial memory performance in a water maze task. J Mol Neurosci 55(1):217–226

Flory JD, Yehuda R (2015) Comorbidity between post-traumatic stress disorder and major depressive disorder: alternative explanations and treatment considerations. Dialogues Clin Neurosci 17(2):141–150

Gamaro GD, Michalowski MB, Catelli DH, Xavier MH, Dalmaz C (1999) Effect of repeated restraint stress on memory in different tasks. Braz J Med Biol 32(3):341–347

Gold PW (2015) The organization of the stress system and its dysregulation in depressive illness. Mol Psychiatry 20(1):32–47. https://doi.org/10.1038/mp.2014.163

Gould E, Beylin A, Tanapat P, Reeves A, Shors TJ (1999) Learning enhances adult neurogenesis in the hippocampal formation. Nature neurosc 2(3):260–265

Gray JD, Milner TA, McEwen BS (2013) Dynamic plasticity: the role of glucocorticoids, brain-derived neurotrophic factor and other trophic factors. Neuroscience 3(239):214–227

Harlé G, Lalonde R, Fonte C, Ropars A, Frippiat JP, Strazielle C (2017) Repeated corticosterone injections in adult mice alter stress hormonal receptor expression in the cerebellum and motor coordination without affecting spatial learning. Behav Brain Res 30(326):121–131

Hirani K, Khisti RT, Chopde CT (2002) Behavioral action of ethanol in Porsolt's forced swim test: modulation by 3alpha-hydroxy-5alpha-pregnan-20-one. Neuropharmacology 43(8):1339–1350

Jacobson L, Sapolsky R (1991) The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. Endocr Rev 12(2):118–134. https://doi.org/10.1210/edrv-12-2-118

Kim HK, Kim M, Kim S, Kim M, Chung JH (2004) Effects of green tea polyphenol on cognitive and acetylcholinesterase activities. Biosci Biotechnol Biochem 68(9):1977–1979

Kott JM, Mooney-Leber SM, Shoubah FA, Brummelte S (2016) Effectiveness of different corticosterone administration methods to elevate corticosterone serum levels, induce depressive-like behavior, and affect neurogenesis levels in female rats. Neuroscience 312:201–214. https://doi.org/10.1016/j.neuroscience.2015.11.006

Lucassen PJ, Pruessner J, Sousa N, Almeida OF, Van Dam AM, Rajkowska G, Swaab DF, Czéh B (2014) Neuropathology of stress. Acta Neuropathol 127(1):109–135. https://doi.org/10.1007/s00401-013-1223-5

Lucassen PJ, Oomen CA, Naninck EF, Fitzsimons CP, van Dam AM, Czeh B, Korosi A (2015) Regulation of adult neurogenesis and plasticity by (early) stress, glucocorticoids, and inflammation. Cold Spring Harb Perspect Biol 7(9):a021303

Lussier AL, Lebedeva K, Fenton EY, Guskjolen A, Caruncho HJ, Kalynchuk LE (2013) The progressive development of depression-like behavior in corticosterone-treated rats is paralleled by slowed granule cell maturation and decreased reelin expression in the adult dentate gyrus. Neuropharmacology 31(71):174–183

Mazurka R, Wynne-Edwards KE, Harkness KL (2016) Stressful life events prior to depression onset and the cortisol response to stress in youth with first onset versus recurrent depression. J Abnorm Child Psychol 44(6):1173–1184. https://doi.org/10.1007/s10802-015-0103-y

McIntyre RS, Cha DS, Soczynska JK, Woldeyohannes HO, Gallaugher LA, Kudlow P, Alsuwaidan M, Baskaran A (2013) Cognitive deficits and functional outcomes in major depressive disorder: determinants, substrates, and treatment interventions. Depress Anxiety 30(6):515–527. https://doi.org/10.1002/da.22063

McLaughlin KJ, Gomez JL, Baran SE, Conrad CD (2007) The effects of chronic stress on hippocampal morphology and function: an evaluation of chronic restraint paradigms. Brain Res 1161:56–64. https://doi.org/10.1016/j.brainres.2007.05.042

Mehta V, Parashar A, Sharma A, Singh TR, Udayabanu M (2017) Quercetin ameliorates chronic unpredicted stress-mediated memory dysfunction in male Swiss albino mice by attenuating insulin resistance and elevating hippocampal GLUT4 levels independent of insulin receptor expression. Horm Behav 31(89):13–22

Morris R (1994) Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 11(1):47–60

Murrough JW, Iacoviello B, Neumeister A, Charney DS, Iosifescu DV (2011) Cognitive dysfunction in depression: neurocircuitry and new therapeutic strategies. Neurobiol Learn Mem 96(4):553–563. https://doi.org/10.1016/j.nlm.2011.06.006

Ngoupaye GT, Bum EN, Daniels WM (2013) Antidepressant-like effects of the aqueous macerate of the bulb of Gladiolus Dalenii van Geel (Iridaceae) in a rat model of epilepsy-associated depression. BMC Complement Altern Med 13(1):272. https://doi.org/10.1186/1472-6882-13-272

Ngoupaye GT, Bum EN, Taiwe GS, Moto FC, Talla E (2014) Antidepressant properties of aqueous macerate from Gladiolus Dalenii corms. AJTCAM 11(1):53–61

O'Keane V, Frodl T, Dinan TG (2012) A review of atypical depression in relation to the course of depression and changes in HPA axis organization. Psychoneuroendocrinology 37(10):1589–1599. https://doi.org/10.1016/j.psyneuen.2012.03.009

Reis J, Schambra HM, Cohen LG, Buch ER, Fritsch B, Zarahn E, Celnik PA, Krakauer JW (2009) Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation. Proc Natl Acad Sci U S A 106(5):1590–1595. https://doi.org/10.1073/pnas.0805413106

Risch N, Herrell R, Lehner T, Liang KY, Merikangas KR (2009) Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression. JAMA 301(23):2462–2471. https://doi.org/10.1001/jama.2009.878

Robinson SA, Brookshire BR, Lucki I (2016) Corticosterone exposure augments sensitivity to the behavioral and neuroplastic effects of fluoxetine in C57BL/6 mice. Neurobiol Stress 30(3):34–42

Rock PL, Roiser JP, Riedel WJ, Blackwell AD (2014) Cognitive impairment in depression: a systematic review and meta-analysis. Psychol Med 44(10):2029–2040. https://doi.org/10.1017/S0033291713002535

Rubin RD, Watson PD, Duff MC, Cohen NJ (2014) The role of the hippocampus in flexible cognition and social behavior. Front Hum Neurosci 8:742. https://doi.org/10.3389/fnhum.2014.00742

Sapolsky RM, Krey LC, McEwen BS (1984) Glucocorticoid-sensitive hippocampal neurons are involved in terminating the adrenocortical stress response. Proc Natl Acad Sci 81(19):6174–6177. https://doi.org/10.1073/pnas.81.19.6174

Snyder JS, Soumier A, Brewer M, Pickel J, Cameron HA (2011) Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. Nature 476(7361):458–461

Song L, Che W, Min-Wei W, Murakami Y, Matsumoto K (2006) Impairment of the spatial learning and memory induced by learned

helplessness and chronic mild stress. Pharmacol Biochem Behav 2: 186–193

Sprowles JL, Hufgard JR, Gutierrez A, Bailey RA, Jablonski SA, Williams MT, Vorhees CV (2016) Perinatal exposure to the selective serotonin reuptake inhibitor citalopram alters spatial learning and memory, anxiety, depression, and startle in Sprague-Dawley rats. Int J Dev Neurosci 54:39–52. https://doi.org/10.1016/j.ijdevneu.2016.08.007

Travis SG, Coupland NJ, Hegadoren K, Silverstone PH, Huang Y, Carter R, Fujiwara E, Seres P, Malykhin NV (2016) Effects of cortisol on hippocampal subfields volumes and memory performance in healthy control subjects and patients with major depressive disorder. J Affect Disord 201:34–41. https://doi.org/10.1016/j.jad.2016.04.049

Vinkers CH, Joëls M, Milaneschi Y, Kahn RS, Penninx BW, Boks MP (2014) Stress exposure across the life span cumulatively increases depression risk and is moderated by neuroticism. Depress Anxiety 31(9):737–745. https://doi.org/10.1002/da.22262

Vorhees CV, Williams MT (2006) Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat Protoc 1(2):848–858. https://doi.org/10.1038/nprot.2006.116

W.H.O. (2012) Depression: a global public health concern. WHO, Geneva

Wang S, Blazer DG (2015) Depression and cognition in the elderly. Annu Rev Clin Psychol 11(1):331–360. https://doi.org/10.1146/annurev-clinpsy-032814-112828

Wang C, Guo J, Guo R (2017) Effect of XingPiJieYu decoction on spatial learning and memory and cAMP-PKA-CREB-BDNF pathway in rat model of depression through chronic unpredictable stress. BMC Complement Altern Med 17(1):73

Wong EY, Herbert J (2005) Roles of mineralocorticoid and glucocorticoid receptors in the regulation of progenitor proliferation in the adult hippocampus. Eur J Neurosci 22(4):785–792. https://doi.org/10.1111/j.1460-9568.2005.04277.x

Yau SY, Li A, Tong JB, Bostrom C, Christie BR, Lee T, So KF (2016) Chronic corticosterone administration reduces dendritic complexity in mature, but not young granule cells in the rat dentate gyrus. Restor Neurol Neurosci 34(5):849–857. https://doi.org/10.3233/RNN-160662

Zoladz PR, Fleshner M, Diamond DM (2012) Psychosocial animal model of PTSD produces a long-lasting traumatic memory, an increase in general anxiety and PTSD-like glucocorticoid abnormalities. Psychoneuroendocrinology 37(9):1531–1545. https://doi.org/10.1016/j.psyneuen.2012.02.007