Pharmacology, Biochemistry and Behavior 97 (2010) 213–221

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Comparison of the antidepressant sertraline on differential depression-like behaviors elicited by restraint stress and repeated corticosterone administration

J.L. Ulloa [a,1], P. Castañeda [a,1], C. Berríos [a,1], G. Díaz-Veliz [b], S. Mora [b], J.A. Bravo [a,2], K. Araneda [a], C. Menares [a], P. Morales [b], J.L. Fiedler [a,*]

[a] Laboratory of Neuroplasticity and Neurogenetics, Department of Biochemistry and Molecular Biology, Faculty of Chemistry and Pharmaceutical Sciences, Universidad de Chile, P.O. BOX. 233, Santiago 1, Chile
[b] ICBM, School of Medicine, Universidad de Chile, Santiago, Chile

ARTICLE INFO

Article history:
Received 16 February 2010
Received in revised form 27 July 2010
Accepted 2 August 2010
Available online 10 August 2010

Keywords:
Stress
Restraint
Depression
Anxiety
Behavior
Corticosterone
Antidepressant
Sertraline

ABSTRACT

Depressive disorder involves emotional, cognitive, autonomic and endocrine alterations and also evidences support the role of stress in the development of this disorder. Because the hypothalamic-pituitary-adrenal axis is involved in the stress response with a concomitant rise in plasma corticoids, the present study compares the antidepressant effects of sertraline (10 mg/kg, i.p.) on behavioral changes elicited by (i) restraint stress (2.5 h/day for 13 days) and (ii) corticosterone injections (30 mg/kg, s.c., for 13 days). Stressed animals, but not corticosterone-treated animals displayed anxiety behavior and a reduction in the acquisition of a conditioned avoidance response to 25% of control levels (8.0 ± 2.2 vs. 31.7 ± 3.2), being this effect partly sensitive to sertraline. Stressed, but not corticosterone-treated, animals displayed an increased escape failure compared with the control group ($24.6\% \pm 3.5$ vs. 1.6 ± 0.7), an effect partly prevented by sertraline treatment ($7.3\% \pm 2.0$). Both stressed rats and corticosterone-treated rats showed an increase in immobility in the forced swim test, an effect prevented by sertraline. These results suggest that the altered behaviors elicited by stress and corticosterone can be explained by neural modifications that are sensitive to the sertraline antidepressant.

1. Introduction

Major depression is one of the most prevalent forms of mood disorder and can be life-threatening due to the risk of suicide (Lecrubier, 2001; Murray et al., 1996). Moreover, major depression shows a high comorbidity with anxiety disorders (Millan, 2009; Mineka and Zinbarg, 2006). Although genetic research has revealed that the vulnerability to depression is partly heritable (Lesch, 2004), there is also strong support for the role of stress in the development and manifestation of this disorder (Caspi et al., 2003; Nestler et al., 2002). Stress is characterized by physiological changes that occur in response to novel or threatening stimuli. These changes comprise a cascade of neuroendocrine events mediated by stress systems such as the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis. Activation of the HPA axis results in the release of hypothalamic corticotropin-releasing hormone (CRH), which in turn releases pituitary adrenocorticotropin-releasing hormone (ACTH), which triggers the synthesis and secretion of adrenal glucocorticoids (cortisol in humans and corticosterone [CORT] in rodents) into the circulatory system (Brown et al., 1999). Glucocorticoids then act at target tissues to evoke physiological changes that enable an organism to deal with stressors. Normal HPA axis activation is thus essential for survival because it acts to maintain homeostasis. Nonetheless, there is a relationship between the prevalence of major depressive disorder and dysregulation of the HPA axis. Certainly, a significant percentage of depressed patients has elevated plasma cortisol levels at the usual afternoon nadir (Nasr and Gibbons, 1983), and a flattened diurnal rhythm of cortisol secretion (Deuschle et al., 1997; Wong et al., 2000). These alterations are related to impairment in the negative feedback of the HPA axis through the glucocorticoid receptor (Pariante and Miller, 2001). In line with this, the effect of tricyclic antidepressants is associated with a reduction in stress-induced HPA axis hyperactivity (Bravo et al., 2009; Pariante and Miller, 2001; Pepin et al., 1992a,b), suggesting that depressive symptoms are in part related to the rise in circulating levels of glucocorticoids that may affect brain functioning (Kim and Haller, 2007). Chronic or severe stressors, as well as high levels of corticoids, are associated with a decline in hippocampal-dependent memory (Brown et al., 2005; Fiedler et al., 2007; Lucassen et al., 2001; Magarinos et al., 1996; Pittenger and Duman, 2008). There are several lines of evidence showing that

* Corresponding author. Tel.: +56 2 9782923; fax: +56 2 737 920.
E-mail addresses: joseluisulloafulgeri@gmail.com (J.L. Ulloa), patricia.castaneda@umce.cl (P. Castañeda), claudiorogelio@gmail.com (C. Berríos), gdiaz@med.uchile.cl (G. Díaz-Veliz), smora@med.uchile.cl (S. Mora), J.Bravo@ucc.ie (J.A. Bravo), karina.araneda@gmail.com (K. Araneda), clarita_andrea07@hotmail.com (C. Menares), pmorales@med.uchile.cl (P. Morales), jfiedler@ciq.uchile.cl (J.L. Fiedler).
[1] Authors contributed equally to this work.
[2] Current address: Laboratory of Neurogastroenterology, Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland.

0091-3057/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2010.08.001

depressive disorder is associated with a significant deficit in episodic memory and learning (see (Austin et al., 2001) for review). Cognitive impairments, especially those related to hippocampal and prefrontal function, are related to cortisol levels in depressed patients (Brown et al., 2004; Hinkelmann et al., 2009; Sheline, 1996; Sheline et al., 1999). In line with this, acute and subchronic administration of corticoids in healthy volunteers produces deficits in certain tests of cognitive function sensitive to frontal lobe dysfunction (Young et al., 1999).

Animal models that display depression-like behavior accompanied by anxiety and cognitive impairments are very useful in evaluating the effects of antidepressant drugs. There are some reports of the effects of restraint stress and corticosterone administration on depressive and anxiety-like behaviors (Ardayfio and Kim, 2006; Bondi et al., 2008; David et al., 2009; Gourley et al., 2008a; Gourley and Taylor, 2009; Gourley et al., 2008b; Gregus et al., 2005; Grippo et al., 2005; Johnson et al., 2006; Marks et al., 2009; Murray et al., 2008). However, a subset of these studies explored the effect of antidepressant drugs administrated prior, during or few days after the stress procedure or corticosterone injection (Bondi et al., 2008; Bravo et al., 2009; David et al., 2009; Gourley et al., 2008a; Gourley et al., 2008b). The stress manipulation followed by antidepressant treatment probably mimics the clinical condition of depressed patients under antidepressant treatments and the reversal of the stress-induced cognitive impairment and neurochemical dysfunctions should occur during the treatment. On the other hand, the administration of antidepressant minutes prior to the stress protocol or CORT administration should allow the dissection of the behavioral responses to stress or CORT which are sensitive to antidepressant treatment. Moreover, the comparison of antidepressant drug effects on stressed animals and CORT treated animals might help to elucidate whether some symptoms in depressive illness are related to stress and/or to the rise in glucocorticoid levels and whether depression-like behavior induced by stress and CORT administration is equally sensitive to antidepressants.

The aim of the present study was to compare in rats the effects of 13 days of restraint stress (2.5 h/day) with 13 days of repeated CORT injections, in terms of anxiety-like and depression-like behaviors. To analyze whether an antidepressant treatment modifies these behaviors, we also treated rats during the stress period and CORT administration with sertraline. This selective serotonin reuptake inhibitor (SSRI) antidepressant has shown a higher efficacy (fluoxetine) and tolerability than other antidepressants (amitriptyline, imipramine, paroxetine and mirtazapine) (Cipriani et al., 2010). Moreover, the normalization of the HPA axis function and clinical recovery are observed during some antidepressant treatments; however it is likely that these effects are either directly or indirectly related.

2. Methods

2.1. Subjects

Adult male Sprague–Dawley rats (200–250 g) were housed in groups of four per cage at a temperature (22–23 °C) and humidity (55–65%) controlled room with a standard light:dark cycle (12 h:12 h). Food (standard rat chow) and water were freely provided, except when restraint stress was applied. Food and water intake was monitored and all rats were weighed daily. Blind observations were used throughout all behavioral testing, which was carried out from 13:00–17:00 h in a quiet room. Efforts were made to minimize both the number of animals used and their suffering. All procedures were approved by the Ethical Committee of the Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile and were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2. General procedures

The rats were handled once per day for 7 days prior to initiating experimental procedures. The handling procedure consisted of picking up the rat by its body, then weighing it and finally returning it to its home cage. Rats were randomly assigned to weight-matched groups that received one of the following six treatments: (i) unstressed animals injected i.p. every day for 13 days with saline (0.9% NaCl) or vehicle (propylene glycol) (CONTROL group, n = 13), (ii) restraint-stressed animals injected i.p. daily with saline (REST group, n = 8) prior to restraint protocol for 13 days, (iii) unstressed control injected i.p. every day for 13 days with 10 mg/kg sertraline (SERT group, n = 14), (iv) restraint-stressed animals injected i.p. daily with saline 10 mg/kg sertraline (REST/SERT group, n = 7) prior to restraint protocol for 13 days, (v) animals injected s.c. every day for 13 days with 30 mg/kg/day corticosterone (Sigma-Aldrich, St Louis MO) suspended in propylene glycol (CORT group, n = 7) and (vi) corticosterone-treated animals also injected i.p. with 10 mg/kg sertraline for 13 days (CORT/SERT group, n = 7). All treatment procedures were carried out during the light phase of the light:dark cycle and antidepressant administration or vehicle were carried out just prior to the injection of corticosterone or stress procedure. Because animals injected with saline or propylene glycol showed no differences in behavioral and physiological parameters, these data were pooled and the two groups were together considered control animals.

All animals were restrained at the same time, and the injections with saline or antidepressants were performed prior to the stress procedure. The restraint stress procedure was carried out between 9:00 and 12:00 h in a different room, as previously described (Bravo et al., 2009). In brief, rats were placed in a transparent plexiglass tube (25 × 8 cm) for 2.5 h a day for 13 consecutive days. The length of each restrainer was adjusted to limit limb movements. Following restraint, rats were returned to their home cages. Unstressed animals were injected with a vehicle (saline or propylene glycol) or sertraline and left undisturbed in their home cage in another room. The following parameters were measured to monitor the effects of the stress and corticosterone treatment: percentage of body weight gain (daily weight determination × 100/weight at the beginning of experiment), and adrenal weight and serum corticosterone levels (at the end point of the experiment).

2.3. Behavioral testing

During the 13th and 14th days of treatment, all rats were submitted to the forced swim test (FST) as described below. Twenty-four hours after that, animals were injected (saline, sertraline or corticosterone) and, 30 min later, submitted to elevated plus-maze and active avoidance conditioning tests as previously described (Bravo et al., 2009). All behavioral testing occurred between 10:00 and 14:00 and were conducted by two highly trained observers who were blind to the treatments.

2.3.1. Forced swimming test (FST)

The forced swimming test (FST) is a behavioral test used frequently to evaluate the efficacy of potential antidepressant drugs in rats. Immersion of rats in water for an extended period of time produces a characteristic behavior of immobility (learned helplessness) (Lucki, 1997). Antidepressant treatments decrease the immobility behavior, a change accompanied by an increase in the escape response (climbing plus swimming) (Lucki, 1997). This test was performed according to Lucki (Lucki, 1997). A transparent Plexiglas cylinder (50 cm high × 20 cm wide) was filled up to a depth of 40 cm with water at 24 °C. At this depth, rats could not touch the bottom of the cylinder with their tails or hind limbs. On day 13 after 5 h of treatments, all groups of animals were trained for 15 min by placing

them in the water-filled cylinder. On day 14, animals were subjected to 5 min of forced swim, and escape behaviors (climbing and swimming) were determined. Climbing was defined as upward-directed movements of the forepaws along the side of the swim chamber, while swimming was considered as movements throughout the swim chamber including crossing into another quadrant. Immobility behavior was calculated as the length of time in which the animal did not show escape responses (e.g., total time of the test minus time spent in climbing and swimming behaviors). After the test, the rat was removed from the tank, dried with a towel and placed back in its home cage. The water in the swim tank was changed between rats.

2.3.2. Elevated plus-maze

The elevated plus-maze is a widely used behavioral test to assess anxiety and the anxiogenic or anxiolytic effects of pharmacological agents (Pellow et al., 1985; Walf and Frye, 2007). Animals displaying anxiety-like behaviors in the elevated plus-maze usually show reductions both in the number of entries and in the time spent in the open arms, along with an increase in the amount of time spent in the closed arms. On day 15, the elevated plus-maze test was conducted. Animals were injected with sertraline or corticosterone and then individually placed in an elevated plus-maze. This apparatus consisted of two open arms (50×10 cm each), two closed arms (50×10×20 cm each) and a central platform (10×10 cm), arranged in a way such that the two arms of each type were opposite to each other. The maze was made from black Plexiglas and elevated 100 cm above the floor. At the beginning of each trial, animals were placed at the centre of the maze, facing a closed arm. During a 5-min test period, the following was recorded: a) number of open arm entries, b) number of closed arm entries, c) time spent in open arms, and d) time spent in closed arms. Entry into an arm was defined as the animal placing all four limbs onto the arm. Open to total ratios (OTR) for arm entries was calculated as an indicator of open arm exploration and defined as the percentage of entries into open arms relative to the total entries (open + closed). Also OTR as a function of time was calculated as the percentage of time spent in open arms relative to the total time (open + closed). Total arm entries were analyzed as a measure of non-specific change in locomotor activity. The maze was cleaned thoroughly with a 5% ethanol solution after each animal.

2.3.3. Active avoidance conditioning

The active avoidance task measures the ability of an animal to avoid an aversive event and provides a way to assess associative learning and memory. Active avoidance conditioning was performed as previously described (Bravo et al., 2009; Mora and Díaz-Véliz, 1993) after the elevated plus-maze assay. Briefly, each rat was individually placed in a two-way shuttle box (Lafayette Instrument Co., Lafayette, IN) composed of two stainless steel modular testing units. Each unit was equipped with an 18-bar insulated shock grid floor, two 28 V DC lights and a tone generator (Mallory Sonalert 2800 Hz, Lafayette Instrument Co., Lafayette, IN). Electric shocks were transmitted to the grid floor by a Master shock supply (Lafayette Instrument Co., Lafayette, IN). After a 5 min period of habituation to the shuttle box, the rats were subjected to 50 avoidance trials (inter-trial interval: 30 s). Each trial consisted of the presentation of a tone (conditioning stimulus) that after 5 s was overlapped with a 0.20-mA foot-shock (unconditioned stimulus) until the animal escaped to the opposite chamber; the maximum shock duration was 10 s. A conditioned avoidance response (CAR) was defined as crossing to the opposite chamber within the first 5 s (tone alone). If no escape response to the shock occurred within 10 s, the shock and tone-conditioning stimulus were discontinued and an escape failure (EF) was recorded.

2.4. Blood sampling and tissue extraction

Animals were killed by decapitation one day after behavioral measurements (i.e., 48 h after the last session of stress protocol or 24 h the last injection of corticosterone) between 9:00 and 11:00. Trunk blood samples were collected for the determination of serum corticosterone levels. Special care was taken to avoid pre-decapitation stress—while decapitation took place the other animals were left outside the room and handled for a few minutes prior to sampling. Blood was then centrifuged at 4000 g for 10 min, and serum was collected and stored at −20 °C. The adrenals were dissected and weighed.

2.5. Determination of serum corticosterone levels

Hormone levels were carried out using the corticosterone-correlate-EIA™ kit, (Assay designs, Inc. Michigan, USA) according to the manufacturer's instructions. The assay has a detection limit of <0.003 μg/dL with intra- and inter-assay variations of 8%.

2.6. Data analysis

All the values are expressed as the mean ± SEM. The data were analyzed with GraphPad (GraphPad software Inc., San Diego, CA, USA). First, we evaluated the normality of data using Kolmogorov–Smirnov test and Shapiro–Wilk normality test, and the Bartlett's test for equal variances. In order to compare differences in groups, one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test was done. The effect of treatments on weight was analyzed by two-way ANOVA for repeated measurements, followed by Bonferroni's post hoc test. In both cases, statistical differences between groups were considered significant when p was equal to or less than 0.05.

3. Results

3.1. Effects of chronic restraint on body weight gain and corticosterone levels

Because preclinical studies have shown that stress affects physiological parameters related to HPA activation (Dhabhar et al., 1997), we tested whether repeated restraint stress (REST group) and corticosterone administration (CORT group) promote changes in body weight gain (difference between daily weight and starting weight) and serum corticosterone levels. Fig. 1A shows body weight gain values during the 13 days of treatments. The REST and CORT groups gained less weight over the treatment period than did controls. Indeed, ANOVA tests for repeated measurements revealed significant main effects of time ($F(13, 663) = 83.63$, $p<0.0001$) and of treatment ($F(5, 663) = 16.63$, $p<0.0001$) and a significant time × treatment interaction ($F(65, 663) = 7.21$, $p<0.0001$). Post hoc tests showed a significant reduction in weight gain after 7 days for the REST group in comparison with the CONTROL group ($p<0.05$ day 7, $p<0.01$ day 8, $p<0.001$ days 9–14) (Fig. 1A). Similarly, the CORT group showed a reduction in weight gain in relation to controls, which was statistically significant after 6 days of treatment ($p<0.01$ days 6–7; $p<0.001$ days 8–14). Administration of sertraline to unstressed (SERT), restraint-stressed (REST/SERT) or CORT groups produced a significant reduction in weight gain as compared with their respective controls throughout the experimental period, these differences being significant after 3 days of drug administration ($p<0.05$; Fig. 1A). These differences can also be clearly observed as a total change in weight gain expressed as percentage of starting weight (Fig. 1B). ANOVA showed a significant effect of treatment ($F(5, 56) = 10.04$, $p<0.0001$) on body weight gain (CONTROL vs. REST $p<0.0001$; CONTROL vs. CORT $p<0.0001$).

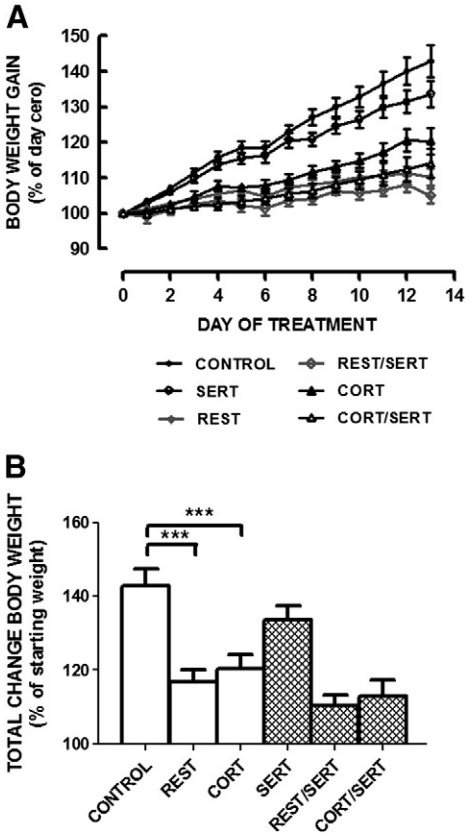

Fig. 1. Effect of repeated restraint stress and corticosterone injections on body weight gain. Animals were either restraint-stressed (2.5 h/day) (REST group) or corticosterone-treated (30 mg/kg/day) (CORT group) for 13 consecutive days. Other animals subject to these treatments were also injected with sertraline (10 mg/kg/day) (REST/SERT and CORT/SERT groups). Control animals were injected with saline (CONTROL group) or with sertraline (SERT group). (A) Data represent the mean ± SEM for each group during the days of treatments. Comparisons were made by two-way ANOVA for repeated measurements followed by the Bonferroni post hoc test. REST vs. CONTROL: *$p<0.05$ day 7, **$p<0.01$ day 8, ***$p<0.001$ days 9–14. CORT vs. CONTROL: $^{++}p<0.01$ days 6–7, $^{+++}p<0.001$ days 8–14. (B) Data represent the mean ± SEM of the total percentage change of weight in each group. REST vs. CONTROL: ***$p<0.0001$, CORT vs. CONTROL: ***$p<0.0001$.

Changes in adrenal weight and serum corticosterone levels were determined 48 h (9:00–11:00) after the last treatment. Table 1 shows that treatments promote differences in adrenal gland weight. ANOVA showed a significant effect of treatment ($F(5, 53) = 16.95$, $p<0.0001$) on adrenal weight. Pos hoc analysis showed that restraint stress promotes a significant increase in adrenal gland weight (30%) in comparison with controls ($p<0.0001$), an effect insensitive to sertraline administration. In contrast, the CORT group showed a decrease in the adrenal gland weight (49%) compared with controls ($p<0.0001$) (Table 1). Because stress and corticosterone treatments induced a decrease in weight gain, the adrenal weights were not corrected for body weight. No significant effect on corticosterone levels was observed after treatment in any of the experimental groups (($F(5, 59) = 0.794$, $p = 0.56$) (Table 1).

Table 1
Effect of restraint stress and CORT administration on adrenal weight and CORT serum levels.

Treatment	Adrenal weight (mg)	CORT (μg/dL)
Control	20.47 ± 1.22^{a}	2.02 ± 0.71
SERT (10 mg/Kg)	24.86 ± 1.73^{b}	3.99 ± 2.15
REST	26.92 ± 1.47^{c}	6.14 ± 1.80
REST/SERT	25.46 ± 1.21	4.68 ± 1.38
CORT (30 mg/Kg/)	9.95 ± 0.81^{d}	6.31 ± 2.35
CORT/SERT	11.17 ± 0.53	5.24 ± 2.00

Data were analyzed with ANOVA followed Newman–Keuls Multiple Comparison Test. Adrenal weight, ANOVA $p<0.0001$, $^{a,b}p<0.05$, $^{a,c}p<0.01$, and ^{a,d} $p<0.001$. Data represent the mean ± SD.

3.2. Effect of repeated restraint stress or corticosterone injections on behavior displayed in the forced swimming test and its modification by sertraline administration

Fig. 2 shows the time spent in immobility or in active behaviors scored during 300 s of testing. The CONTROL group spent similar lengths of time in immobility, climbing and swimming behaviors, showing active behaviors for approximately 60% of the time (Fig. 2). An overall one-way ANOVA revealed a significant effect of treatment on immobility ($F(5, 52) = 13.19$, $p<0.0001$). The REST and CORT groups spent significantly more time in immobility (126.8 ± 7.7 s and 151 ± 5.99 s, respectively) during the FST than did the CONTROL group (83.6 ± 6.51 s) (Fig. 2A). These observations were correlated with a modification in active behaviors ($F(5, 52) = 13.19$, $p<0.0001$). The REST and CORT groups showed similar climbing behavior to CONTROL (Fig. 2B). Although the REST group showed time spent in swimming similar to the CONTROL group, the increase in immobility observed in this group was returned to control values by sertraline administration (REST/SERT, $p<0.001$), an effect that occurs at the expense of an increase in swimming behavior ($p<0.01$, Fig. 2C). In contrast, the immobility observed in the CORT group was correlated with a significant reduction in swimming behavior ($p<0.0001$; Fig. 2C). The CORT/SERT group showed a reduction in the duration of immobility behavior (Fig. 2A). These differences in active behavior compared with the CORT group were statistically significant when time spent in both behaviors were considered (climbing plus swimming, $p<0.001$).

3.3. Effect of repeated restraint stress or corticosterone injections on anxiety behavior evaluated by elevated plus-maze: modification by SERT administration

In relation to the effect of treatments on anxiety-like behavior assessed in the elevated plus-maze, ANOVA revealed a significant main effect of treatments ($F(5, 50) = 2.8$, $p = 0.026$) on the open to total ratios (OTR) for arm entries (Fig. 3A). In contrast to the CORT group, the REST group showed a significant reduction in OTR for arm entries ($p<0.001$, Fig. 3A). Moreover, the REST animals injected with sertraline showed a percentage of OTR for arm entries similar to the CONTROL group; however this difference was not statistically significant from the REST group. In addition, we observe a significant effect of treatments on OTR as a function of time ($F(5, 50) = 2.4$, $p<0.05$). Post hoc comparison indicates that the REST group spent significantly less time in open arms (Fig. 3B, $p<0.001$), and this effect was not prevented by the antidepressant (REST/SERT group). As shown in Fig. 3, antidepressant administration to SERT and SERT/CORT groups did not elicit anxiolytic or anxiogenic effects. Moreover, animals under different treatments showed similar numbers of arm entries suggesting that treatments did not alter locomotor activity (Fig. 3C). These data indicate that restraint promotes anxiety behavior that is insensitive to sertraline administration.

3.4. Effect of repeated restraint stress or corticosterone injections on conditioned avoidance responses (CAR) and escape failures (EF): modification by sertraline administration

ANOVA showed that there was a significant effect of treatment ($F(5, 49) = 11.26$, $p<0.0001$) on CAR ($F(5, 49) \equiv = 11.26$, $p<0.0001$). Fig. 4A shows a significant decrease of CAR in the REST group (8 ± 1.7% $p<0.001$) compared with the CONTROL group (31.7 ± 3.2%). In the SERT group we observed that daily

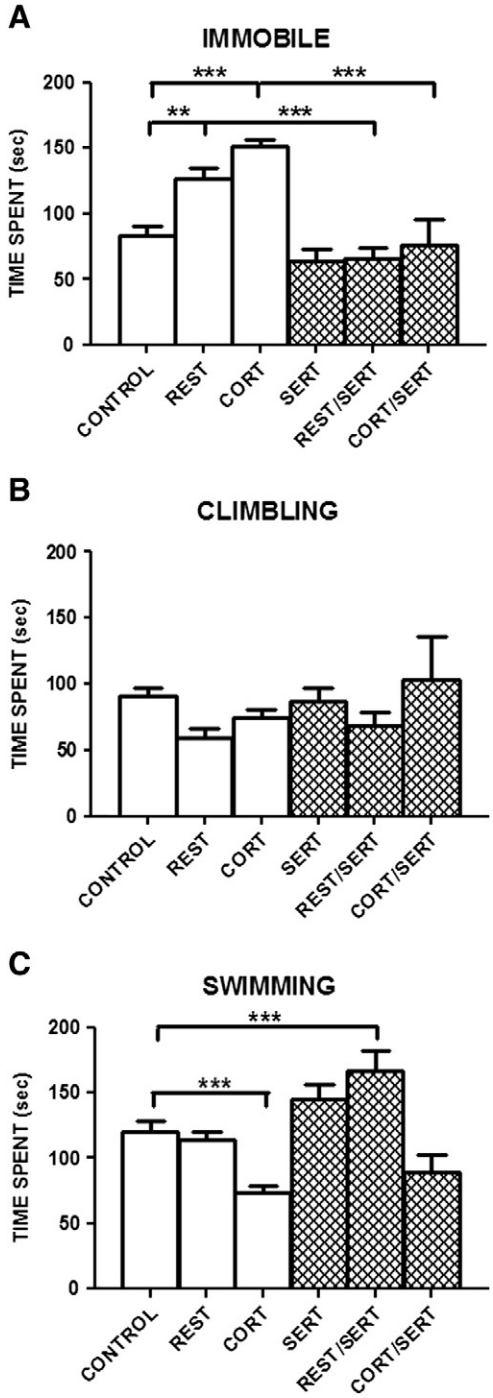

Fig. 2. Effect of repeated restraint stress or corticosterone injections on behaviors displayed in the forced swim test and its modification by sertraline administration. Active behaviors (climbing and swimming) were measured for 5 min and data represent mean ± SEM of time. (A) Time spent immobile. REST and CORT administration increased the time spent in immobility, behavior prevented by SERT administration. (B) Time spent climbing. Treatment did not elicit significant variation in the time spent climbing. (C) Time spent swimming. REST did not elicit variation in time spent swimming, but the administration of SERT to REST rats increases the swimming behavior. In contrast, CORT administration reduced time spent swimming, an effect not prevented by SERT. Nonetheless, CORT/SERT rats showed an increase in both active behaviors (climbing + swimming, $p<0.01$ vs. REST). Comparisons were made by one-way ANOVA followed by the Bonferroni post hoc test. $^{**}p<0.001$, $^{***}p<0.0001$.

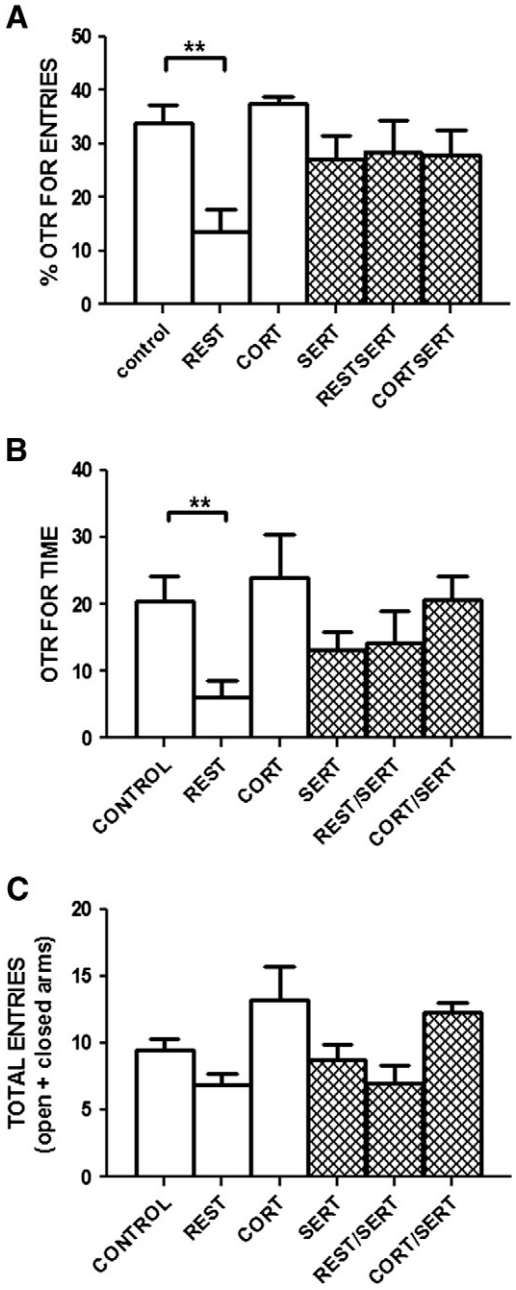

Fig. 3. Effect of repeated restraint stress or corticosterone injections on anxiety behavior evaluated by elevated plus-maze and its modification by sertraline administration. (A) In the REST group, but not in the CORT or SERT groups, anxiety behaviors were observed as a reduction in open to total ratios (OTR) for entries. (B) The REST, but not the CORT or SERT groups, displayed reduction in OTR as a function of time. (C) Treatments did not elicit modifications in total entries (open + closed arms). The data represent the mean ± SEM. Comparisons were made by one-way ANOVA followed by the Bonferroni post hoc test. $^{**}p<0.001$.

antidepressant administration promoted a reduction by nearly 50% in CAR observed in the CONTROL group (18.0 ± 2.3%, vs. 31.7 ± 3.2% $p<0.05$) (Fig. 4A). In contrast, the REST/SERT group did not display the reduction in CAR to the percentage observed in the SERT group (20.3 ± 2.9% vs. 18 ± 2.3%) or the CONTROL group. The CORT and CORT/SERT groups displayed CAR similar to that of CONTROL (27 ± 4.7%, 41.7 ± 5.0, 31.7 ± 3.2% respectively, Fig. 4A).

Fig. 4B shows the EF for all groups. ANOVA shows significant differences for the experimental conditions (F (5, 49) = 11.26, $p<0.0001$). The CONTROL group showed a 1.6% ± 0.7 of EF, and antidepressant treatment in the SERT group increased EF by nearly 5 fold (7.4% ± 1.3, $p<0.05$). The REST group showed significant increase in EF (24.6% ± 3.5, $p<0.001$) compared with the CONTROL group, an effect prevented by sertraline treatment (7.3% ± 2.0, $p<0.001$) (Fig. 4B). In contrast, the CORT and CORT/SERT groups displayed

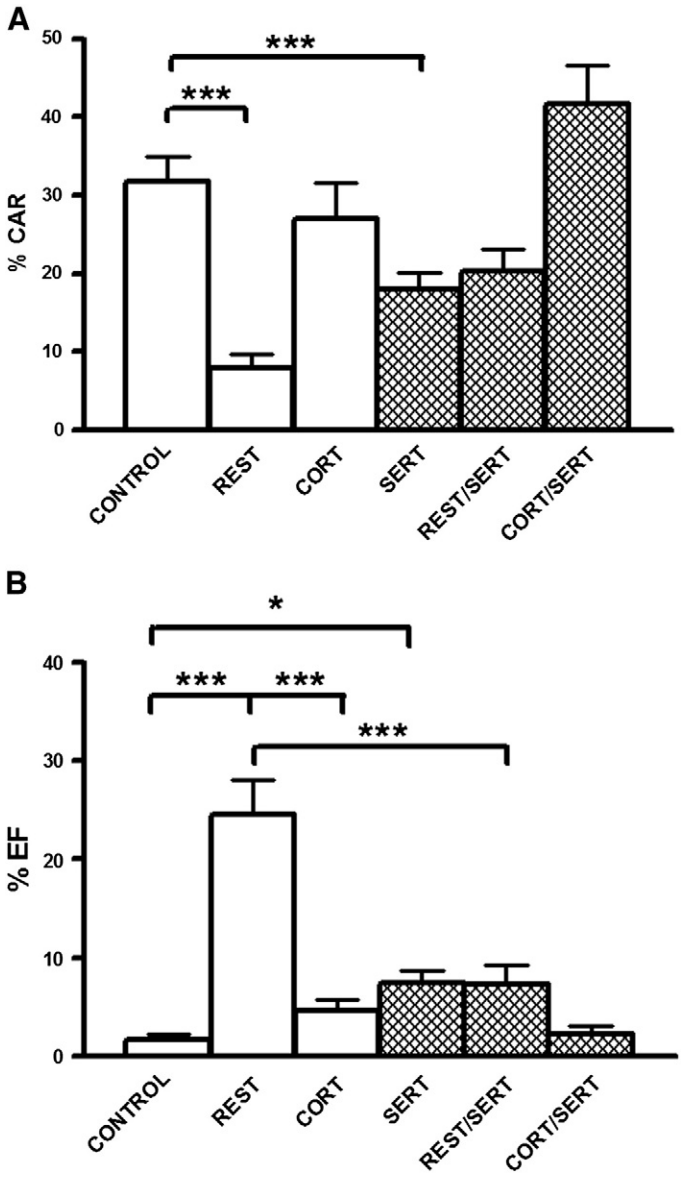

Fig. 4. Effect of repeated restraint stress or corticosterone injections on conditioned avoidance responses and escape failures and their modification by sertraline administration. (A) Conditioned avoidance response (CAR) was defined as a crossing to the opposite chamber within the first 5 s during the active avoidance conditioning. The REST but not the CORT group displayed reduction in CAR compared with the CONTROL group. Antidepressant administration to the SERT group reduces the CAR, but in the REST/SERT group the drug prevents the reduction of CAR. The CORT and CORT/SERT groups displayed CAR similar to that of the CONTROL group. (B) Escape failure (EF) was defined as no escape response to the shock within 10 s during the active avoidance conditioning. Antidepressant administration increases the EF in the SERT group but prevents the increase of EF in the REST group. Antidepressant administration does not modify EF in the CORT/SERT group. The data represent mean ± SEM. Comparisons were made by one-way ANOVA followed by the Bonferroni post hoc test. $^{***}p<0.001$, $^{*}p<0.05$.

EF similar to that of the CONTROL group (4.7% ± 1.2; 2.3% ± 0.8 and 1.6% ± 0.7, respectively).

4. Discussion

The present study compared the effect of repeated restraint stress and repeated corticosterone injections on anxiety- and depression-like behaviors. Furthermore, we evaluated the effect of chronic treatment with the SSRI sertraline on both experimental models. The results obtained in this study can be summarized as follows. First, REST and CORT groups gained less weight over 13 days of treatment than the vehicle-treated rats, an effect related to adrenal weight. Second, restraint induced an increase in adrenal gland weight compared with the CONTROL group, in contrast to the reduction observed in animals under corticosterone administration. Third, repeated corticosterone administration and restraint stress increased the time spent in immobility in the FST, considered a depression-like behavior (Cryan et al., 2002), a behavior that was fully sensitive to antidepressant treatment. Fourth, the REST group but not the CORT group showed an increase in anxiety-like behavior that was not prevented by sertraline administration. Fifth, the REST group but not the CORT group showed a reduction in associative learning accompanied by an increase in escape failures, effects partly prevented by sertraline administration.

Several lines of evidence have indicated that chronically stressed animals often exhibit suppressed or decreased HPA responses upon re-exposure to the same stressor including restraint. This habituation has been observed with various stress paradigms, including restraint stress (Cole et al., 2000; Dhabhar et al., 1997; Girotti et al., 2006; Melia et al., 1994). Viau et al, have proposed a lack of homogeneity among stress-responsive parvicellular neurosecretory neurons, suggesting that distinct complements of CRH cells may be preferentially involved

in initiating HPA responses to acute stress and sustaining the chronic condition (Viau and Sawchenko, 2002). Even though we did not observe changes in corticosterone levels 48 h after the last restraint session, the stress procedure used in the current study increased the adrenal gland weight with a concomitant reduction in weight gain, suggesting an increase in adrenal hormone production during daily stress procedure. Similarly, corticosterone-treated animals showed a reduction in a 50% of adrenal weight. This variation suggests a reduction in glucocorticoid adrenal synthesis. Murray et al. (Murray et al., 2008) showed that 1–2 h after i.p. administration of 40 mg/kg of corticosterone, the hormone in plasma increased in the range of 13 μg/dL. Also, they showed that the chronic administration of corticosterone reduced adrenal weight; an effect similar to our data. This finding is in accordance with the notion that chronic corticosterone administration blunts the HPA axis activity (Gadek-Michalska and Bugajski, 2003). Although we did not observe an elevation in corticosterone serum levels in the CORT group, we did observe a significant decrease in adrenal weight and body weight gain, both parameters indicative of hormone elevation during the period of treatment.

Several investigations have suggested that in major depression there are functional alterations of the prefrontal cortex and the limbic system, both of which are associated with cognitive dysfunction and anxiety (Millan, 2009; Mineka and Zinbarg, 2006). In the present study, anxiety-like behavior was observed in the REST group but not in the CORT group. Studies using similar treatments showed no significant effect on anxiety in the open-field test (Fernandes et al., 1997; Gregus et al., 2005; Kalynchuk et al., 2004), a discrepancy that could be related to the quality and severity of the stressor used. Although the REST/SERT animals displayed OTR for arm entries similar to CONTROL, we observed that sertraline administration did not prevent anxiety-like behaviors in the REST animals. In agreement with our results, it has been shown that chronic unpredictable stress elicits anxiety-like behaviors that can be prevented by desipramine, a norepinephrine reuptake inhibitor, but not by an SSRI like escitalopram (Bondi et al., 2008). Thus, it is probable that the anxious behavior elicited by restraint is insensitive to SSRI drugs. These data also suggest that the anxiogenic component observed in stressed animals probably is not related to glucocorticoid levels. Nonetheless, other behavioral test to observe anxiety component and experiments with higher doses and length of the corticosterone treatment need be explored in order to confirm this notion.

It is important to emphasize that stress triggers not only endocrine responses but also activates the limbic system (Ulrich-Lai and Herman, 2009). Interestingly, we observed differences in the effects of restraint and corticosterone treatments on associative learning assessed in the active avoidance task. The REST group but not the CORT group showed a reduction in the acquisition of CAR and an increase in EF. The stress-induced cognitive impairment was partly prevented by sertraline administration. Moreover, the percentage of CAR or EF observed in the REST/SERT group was similar to those observed in the SERT group. These observations reinforce the idea that sertraline treatment to some extent impairs associative learning and suggests that the appropriate antidepressant action of sertraline might depend on the presence of an altered neuronal substrate.

Glucocorticoids are not essential for the formation of memories, but evidence shows that they are potent modulators of cognitive processes such as learning, memory and retrieval (Joels et al., 2006). In particular, it has been documented that a stress response induced by learning a new task, with the consequent release of corticoids, is critically involved in memory consolidation processes (de Kloet et al., 1999). However, when high levels of glucocorticoids are applied outside the context of learning, they may interfere with memory acquisition and retrieval, facilitating memory extinction (Joels et al., 2006). The effect of stress on CAR might be explained by a rise of corticosterone in a period that was not coincident with the conditioning, in contrast to the CORT group to which the hormone was administrated prior to the test. Surprisingly, sertraline administration to the CORT/SERT group improved CAR in comparison with the SERT group, suggesting that impairment of associative learning elicited by sertraline alone could be counteracted by corticosterone administration. In summary, restraint stress and corticosterone administration have differing effects on the acquisition of CAR, and sertraline produces a dissimilar effect on restraint stress and chronic corticosterone injection paradigms.

The immobility observed in the FST has been used to infer learned helplessness as depression-like behavior in rats (Cryan and Holmes, 2005). Moreover, the prevention of this behavior is commonly used to determine the efficacy of several antidepressant treatments in animals (Porsolt et al., 1977). It has been shown that selective norepinephrine reuptake inhibitors, such as desipramine, increase climbing without altering swimming (Bravo et al., 2009; Detke et al., 1997; Lucki, 1997). In contrast SSRIs, such as fluoxetine, increase swimming without affecting climbing, and drugs with dual effects at norepinephrine and serotonin transporters increase both patterns of active behavior (Detke et al., 1995; Reneric and Lucki, 1998). Based on these effects, swimming behavior has been related to the serotonergic system, and climbing behavior has been related to the noradrenergic system (Cryan et al., 2005). In the FST we observed that the REST group spent more time immobile and showed reduced climbing and swimming behaviors suggesting alterations in norepinephrine and serotonin neurotransmission. In contrast, the CORT group increased immobility by reducing swimming time in comparison with the CONTROL group, an effect similar to that reported by others (Gregus et al., 2005; Johnson et al., 2006; Kalynchuk et al., 2004). This finding suggests that corticosterone administration can modify the serotonin neurotransmission. In line with this, it has been reported that exposure to glucocorticoid attenuates serotonin responses in rat hippocampal neurons (Karten et al., 1999) and enhance serotonin uptake both *in vitro* and *in vivo* by increasing the expression of the serotonin transporter (Tafet et al., 2001a; Tafet et al., 2001b). On the other hand, it has been evaluated the effect of flat glucocorticoid rhythm on serotonin overflow by frontal cortex microdialysis (Gartside et al, 2003). This study showed an increase in extracellular serotonin in comparison to control animals but the mechanism involved is still unclear (Gartside et al, 2003). Additionally, it should be important to evaluate whether this effect is related or not to the flattened corticosterone rhythm.

Sertraline administration to stressed animals prevents stress-induced immobility by increasing swimming behavior. In contrast, antidepressant administration to the CORT/SERT group promotes an increase in both swimming and climbing behaviors. The present study failed to show an increase in active behaviors in FST following chronic sertraline administration. However, our data are in line with the antidepressant-like property of sertraline in both the REST and CORT groups. These results suggest that sertraline is able to exert its antidepressant effect only on altered brain substrates, i.e., under neural modifications elicited by stress and corticosterone administration in the present work.

These modifications could be related to changes in the firing of 5-HT neurons (Bambico et al., 2009), expression and responsiveness of serotonergic receptors (Cyr et al., 2001; Deakin, 1988; Fernandes et al., 1997; Filipenko et al., 2002; Maj and Moryl, 1992) or in the expression of serotonin transporter (Zhao et al., 2008). Further investigation on altered neuronal substrates in restraint-stressed and corticosterone-treated animals should provide new insights on molecular mechanisms not addressed in the present work.

In this work, we show that chronic restraint stress produces cognitive deficits and anxiety-like behaviors in rats similar to those seen in patients with major depressive disorder (Hinkelmann et al., 2009), and that cognitive impairment is prevented by sertraline treatment. The present study favors the notion that restraint stress

evokes different modifications; ones related to the raise in corticosterone, and others probably due to context-induced stress on emotional response as evidenced in the behavioral tests. This could explain the behavioral differences observed between stressed and corticosterone-treated animals. Also, we can emphasize that not all of the neural changes induced by restraint stress can be elicited by chronic corticosterone administration. Moreover, the antidepressant effect of sertraline can only be observed when the behavioral test assesses the functionality of an area sensitive to both restraint stress and corticosterone administration.

Finally, based on the current behavioral tests, we can emphasize that not all of the neural changes induced by restraint stress can be elicited by chronic corticosterone administration. Moreover, the antidepressant effect of sertraline can only be observed when the behavioral test assesses the functionality of an area sensitive to both restraint stress and corticosterone administration. Further investigation on altered neuronal substrates in restraint-stressed and corticosterone-treated animals should provide new insights to understand molecular mechanisms not addressed in the present study potentially useful in managing human disorders such as depressive disorder.

Acknowledgments

This study was supported by ENL-07/11 Universidad de Chile and FONDECYT 104-0937 and 108-0489.

References

Ardayfio P, Kim KS. Anxiogenic-like effect of chronic corticosterone in the light–dark emergence task in mice. Behav Neurosci 2006;120:249–56.
Austin MP, Mitchell P, Goodwin GM. Cognitive deficits in depression: possible implications for functional neuropathology. Br J Psychiatry 2001;178:200–6.
Bambico FR, Nguyen NT, Gobbi G. Decline in serotonergic firing activity and desensitization of 5-HT1A autoreceptors after chronic unpredictable stress. Eur Neuropsychopharmacol 2009;19:215–28.
Bondi CO, Rodriguez G, Gould GG, Frazer A, Morilak DA. Chronic unpredictable stress induces a cognitive deficit and anxiety-like behavior in rats that is prevented by chronic antidepressant drug treatment. Neuropsychopharmacol 2008;33:320–31.
Bravo JA, Diaz-Veliz G, Mora S, Ulloa JL, Berthoud VM, Morales P, et al. Desipramine prevents stress-induced changes in depressive-like behavior and hippocampal markers of neuroprotection. Behav Pharmacol 2009;20:273–85.
Brown ES, Varghese FP, McEwen BS. Association of depression with medical illness: does cortisol play a role? Biol Psychiatry 2004;55:1–9.
Brown ES, Rush AJ, McEwen BS. Hippocampal remodeling and damage by corticosteroids: implications for mood disorders. Neuropsychopharmacology 1999;21:474–84.
Brown SM, Henning S, Wellman CL. Mild, short-term stress alters dendritic morphology in rat medial prefrontal cortex. Cereb Cortex 2005;15:1714–22.
Caspi A, Sugdin K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, Poulton R. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 2003;301:386–9.
Cipriani A, La Ferla T, Furukawa TA, Signoretti A, Nakagawa A, Churchill R, McGuire H, Barbui C. Sertraline versus other antidepressive agents for depression. Cochrane Database Syst Rev. 2010 CD006117.
Cole MA, Kalman BA, Pace TW, Topczewski F, Lowrey MJ, Spencer RL. Selective blockade of the mineralocorticoid receptor impairs hypothalamic-pituitary-adrenal axis expression of habituation. J Neuroendocrinol 2000;12:1034-42.
Cryan JF, Holmes A. The ascent of mouse: advances in modelling human depression and anxiety. Nat Rev Drug Discov 2005;4:775–90.
Cryan JF, Markou A, Lucki I. Assessing antidepressant activity in rodents: recent developments and future needs. Trends Pharmacol Sci 2002;23:238–45.
Cryan JF, Valentino RJ, Lucki I. Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. Neurosci Biobehav Rev 2005;29:547–69.
Cyr M, Charbonneau C, Morissette M, Rochford J, Barden N, Di Paolo T. Central 5-hydroxytryptamine-2A receptor expression in transgenic mice bearing a glucocorticoid receptor antisense. Neuroendocrinology 2001;73:37–45.
David DJ, Samuels BA, Rainer Q, Wang JW, Marsteller D, Mendez I, et al. Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. Neuron 2009;62:479–93.
Deakin JF. 5HT2 receptors, depression and anxiety. Pharmacol Biochem Behav 1988;29:819–20.
de Kloet ER, Oitzl MS, Joels M. Stress and cognition: are corticosteroids good or bad guys? Trends Neurosci 1999;22:422–6.
Detke MJ, Johnson J, Lucki I. Acute and chronic antidepressant drug treatment in the rat forced swimming test model of depression. Exp Clin Psychopharmacol 1997;5:107–12.
Deuschle M, Schweiger U, Weber B, Gotthardt U, Korner A, Schmider J, Standhardt H, Lammers CH, Heuser I. Diurnal activity and pulsatility of the hypothalamus-pituitary-adrenal system in male depressed patients and healthy controls. J Clin Endocrinol Metab 1997;82:234–8.
Detke MJ, Rickels M, Lucki I. Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. Psychopharmacology (Berl) 1995;121:66–72.
Dhabhar FS, McEwen BS, Spencer RL. Adaptation to prolonged or repeated stress — comparison between rat strains showing intrinsic differences in reactivity to acute stress. Neuroendocrinology 1997;65:360–8.
Fernandes C, McKittrick CR, File SE, McEwen BS. Decreased 5-HT1A and increased 5-HT2A receptor binding after chronic corticosterone associated with a behavioural indication of depression but not anxiety. Psychoneuroendocrinology 1997;22:477–91.
Fiedler JL, Bravo J, Ulloa JL. What are the possible consequences of chronic stress in central nevous system? In: Aboitiz F, Dagnino-Subiabre A, editors. The Neurobiology of Stress: An Evolutionary Approach. Kerala: Research Signpost, 2007, p 79–118.
Filipenko ML, Beilina AG, Alekseyenko OV, Dolgov VV, Kudryavtseva NN. Repeated experience of social defeats increases serotonin transporter and monoamine oxidase A mRNA levels in raphe nuclei of male mice. Neurosci Lett 2002;321:25–8.
Gadek-Michalska A, Bugajski J. Repeated handling, restraint, or chronic crowding impair the hypothalamic-pituitary-adrenocortical response to acute restraint stress. J Physiol Pharmacol 2003;54:449–59.
Gartside SE, Leitch MM, Young AH. Altered glucocorticoid rhythm attenuates the ability of a chronic SSRI to elevate forebrain 5-HT: implications for the treatment of depression. Neuropsychopharmacology 2003;28:1572–8.
Girotti M, Pace TW, Gaylord RI, Rubin BA, Herman JP, Spencer RL. Habituation to repeated restraint stress is associated with lack of stress-induced c-Fos expression in primary sensory processing areas of the rat brain. Neuroscience 2006;138:1067–81.
Gourley SL, Kiraly DD, Howell JL, Olausson P, Taylor JR. Acute hippocampal brain-derived neurotrophic factor restores motivational and forced swim performance after corticosterone. Biol Psychiatry 2008a;64:884–90.
Gourley SL, Taylor JR. Recapitulation and reversal of a persistent depression-like syndrome in rodents. Curr Protoc Neurosci 2009 Chapter 9:Unit 9:32.
Gourley SL, Wu FJ, Kiraly DD, Ploski JE, Kedves AT, Duman RS, Taylor JR. Regionally specific regulation of ERK MAP kinase in a model of antidepressant-sensitive chronic depression. Biol Psychiatry 2008b;63:353–9.
Gregus A, Wintink AJ, Davis AC, Kalynchuk LE. Effect of repeated corticosterone injections and restraint stress on anxiety and depression-like behavior in male rats. Behav Brain Res 2005;156:105–14.
Grippo AJ, Sullivan NR, Damjanoska KJ, Crane JW, Carrasco GA, Shi J, et al. Chronic mild stress induces behavioral and physiological changes, and may alter serotonin 1A receptor function, in male and cycling female rats. Psychopharmacology (Berl) 2005;179:769–80.
Hinkelmann K, Moritz S, Botzenhardt J, Riedesel K, Wiedemann K, Kellner M, Otte C. Cognitive impairment in major depression: association with salivary cortisol. Biol Psychiatry 2009;66:879–85.
Joels M, Pu Z, Wiegert O, Oitzl MS, Krugers HJ. Learning under stress: how does it work? Trends Cogn Sci 2006;10:152–8.
Johnson SA, Fournier NM, Kalynchuk LE. Effect of different doses of corticosterone on depression-like behavior and HPA axis responses to a novel stressor. Behav Brain Res 2006;168:280–8.
Kalynchuk LE, Gregus A, Boudreau D, Perrot-Sinal TS. Corticosterone increases depression-like behavior, with some effects on predator odor-induced defensive behavior, in male and female rats. Behav Neurosci 2004;118:1365–77.
Karten YJ, Nair SM, van Essen L, Sibug R, Joels M. Long-term exposure to high corticosterone levels attenuates serotonin responses in rat hippocampal CA1 neurons. Proc Natl Acad Sci USA 1999;96:13456–61.
Kim JJ, Haller J. Glucocorticoid hyper- and hypofunction: stress effects on cognition and aggression. Ann N Y Acad Sci 2007;1113:291–303.
Lecrubier Y. The burden of depression and anxiety in general medicine. J Clin Psychiatry 2001;62 Suppl 8:4–9.
Lesch KP. Gene-environment interaction and the genetics of depression. J Psychiatry Neurosci 2004;29:174–84.
Lucassen PJ, Vollmann-Honsdorf GK, Gleisberg M, Czeh B, De Kloet ER, Fuchs E. Chronic psychosocial stress differentially affects apoptosis in hippocampal subregions and cortex of the adult tree shrew. Eur J Neurosci 2001;14:161–6.
Lucki I. The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. Behav Pharmacol 1997;8:523–32.
Magarinos AM, McEwen BS, Flugge G, Fuchs E. Chronic psychosocial stress causes apical dendritic atrophy of hippocampal CA3 pyramidal neurons in subordinate tree shrews. J Neurosci 1996;16:3534–40.
Maj J, Moryl E. Effects of sertraline and citalopram given repeatedly on the responsiveness of 5-HT receptor subpopulations. J Neural Transm Gen Sect 1992;88:143–56.
Marks W, Fournier NM, Kalynchuk LE. Repeated exposure to corticosterone increases depression-like behavior in two different versions of the forced swim test without altering nonspecific locomotor activity or muscle strength. Physiol Behav 2009;98:67–72.
Melia KR, Ryabinin AE, Schroeder R, Bloom FE, Wilson MC. Induction and habituation of immediate early gene expression in rat brain by acute and repeated restraint stress. J Neurosci 1994;14:5929–38.
Millan MJ. Dual- and triple-acting agents for treating core and co-morbid symptoms of major depression: novel concepts, new drugs. Neurotherapeutics 2009;6:53–77.
Mineka S, Zinbarg R. A contemporary learning theory perspective on the etiology of anxiety disorders: it's not what you thought it was. Am Psychol 2006;61:10–26.

Mora, S, Díaz-Véliz, G. Intracerebral administration of neuropeptides: an assessment of behavioral change. In: Conn, M, editor. Paradigms for the study of behavior. Methods in neurosciences. San Diego, CA: Academic Press, 1993, p 180–93.
Murray F, Smith DW, Hutson PH. Chronic low dose corticosterone exposure decreased hippocampal cell proliferation, volume and induced anxiety and depression like behaviours in mice. Eur J Pharmacol 2008;583:115–27.
Murray, CJL, Lopez, AD, Harvard School of Public Health, World Health Organization, World Bank. The global burden of disease: a comprehensive assessment of mortality and disability from diseases, injuries, and risk factors in 1990 and projected to 2020. Cambridge, MA: Published by the Harvard School of Public Health on behalf of the World Health Organization and the World Bank; Distributed by Harvard University Press, 1996.
Nasr SJ, Gibbons RD. Depressive symptoms associated with dexamethasone resistance. Psychiatry Res 1983;10:183–9.
Nestler EJ, Gould E, Manji H, Buncan M, Duman RS, Greshenfeld HK, Hen R, Koester S, Lederhendler I, Meaney M, Robbins T, Winsky L, Zalcman S. Preclinical models: status of basic research in depression. Biol Psychiatry 2002;52:503–28.
Pariante CM, Miller AH. Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. Biol Psychiatry 2001;49:391–404.
Pellow S, Chopin P, File SE, Briley M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 1985;14: 149–67.
Pepin MC, Govindan MV, Barden N. Increased glucocorticoid receptor gene promoter activity after antidepressant treatment. Mol Pharmacol 1992a;41:1016–22.
Pepin MC, Pothier F, Barden N. Antidepressant drug action in a transgenic mouse model of the endocrine changes seen in depression. Mol Pharmacol 1992b;42:991–5.
Pittenger C, Duman RS. Stress, depression, and neuroplasticity: a convergence of mechanisms. Neuropsychopharmacology 2008;33:88-109.
Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. Nature 1977;266:730–2.
Reneric JP, Lucki I. Antidepressant behavioral effects by dual inhibition of monoamine reuptake in the rat forced swimming test. Psychopharmacology (Berl) 1998;136: 190–7.
Sheline YI. Hippocampal atrophy in major depression: a result of depression-induced neurotoxicity? Mol Psychiatry 1996;1:298–9.
Sheline YI, Sanghavi M, Mintun MA, Gado MH. Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. J Neurosci 1999;19:5034–43.
Tafet GE, Idoyaga-Vargas VP, Abulafia DP, Calandria JM, Roffman SS, Chiovetta A, et al. Correlation between cortisol level and serotonin uptake in patients with chronic stress and depression. Cogn Affect Behav Neurosci 2001a;1:388–93.
Tafet GE, Toister-Achituv M, Shinitzky M. Enhancement of serotonin uptake by cortisol: a possible link between stress and depression. Cogn Affect Behav Neurosci 2001b;1:96-104.
Ulrich-Lai YM, Herman JP. Neural regulation of endocrine and autonomic stress responses. Nat Rev Neurosci 2009;10:397–409.
Viau V, Sawchenko PE. Hypophysiotropic neurons of the paraventricular nucleus respond in spatially, temporally, and phenotypically differentiated manners to acute vs. repeated restraint stress: rapid publication. J Comp Neurol 2002;445: 293–307.
Walf AA, Frye CA. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat Protoc 2007;2:322–8.
Wong ML, Kling MA, Munson PJ, Listwak S, Licinio J, Prolo P, Karp B, McCutcheon IE, Geracioti TD Jr., DeBellis MD, Rice KC, Goldstein DS, Veldhuis JD, Chrousos GP, Oldfield EH, McCann SM, Gold PW. Pronounced and sustained central hypernoradrenergic function in major depression with melancholic features: relation to hypercortisolism and corticotropin-releasing hormone. Proc Natl Acad Sci USA 2000;97:325–30.
Young AH, Sahakian BJ, Robbins TW, Cowen PJ. The effects of chronic administration of hydrocortisone on cognitive function in normal male volunteers. Psychopharmacology (Berl) 1999;145:260–6.
Zhao Z, Zhang HT, Bootzin E, Millan MJ, O'Donnell JM. Association of changes in norepinephrine and serotonin transporter expression with the long-term behavioral effects of antidepressant drugs. Neuropsychopharmacology 2008;34: 1467–81.