Available online at www.sciencedirect.com
ScienceDirect
Pharmacology, Biochemistry and Behavior 85 (2006) 522–534
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

The effects of acute and chronic administration of corticosterone on rat behavior in two models of fear responses, plasma corticosterone concentration, and c-Fos expression in the brain structures

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Received 16 February 2006; received in revised form 2 October 2006; accepted 3 October 2006
Available online 14 November 2006

Abstract

The aim of this paper was to examine changes in rat emotional behavior, and to find the brain structures, which are involved in the mediation of behavioral effects, related to the repeated administration of glucocorticoids. The effects of acute and chronic pretreatment of rats with two doses of corticosterone (5 and 20 mg/kg) were analyzed in two models of fear responses: neophobia-like behavior in the open field test, and freezing reaction in the conditioned fear test. Behavioral effects of repeated glucocorticoid administration were compared to changes in blood total corticosterone concentration, and expression of immediate early gene (c-Fos) in brain structures. It was found that acute administration of corticosterone (90 min before tests) enhanced rat exploratory behavior, and decreased freezing reaction. On the other hand, repeated administration of corticosterone (for 25 days, the final injection 90 min before contextual fear conditioning training) decreased plasma corticosterone concentration, inhibited exploratory behavior, enhanced freezing responses on retest and produced a complex pattern of changes in c-Fos expression, stimulated by exposure of rats to the aversively conditioned context. Aversive context induced c-Fos in the magnocellular neurons of the hypothalamic paraventricular nucleus (mPVN), dentate gyrus (DG), cingulate cortex area 1 (Cg1), and primary motor cortex (M1). In rats chronically treated with corticosterone this effect was attenuated in the mPVN and DG, enhanced in the M1, and additionally observed in the CA1, CA2 layers of the hippocampus, and in the central nucleus of amygdala (CeA), in comparison to control animals not subjected to contextual fear test. In sum, the present data suggest that chronic corticosterone treatment enhances the activity of primary motor cortex and CeA with subsequent improvement of memory of aversive events, and simultaneously stimulates a negative feedback mechanism operating in PVN with ensuing decrease in blood corticosterone concentration.

Keywords: Corticosterone; Neophobia; Freezing reaction; c-Fos; Brain structures; Rats

1. Introduction

The aim of this paper was to find brain structures, which are involved in the mediation of behavioral effects caused by repeated administration of glucocorticoids. The experiments were also designed to better understand mechanisms responsible for changes in emotional behavior induced by glucocorticoids (e.g. chronic treatment of patients with rheumatoid disorders or after organ transplantation). It is very well recognized that elevated levels of circulating glucocorticoids may provoke many central and peripheral pathological symptoms, including changes in affective behavior (Mitchell and O'Keane, 1998; Korte, 2001; Erickson et al., 2003; McEwen, 2005). In some other diseases, like anxiety and depression, it is suggested that high cortisol concentration contributes directly to the pathology of these mental disorders (Mitchell and O'Keane, 1998; McEwen, 2000, 2005; Erickson

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doi:10.1016/j.pbb.2006.10.001

et al., 2003; Swaab et al., 2005). In spite of many years of research in this field, many questions still remain unanswered. It is not clear, for example, which structures in the brain are the targets of high levels of glucocorticoids, and to what extent, and in which way, their activity, modified by steroid hormones, is correlated with changes in behavior. It is not well recognized whether behavioral effects of enhanced levels of glucocorticoids are related to their genomic effects, or are linked to their rapid, transient, influence on brain structures activity. One of the important problems in this area relates to the effects of chronic administration of glucocorticoids, in animal model of hypercorticosolemia, on the acquisition and retention of fear reactions, likely mechanisms of emotional disturbances appearing in depression and anxiety disorders. In the scientific literature, both inhibition and enhancement of fear responses after repeated administration of corticosterone, were reported (Ohl and Fuchs, 1999; Wolf, 2003; Conrad et al., 2004; Thompson et al., 2004). These effects were found to depend on the dose of glucocorticoid, time of administration, time of final injection (pre or post-training or test session), and intensity of aversive stimulus. Some of the authors claim that repeated glucocorticoid treatment-induced decrease in neurogenesis and increase in dendritic atrophy in prefrontal cortex and hippocampus, may be responsible for memory deficits and ensuing behavioral pathology (Woolley et al., 1990; Gould and Tanapat, 1999; Sapolsky, 2000; Wellman, 2001; McEwen, 2005). Other authors underline the role of local changes in corticotropin releasing factor (CRF) concentration in the brain as an important component modulating central processes leading to fear and anxiety (Holsboer, 1999; Koob and Heinrichs, 1999; Shepard et al., 2000).

In the present study we have further explored some of the above mentioned problems by using behavioral, biochemical and histochemical approach. The influence of acute and repeated pretreatment of rats with two doses of corticosterone was analyzed in two models of fear responses: neophobia-like behavior in the open field test (OFT), and freezing reaction in the conditioned fear test (CFT). The effects of acute treatment of animals with a glucocorticoid were included to the study, to show different patterns of behavioral changes after acute vs. chronic hormone administration, and also to validate our models of fear reactions as reflecting human pathology, related to persistently elevated levels of glucocorticoids. Two tests were selected to examine the effects of corticosterone in the unconditioned and conditioned models of fear responses. In this way the role of possible changes in learning and memory processes, contributing to animal behavior could be better controlled. The relationships between behavioural effects, changes in blood total corticosterone concentration, and expression of c-Fos in brain structures, were also examined. c-Fos protein is a product of expression of the *c-fos* gene, and a well recognized marker of rapid changes in neuronal activity, widely used in psychopharmacological studies (Bullitt, 1990; Campeau et al., 1997). The interval of 90 min between the last corticosterone administration and the test procedures was selected purposefully, to examine the fast adaptive, non-genomic effects after single injection, and long-lasting changes after repeated hormone injections, but not the influence of acutely elevated levels of the steroid hormone. This was especially important in case of chronic hormone administration, when its influence on conditioned fear acquisition was studied. Because of that, we have used the same time interval also in case of acute injections. Such experimental paradigm allowed to directly compare the effects of acute and chronic corticosterone administration.

To sum up, the aim of the study was to examine changes in rat emotional behavior, and in activity of brain structures after repeatedly administered steroid hormone.

2. Materials and methods

2.1. Animals

Adult male Wistar rats weighting 200±20 g at the beginning of the experiment were used in the study. The animals were housed in groups of 5 per cage in standard laboratory conditions under 12 h: 12 h light:dark cycle (lights on at 7 a.m.) in a constant temperature (21±2 °C) and 70% humidity. The rats were given free access to food and water. All experiments were performed between 8.00 a.m. and 1.00 p.m. Body weights were recorded periodically. The experiments were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609 EEC). All experimental procedures using animal subjects were approved by the Local Committee for Animal Care and Use at Warsaw Medical University, Poland.

2.2. Drugs

Corticosterone and sesame oil (d=0.92 g/cm^3, heavy metals content <0.001%) were purchased from Sigma–Aldrich (Poland). The corticosterone was suspended in sesame oil at a volume adjusted to 1 ml/kg body weight and administered subcutaneously in the nape of the neck.

2.3. Treatments

Rats were injected with either corticosterone or vehicle (sesame oil). Depending on the experiment, corticosterone injections were given either acutely (single injection of 5 or 20 mg/kg) or chronically (daily injections of 5 or 20 mg/kg for 25 consecutive days). The doses of 5 and 20 mg/kg were selected consistently with the previous studies showing changes in animal behavior (Stone et al., 1988; Sandi et al., 1996; Brotto et al., 2001). The dose of 5 mg/kg was found previously to increase plasma levels in a manner similar to the effect of an acute stressor (Sandi et al., 1996). Separate groups of animals were used in the experiment with acute and repeated administration of corticosterone.

In the part of experiment with acutely administered corticosterone, after 4 days of acclimatization to the vivarium, animals were divided into three experimental groups: vehicle-a — sesame oil pretreated animals; CORT 5-a — rats treated with corticosterone, at the dose of 5 mg/kg; CORT 20-a — rats given

corticosterone at the dose of 20 mg/kg. Next, the rats were handled for 7 days prior to experiment. During first 4 days of handling animals were removed from their home cages and held by an experimenter in the same way as during drug administration for 1 min. Days 5–7 of handling included subcutaneous injection of saline (0.9% NaCl) at a volume of 1 ml/kg. On the experimental day rats received subcutaneous injection of corticosterone or vehicle (Fig. 1A).

In part of the experiment with chronically administered corticosterone, after 4 days of habituation to the vivarium, animals were divided into three experimental groups: vehicle-ch — sesame oil pretreated animals; CORT 5-ch — rats given corticosterone at the dose of 5 mg/kg; CORT 20-ch — animals, receiving corticosterone, at the dose of 20 mg/kg. Next, the rats received one injection of corticosterone or vehicle per day, for 25 days. In the final part of the experiment, 2 h after open field test and 3.5 h after drug administration, vehicle-ch group was randomly divided into two experimental groups: vehicle 1-ch — rats pretreated with sesame oil only, and placed threefold in the conditioning box without receiving shock; vehicle 2-ch — rats pretreated with sesame oil, and 24 h later subjected to conditioned fear test (Fig. 1B).

2.4. Open field test (OFT)

The test was performed in a soundproof chamber under dim light and continuos white noise (65 dB) conditions. The open field apparatus consisted of two separate, round arenas (80 cm diameter) with 30-cm height walls. On the experimental day animals received either corticosterone or sesame oil 90 min before test. In open field test rats were examined separately (Fig. 1). Their locomotor activity, the number of central entries,

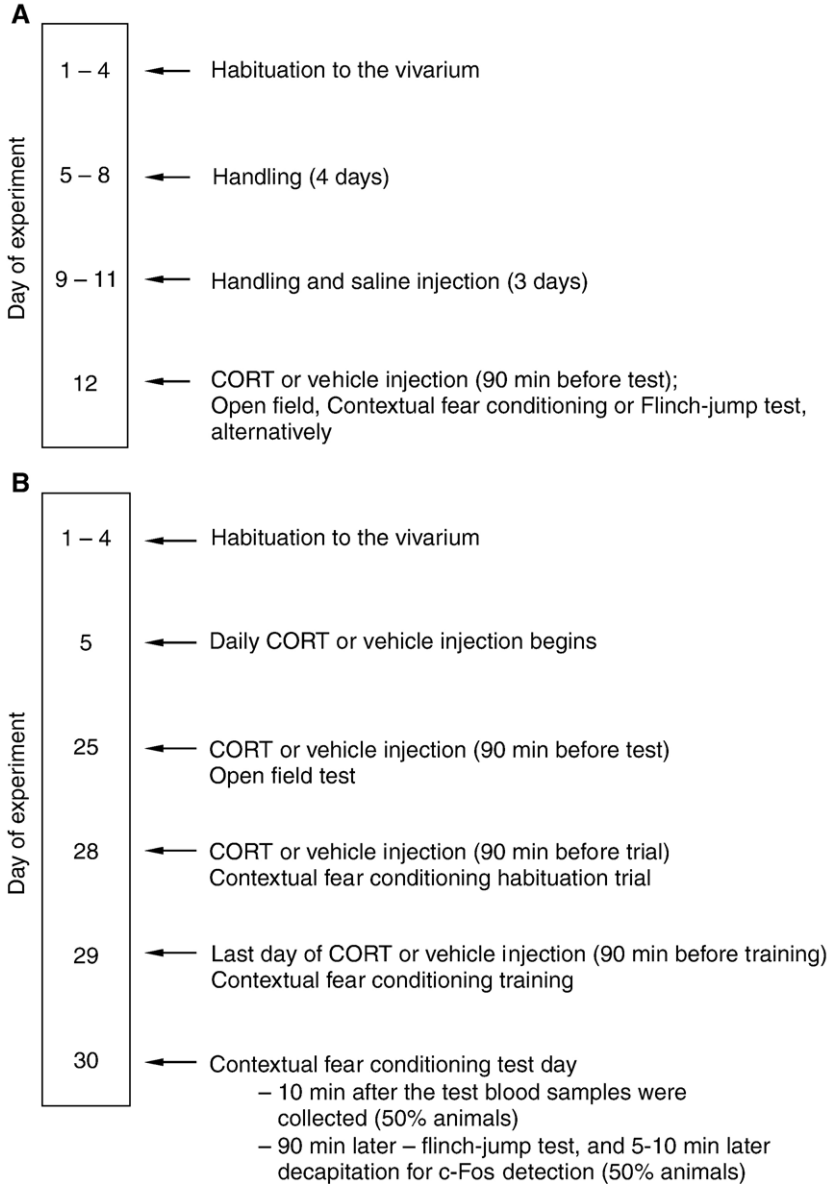

Fig. 1. Treatment scheme of acute (A), and chronic (B) administration of corticosterone. CORT — corticosterone.

and the time spent in the central sector of the open field (50 cm diameter) were recorded on video tape and analyzed by PC-based Videomot System tracking the position of an animal (software — TSE, Bad Homburg, Germany). The parameter of thigmotaxis was calculated as a ratio of the number of entries into central part of testing arena to the rat locomotor activity multiplied by 1000. The higher the value of the score, the lower the thigmotaxis and the more pronounced the anti-emotional effect (Sienkiewicz-Jarosz et al., 2003).

2.5. Conditioned fear test (CFT)

The experiment was performed using a computerized fear-conditioning system (TSE, Bad Homburg, Germany), during three consecutive days, in the same testing box (36 cm×21 cm×20 cm) and experimental chamber, under constant, white noise (65 dB) condition. The box was cleaned after each trial with 95% ethanol. On the first day, the animals received 0.9% NaCl (acute treatment), corticosterone or vehicle (chronic treatment), and 90 min later were placed separately for 2 min in a training box without aversive stimulation, for adaptation to the experimental conditions. On the second day, a training day, animals received either corticosterone or vehicle, and 90 min later were placed for 10 min in a training box. After 5-min pause, the animals received four 1-s long footshocks repeated every 59 s (each consisted of a train of stimuli: 0.8 mA, 150/300 ms). The animals were removed from the testing boxes 1 min after the last shock was delivered. On the third experimental day, the freezing behavior of rats was examined 24 h after drug administration, for 10 min, in the same box (Fig. 1). The freezing was defined as absence of any movements except for those required for respiration (Maciejak et al., 2003).

2.6. Flinch–jump test

The test was performed in the box made of Plexiglas (30 cm×30 cm×60 cm), with a gird floor made of stainless steel bars wired to a shock generator. The floor of the box was cleaned after each trial with 95% ethanol. In the part of experiment with acutely administered corticosterone, rats received drug or vehicle, and 90 min later animals were placed individually into the box. In the part of experiment with chronically administered corticosterone, 90 min after the conditioned fear test and 24 h after drug administration, animals were placed individually into the box. Shocks were delivered to the gird floor of the box through a shock generator. After a 3-min period of habituation to the test box, shocks titrations were continued upwards in a stepwise manner (0.05 mA, 0.05–1.0 mA range) depending upon responsiveness of the rat. The flinch threshold was defined as the lowest shock intensity that elicited any detectable response. The jump threshold was defined as the lowest shock intensity that elicited simultaneous removal of at least three paws (both hindpaws) from the gird. To avoid foot damage, the cut-off=1.0 mA was established. The time gap between shocks was 10 s, and each animal was tested only once (Szyndler et al., 2002).

2.7. Corticosterone assay

In the part of experiment with chronic administration of corticosterone, blood samples were collected from 50% animals, on the 3rd day of contextual fear conditioning test, 10 min after exposure to the aversive context and 24 h after the last dose of corticosterone or vehicle (Fig. 1B). After testing the rat was transported to the home cage, and 10 min later the blood samples were taken in a different room. After decapitation, 500 μl samples of blood were collected into heparinised tubes (Mercier et al., 2003). Additionally, two another control groups of animals were included in the experiment: vehicle (rats chronically pretreated with sesame oil, without contextual fear conditioning), and CORT 20 (rats given chronically corticosterone at the dose of 20 mg/kg, without contextual fear conditioning). 24 h after the last dose of corticosterone (26 day) animals were decapitated and 500 μl samples of blood were collected into heparinised tubes. The samples were immediately centrifuged (2600 ×*g* at 4 °C for 15′). Plasma was immediately extracted and stored at −70 °C, until analysis. Plasma was diluted (1:500) and analysed by radioimmunoassay [^{3}H] RIA kit, MP Biomedicals Inc. The concentration range for this assay was 0.025–1 ng/0.5 ml with a six point calibration curve 0.025; 0.05; 0.1; 0.25; 0.5; 1 ng/0.5 ml. Total plasma corticosterone averages usually between 100 and 500 ng/1 ml (0,289–1,445 nmol/ml).

2.8. Immunocytochemistry

100 min after the experiments and 24 h after last dose of corticosterone, the animals (50%) were decapitated, their brains removed, frozen in dry-ice cooled cyclopenthane, and stored at −70 °C. It is important to underline that rats were sacrificed 90 min after conditioned fear test, and 5–10 min after flinch–jump test, thus only the influence of a contextual fear could be revealed by the immunocytochemical reaction. The immunocytochemical reaction was performed on slide-mounted brain sections, according to the procedure described previously (Johnstone et al., 2000; Lehner et al., 2004). Two coronal 15 μm cryostat sections (based on the atlas of Paxinos and Watson, 1986) from each animal were cut and mounted on silan-coated slides, and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH=7.4) for 15 min. The specimens were then washed twice (2×15 min) in 0.01 M PBS solution (pH=7.4), incubated in 3% H_2O_2 solution for 30 min to block the activity of endogenous peroxidase, then washed again in 0.01 M PBS solution (pH=7.4) twice (2×15 min), and incubated in a 3% normal goat serum (NGS) blocking solution. Subsequently, slide-mounted brain sections were incubated in rabbit polyclonal c-Fos IgG diluted at 1:1000 (Oncogene) in temperature 4–8 °C for 72 h, washed in 0.01 M PBS solution (pH=7.4) three times (3×15 min), then incubated with biotynylated anti-rabbit IgGs (Vector Laboratories, CA) for 2 h, rinsed in 0.01 M PBS solution (pH=7.4) twice (2×15 min), and incubated with avidine–biotine–peroxydase complex (Vector Laboratories, CA) for 1 h. Finally, after being washed in 0.01 M PBS solution (pH=7.4) twice (2×15 min) slide-

Table 1
The effects of acute administration of corticosterone on rat behavior in the open field test

Groups	N	Total distance (cm)	Central distance (cm)	Central entries	Time in central area (s)	Anti-thigmotactic effect
5 min						
Vehicle-a	6	881.16±101.5	26.9±17.3	1.0±0.6	1.9±1.2	0.99±0.6
CORT 5-a	6	1114.49±227.2	16.4±14.3	0.8±0.5	1.4±1.2	1.10±0.7
CORT 20-a	7	1065.20±159.98	90.5±26.0*	2.1±0.8	9.0±2.7 *,#	1.95±0.54
15 min						
Vehicle-a	6	1670.84±366.1	75.9±40.1	3.3±1.8	8.3±4.8	1.35±0.7
CORT 5-a	6	1923.54±476.6	64.0±44.4	2.3±1.4	8.3±5.9	1.21±0.7
CORT 20-a	7	2238.63±282	158.4±46.9	4.7±1.8	15.4±5.1	1.82±0.4

The data are shown as means±S.E.M. *N* — number of rats. Vehicle-a — sesame oil pretreated animals; CORT 5-a — corticosterone at the dose of 5 mg/kg; CORT 20-a — corticosterone at the dose of 20 mg/kg. * — differs from vehicle-a; # — differs from 5 mg/kg. *, # $p<0.05$.

mounted brain sections were immunoreacted with a solution containing Tris, 0.03% diaminobenzidine hydrochloride (DAB) and 0.003% H_2O_2. The slides were then dehydrated by serial alcohol rinsing, dewaxed in xylene, and coverslipped in the histofluid mountant. Fos-like immunoreactivity was assessed by light microscopy (Olympus BX-51 light microscope, Camedia Master C-3040 digital camera) at a magnification of ×40. The number of c-Fos-positive nuclei was counted bilaterally with the use of computerized image analysis system (Olympus DP-Soft version 3.2 software) from two sections per rat in the following subregions: AP 0.70; cingulate cortex area 1 and 2 (Cg1, Cg2), primary and secondary motor cortex (M1 and M2), caudate putament (Cpu), nucleus accumbens (Acb); AP −1.88; magnocellular neurons of the paraventricular hypothalamic nucleus (mPVN), parvocellular neurons of the paraventricular hypothalamic nucleus (pPVN), paraventricular thalamic nucleus (PVP), central amygdala (CeA), basolateral amygdala (BLA), medial amygdala (MeA); AP −3.30; CA1, CA2, CA3, DG areas of hippocampus. The brain areas selected for c-Fos evaluation are involved in the regulation of fear, and/or feedback mechanism of the hormonal stress response (Korte, 2001). The area of outlined regions was calculated using DP-Soft, then c-Fos positive nuclei were counted manually for each region of each rat, and expressed as the number of immunopositive neurons per 0.1 mm^2 (Fig. 5).

2.9. Statistical analysis

The data are shown as means±S.E.M. Both behavioral and biochemical data were analyzed by one-way ANOVA followed by post hoc Newman–Keuls test. Corticosterone levels after chronic steroid treatment were analysed by Student's *t*-test or one-way ANOVA followed by post hoc LSD (least significant difference) test. A probability value of $P<0.05$ was considered significant in this study. Statistical analysis was performed with the use of Stat-Soft Statistica 6.0 for Windows (StatSoft Inc., USA).

3. Results

3.1. Open field test

3.1.1. Effects of acute corticosterone administration

During the first 5 min of the open field test, corticosterone at the dose of 20 mg/kg significantly increased central distance [$F(2,16)=3.92$ ($P<0.05$)], and time spent in the central area of the open field test [$F(2,16)=4.80$ ($P<0.05$)]. Newman–Keuls post hoc showed that corticosterone given at the dose of 20 mg/kg significantly increased central distance and time spent in the central sector ($P<0.05$) Corticosterone at the dose of 5 mg/kg did not produce significant effects ($P>0.05$) (Table 1).

The statistical analysis of rat behavior during the total 15 min of the test did not reveal significant changes between experimental groups.

3.1.2. Effects of administration of corticosterone for 21 days

During the first 5 min of the open field test corticosterone dose-dependently decreased the central distance [$F(2,44)=5.89$ ($P<0.01$)], the number of central entries [$F(2,44)=5.68$ ($P<0.01$)], the total time spent in the central sector [$F(2,44)=4.19$ ($P<0.05$)], and the anti-thigmotactic effect [$F(2,44)=$

Table 2
The effects of chronic administration of corticosterone on rat behavior in the open field test

Groups	N	Total distance (cm)	Central distance (cm)	Central entries	Time in central area (s)	Anti-thigmotactic effect
5 min						
Vehicle-ch	17	1361.60±113.5	122.23±19.6	3.88±0.6	10.10±1.85	3.0±0.4
CORT 5-ch	15	1298.06±87.5	61.86±10.0 *	2.2±0.5 *	4.99±1.0 *	1.6±0.3 *
CORT 20-ch	15	1036.13±84.2	50.92±16.0 **	1.5±0.4 **	4.7±1.4 *	1.5±0.4 *
15 min						
Vehicle-ch	17	2524.22±220.4	194.75±27.1	6.29±0.8	18.19±3.2	2.57±0.3
CORT 5-ch	15	2496.55±281.1	99.76±16.5 **	3.93±0.7 *	10.16±2.6 *	1.72±0.4
CORT 20-ch	15	1966.34±187.3	81.97±22.2 **	2.3±0.6 **	7.86±2.0 *	1.15±0.3 **

The data are shown as means±S.E.M. *N* – number of rats. Vehicle-ch — sesame oil pretreated animals, CORT 5-ch — corticosterone at the dose of 5 mg/kg; CORT 20-ch — corticosterone at the dose of 20 mg/kg pretreated animals. * — differs from vehicle-ch. * $p<0.05$; ** $p<0.01$.

4.54 ($P<0.05$)]. Newman–Keuls post hoc revealed that the statistically significant effect was present after the dose of 5 mg/kg and 20 mg/kg (central distance, $P<0.05$ and $P<0.01$, respectively; the number of central entries, $P<0.05$ and $P<0.01$, respectively; the total time spent in the central sector, $P<0.05$; and the anti-thigmotactic effect, $P<0.05$) (Table 2).

During the 15 min of the open field test corticosterone dose-dependently decreased rat exploratory activity: central distance [$F(2,44)=7.30$ ($P<0.01$)], central entries [$F(2,44)=7.79$ ($P<0.01$)], time in central sector [$F(2,44)=4.12$ ($P<0.05$)], and the anti-thigmotactic effect [$F(2,44)=5.22$ ($P<0.01$)]. Newman–Keuls post hoc test showed that corticosterone given at the dose of 5 mg/kg significantly decreased central distance ($P<0.01$), the number of central entries ($P<0.05$), and time spent by animals in the central area ($P<0.05$). Similar effects appeared after the higher dose of corticosterone (20 mg/kg): central distance ($P<0.01$), central entries ($P<0.01$), time spent in the central area ($P<0.05$), and the anti-thigmotactic effect ($P<0.01$) (Table 2).

3.2. Conditioned fear test

3.2.1. Effects of acute corticosterone administration

One-way ANOVA revealed significant differences in the first 5 min [$F(2,19)=4.69$ ($P<0.05$)], and in the total time of freezing [$F(2,19)=11.94$ ($P<0.01$)] (Fig. 2). Newman–Keuls post hoc test showed that corticosterone at dose of 5 mg/kg and 20 mg/kg decreased freezing duration in comparison to vehicle group (0–5 min, $P<0.05$; total freezing duration, $P<0.01$). The number of freezing episodes during the first 5 min of the test and the total number of freezing episodes were not changed significantly: 0–5 min [$F(2,19)=0.61$ ($P>0.1$)], total freezing episodes [$F(2,19)=1.06$ ($P>0.1$)] (Fig. 2).

3.2.2. Effects of administration of corticosterone for 25 days

In the contextual fear test, corticosterone treated animals showed significant changes in the number of freezing episodes during the first 5 min [$F(2,44)=6.96$ ($P<0.01$)], and in the total number of freezing episodes [$F(2,44)=4.21$ ($P<0.05$)] (Fig. 3). Newman–Keuls post hoc test showed that corticosterone at dose

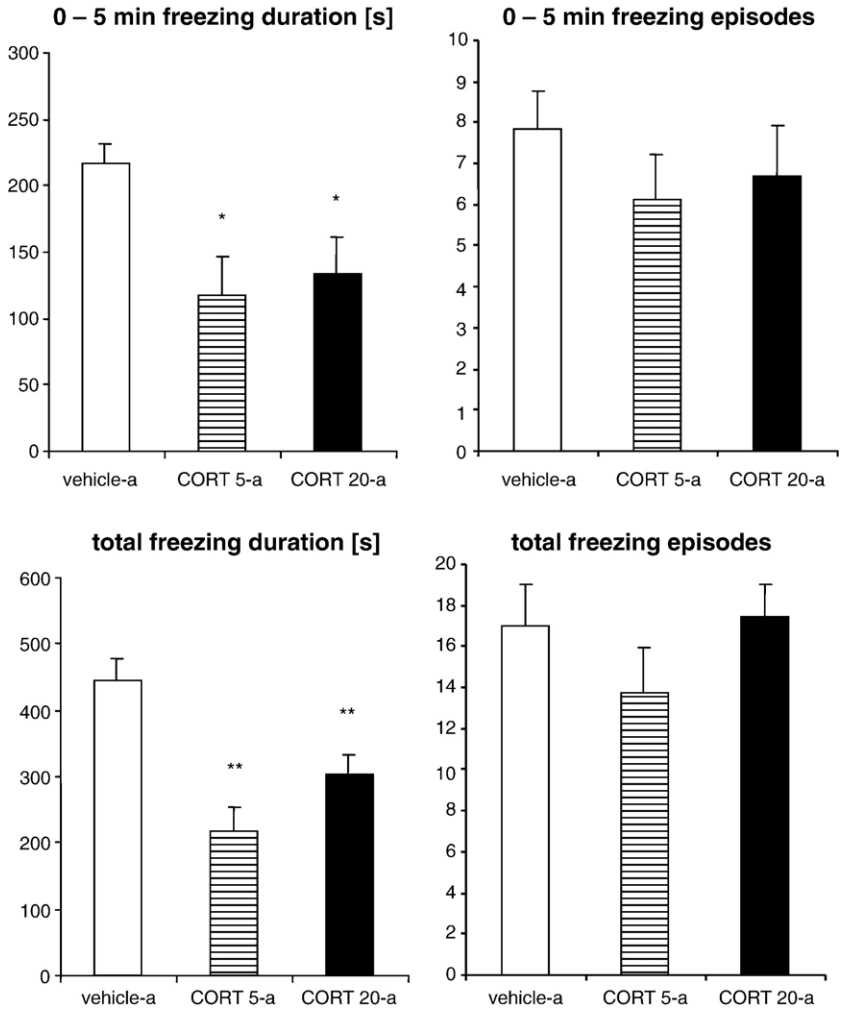

Fig. 2. The influence of acute administration of corticosterone, on rat behavior in the contextual fear test. The ordinates show duration of rat freezing behavior (s) or the number of freezing episodes. The data are shown as means±S.E.M. Vehicle-a — sesame oil pretreated rats (open bars, $n=8$), CORT 5-a — corticosterone at the dose of 5 mg/kg (horizontally striped bars, $n=8$), CORT 20-a — corticosterone at the dose of 20 mg/kg (closed bars, $n=8$). * — differs from vehicle-a. * $P<0.05$; ** $P<0.01$.

of 5 mg/kg and 20 mg/kg increased freezing episodes during the first 5 min ($P<0.01$), and the total number of freezing episodes ($P<0.05$). Freezing duration (during first 5 min, and total time of freezing) were not changed significantly (0–5 min, $F(2,44)=1.30$, $P>0.1$; total freezing duration, $F(2,44)=1.74$, $P>0.1$) (Fig. 3).

3.3. Flinch–jump test

3.3.1. Effects of acute and chronic corticosterone administration

One-way ANOVA did not reveal any significant differences between groups in the rat flinch reaction: acute treatment [$F(2,21)=2.71$ ($P>0.1$)], chronic treatment [$F(3,33)=1.14$ ($P>0.1$)]; and jump reaction: acute treatment [$F(2,21)=0.97$ ($P>0.1$)], chronic treatment [$F(3,33)=1.40$ ($P>0.1$)] (Table 3).

3.4. Radioimmunoassay of corticosterone

3.4.1. Effect of chronic corticosterone administration

Student's *t*-test showed a significant decrease in plasma concentration of corticosterone in CORT 20 group, in comparison to vehicle group ($t=3.43$, $P<0.01$) (Fig. 4A). One-way ANOVA revealed also significant differences between groups in total plasma concentration of corticosterone $F(3,29)=3.43$ ($P<0.05$) (Fig. 4B). LSD post hoc test revealed decreased levels of corticosterone in CORT 5-ch ($P<0.05$) and CORT 20-ch ($P<0.01$) groups, compared to vehicle 1-ch group. In addition, post hoc test showed a significant decrease in the level of the steroid hormone in CORT 20-ch group, in comparison to vehicle 2-ch group ($P<0.05$) (Fig. 4).

3.5. c-Fos immunoreactivity

One-way ANOVA revealed statistically significant differences in the number of c-Fos positive cells, in many examined brain structures: pPVN [$F(3,22)=5.36$ ($P<0.01$)], mPVN [$F(3,22)=34.07$ ($P<0.01$)], CA1 [$F(3,24)=5.21$ ($P<0.01$)], CA2 [$F(3,24)=5.67$ ($P<0.01$)], DG [$F(3,23)=17.55$ ($P<0.01$)], CeA [$F(3,24)=12.25$ ($P<0.01$), MeA [$F(3,23)=4.67$ ($P<0.05$)], Cg1 [$F(3,25)=12.09$ ($P<0.01$)], M1 [$F(3,24)=20.52$ ($P<0.01$)] (Table 4). Newman–Keuls post hoc test

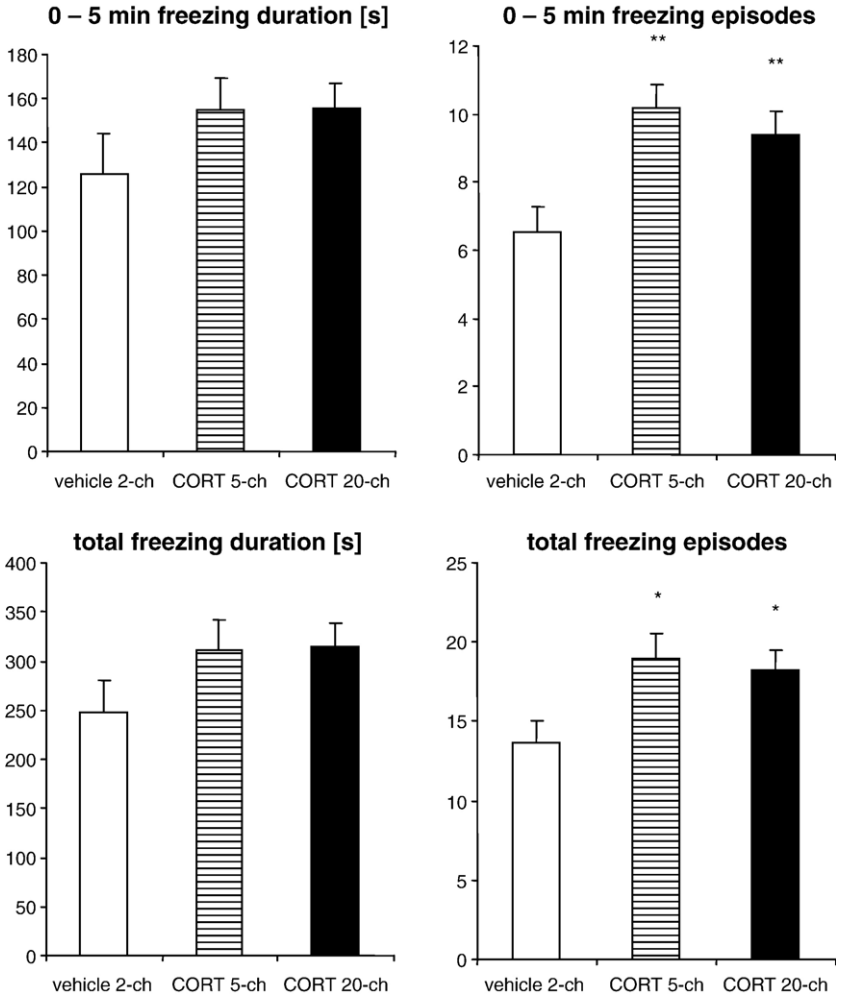

Fig. 3. The influence of chronic administration of corticosterone on rat behavior in the contextual fear test. The ordinates show duration of rat freezing response (s) or the number of freezing episodes. The data are shown as means ± S.E.M. Vehicle 2-ch — sesame oil pretreated rats and subjected to contextual fear conditioning (open bars, $n=16$), CORT 5-ch — corticosterone at the dose of 5 mg/kg (horizontally striped bars, $n=16$), CORT 20-ch — corticosterone at the dose of 20 mg/kg (closed bars, $n=15$). * — differs from vehicle 2-ch. * $P<0.05$; ** $P<0.01$.

Table 3
Pain threshold in the flinch–jump test

Footshock reactivity threshold (mA)							
	Acute			Chronic			
	Vehicle-a	CORT 5-a	CORT 20-a	Vehicle 1-ch	Vehicle 2-ch	CORT 5-ch	CORT 20-ch
	(n=8)	(n=8)	(n=8)	(n=9)	(n=8)	(n=10)	(n=10)
Flinch	0.37±0.05	0.39±0.04	0.27±0.03	0.58±0.07	0.41±0.08	0.56±0.06	0.53±0.07
Jump	0.61±0.05	0.62±0.05	0.53±0.04	0.86±0.07	0.64±0.10	0.80±0.05	0.70±0.07

The data are shown in mA as means±S.E.M. N — number of rats. Vehicle-a — animals pretreated acutely with sesame oil only, vehicle 1-ch — animals pretreated chronically with sesame oil only; vehicle 2-ch — sesame oil pretreated rats subjected to conditioned fear test; CORT 5-a — rats given acutely corticosterone at the dose of 5 mg/kg only; CORT 5-ch — rats chronically administered with corticosterone at the dose of 5 mg/kg and subjected to contextual fear conditioning; CORT 20-a — rats acutely administered with corticosterone at the dose of 20 mg/kg only; CORT 20-ch — rats given chronically corticosterone at the dose of 20 mg/kg and subjected to contextual fear conditioning. For more details see experimental procedures.

revealed a significant increase of c-Fos expression in mPVN (P<0.01), DG (P<0.01), Cg1 (P<0.01) and M1 (P<0.01) in vehicle 2-ch group compared to vehicle 1-ch group. Furthermore, in pPVN, Newman–Keuls post hoc test showed a significant decrease in c-Fos-positive nuclei in rats which received corticosterone at the dose of 20 mg/kg (P<0.01), compared to vehicle 2-ch and CORT 5-ch group. In mPVN, post hoc test revealed a decrease in c-Fos expression in CORT 5-ch and CORT 20-ch group (P<0.01), in comparison to vehicle 1-ch and vehicle 2-ch group (Fig. 6). In CA1, increased c-Fos expression was found in CORT 5-ch and CORT 20-ch group (P<0.05 and P<0.01, respectively), in comparison to vehicle 1-ch group. In CA2, Newman–Keuls post hoc test showed an increase in c-Fos positive nuclei in CORT 5-ch and CORT 20-ch group (P<0.05), compared to vehicle 1-ch and vehicle 2-ch group. Post hoc analysis revealed also a significant decrease of c-*fos* expression in DG in CORT 5-ch and CORT 20-ch animals (P<0.01), compared to vehicle 2-ch group. In addition, post hoc test showed increased c-Fos expression in CORT 5-ch and CORT 20-ch (P<0.01), in comparison to vehicle 1-ch group. Post hoc analysis revealed a strong increase in the number of c-Fos labeled neurons in CeA in the CORT 5-ch and CORT 20-ch groups of animals (P<0.01), compared to vehicle 1-ch and vehicle 2-ch group. In MeA post hoc test showed enhanced c-Fos expression in CORT 5-ch group in comparison to vehicle 2-ch (P<0.05) and CORT 20-ch group (P<0.01). Post hoc test revealed also a significant increase in c-Fos positive nuclei in Cg1 in CORT 5-ch and CORT 20-ch groups (P<0.01), in comparison to vehicle 1-ch. In M1, Newman–Keuls test showed increased c-Fos expression in CORT 5-ch and CORT 20-ch group (P<0.01), compared to vehicle 1-ch and vehicle 2-ch group.

4. Discussion

It was found that acutely administered corticosterone enhanced rat exploratory behavior in the open field test, and decreased freezing reaction in the conditioned fear test. On the other hand, repeatedly administered corticosterone decreased exploratory behavior, enhanced freezing responses, decreased plasma corticosterone concentration, and produced a complex pattern of changes in c-Fos expression stimulated by exposure of rats to the aversively conditioned context. Aversive context induced c-Fos production in the following brain areas: mPVN, DG, Cg1, and M1. Chronic pretreatment of rats with corticosterone attenuated this effect in the mPVN and DG, enhanced it in the M1, and caused it to appear also in the CA1, CA2 and CeA, in comparison to control animals, not subjected to fear conditioning. In these structures aversive context did not enhance c-Fos expression in control animals. In the parvocellular neurons of the hypothalamic paraventricular nucleus (pPVN), pretreatment with corticosterone (20 mg/kg) decreased c-Fos expression in comparison to both control groups, however, the effect of aversive context on c-Fos did not reach the statistically significant level. In other brain structures examined: paraventricular thalamic nucleus (PVP), CA3 area of the hippocampus, basolateral amygdala (BLA), medial amygdala (MeA), cingulate cortex area 2 (Cg2), secondary motor cortex

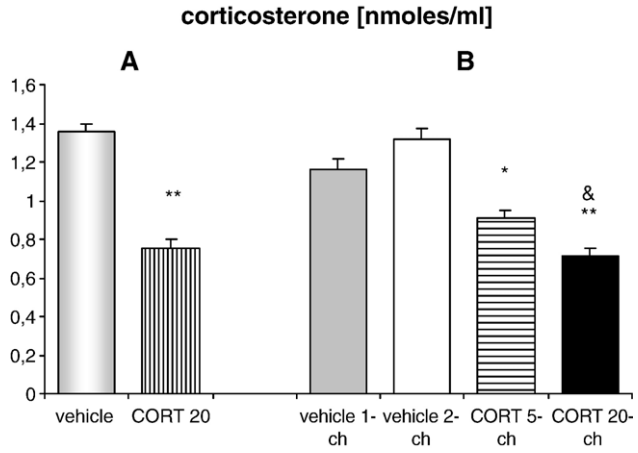

Fig. 4. The effects of repeated administration of corticosterone on plasma levels of corticosterone 24 h after the last dose of corticosterone (nmol/ml), in rats without conditioned fear test (A) and, 10 min after conditioned fear test, 24 h after the last dose of corticosterone (B). The data are shown as means±S.E.M. Vehicle 1-ch — rats pretreated repeatedly with sesame oil only, and placed threefold in the conditioning box without receiving a shock (n=9), vehicle 2-ch — rats pretreated repeatedly with sesame oil, and subjected to conditioned fear test (n=9), vehicle — rats pretreated repeatedly with sesame oil only, without conditioned fear test (n=7), CORT 5-ch — rats given repeatedly corticosterone at the dose of 5 mg/kg, and subjected to conditioned fear test (n=8), CORT 20-ch — rats given repeatedly corticosterone at the dose of 20 mg/kg, and subjected to conditioned fear test (n=7), CORT 20 — rats given repeatedly corticosterone at the dose of 20 mg/kg, without conditioned fear test (n=7). * differs from vehicle or vehicle 1-ch; $^{\&}$ differs from vehicle 2-ch. *, $^{\&}$ P<0.05; ** P<0.01.

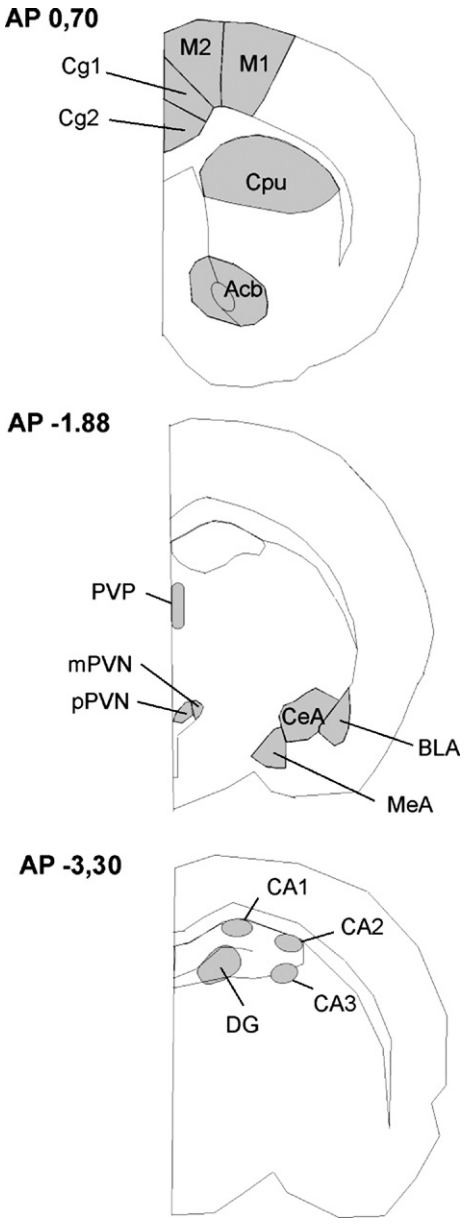

Fig. 5. A scheme showing brain regions analyzed for immunocytochemistry. The areas that were analysed for c-Fos immunoreactivity are outlined according to Paxinos and Watson (1986). The numbers indicate the distance from bregma (mm, caudal to bregma). Cg1, Cg2 — cingulate cortex area 1 and 2; M1, M2 — primary and secondary motor cortex; Cpu — caudate putament; Acb — nucleus accumbens; PVP — paraventricular thalamic nucleus; mPVN — magnocellular hypothalamic nucleus; pPVN — parvocellular hypothalamic nucleus; CeA — central amygdala; MeA — medial amygdala; BLA — basolateral amygdala; CA1, CA2, CA3, DG — areas of hippocampus.

(M2), striatum and nucleus accumbens (Acb), ANOVA did not reveal any significant differences among experimental groups.

The anti-emotional effects of acutely administered corticosterone (2 h before the experiment) have been found previously in the elevated plus maze, in rats (Andreatini and Leite, 1994). It is possible that they are related to the rapid, non-genomic, inhibitory action of this glucocorticosteroid hormone on the synthesis and release of CRF (Hinz and Hirschelmann, 2000; Mikics et al., 2005). Corticosterone has important modulating influence on the adaptive pattern of CRF mRNA expression in the PVN (Pinnock and Herbert, 2001). It was shown, for example, that corticosterone and RU 28362 (the glucocorticoid receptor agonist) when administered intravenously decreased CRF-induced ACTH secretion within 15 min (corticosterone) and 5 min (RU 28362) after steroid administration (Hinz and Hirschelmann, 2000). These data indicate that the release of CRF is under rapid, non-genomic, control by the glucocorticoid receptors. It is conceivable, therefore, that the anti-emotional effect of acutely administered corticosterone may be related to a transient decrease in CRF concentration, taking into consideration its well recognized role in the mediation of reaction to aversive events (Holsboer, 1999; Shepard et al., 2000). The above mentioned data can explain also the short latency of behavioral effects observed in the present study. The other rapid, non-genomic, mechanism of corticosterone action might involve the formation of GABA-A receptor modulating neurosteroids, derived from deoxycorticosterone (Reddy and Rogawski, 2002). In the fear conditioning test, decreased conditioned freezing on the test day could have resulted from anxiolysis on the conditioning day, as in the same conditions it was anxiolytic in the open field. Some studies have shown that blockade of corticosterone action with either metyrapone or steroid receptor antagonists may reduce freezing on fear tasks (Korte et al., 1995; Roozendaal et al., 1996), suggesting that glucocorticoids can mediate also fear responses. However, a mnestic effect during the acquisition phase cannot be ruled out, as it

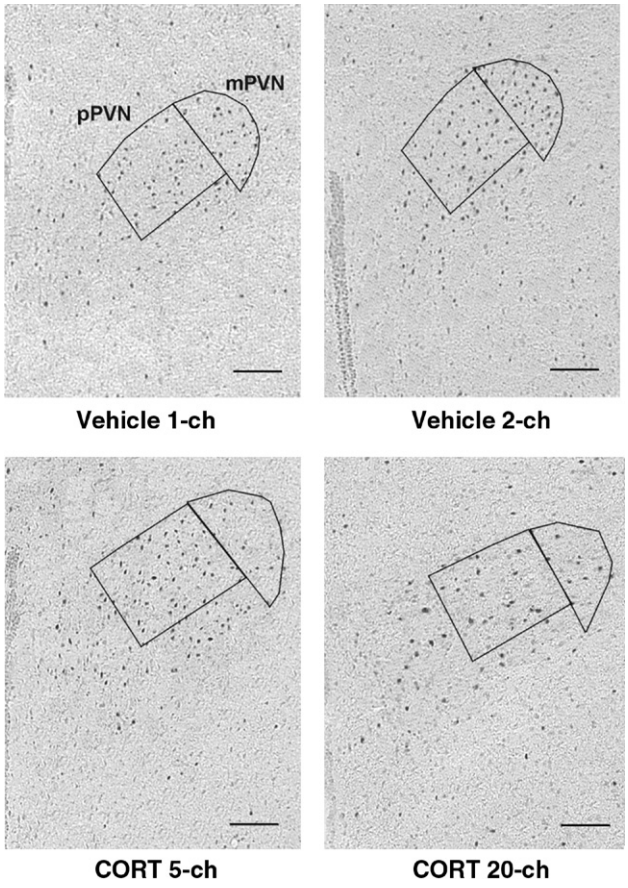

Fig. 6. Photomicrographs, showing representative expression of c-Fos in the hypothalamic nuclei (mPVN and pPVN). Slices were photographed with objective lens 10×, and than magnified digitally. Bar indicates 100 μm. For more details see experimental procedures.

Table 4
Fos expression after chronic administration of corticosterone and contextual fear test

Brain Region	Vehicle 1-ch	Vehicle 2-ch	CORT 5-ch	CORT 20-ch
pPVN	41.9±3.5	53.9±4.7	53.2±5.5	29.2±5.2 **,##
	n=7	n=8	n=6	n=5
mPVN	45.4±5.8	61.6±2.7 &&	18.0±2.3 **,&&	16.9±1.9 **,&&
	n=7	n=7	n=6	n=6
PVP	36.8±2.7	43.6±2.6	52.5±7.3	47.5±6.9
	n=8	n=8	n=6	n=6
CA1	12.4±1.5	17.0±2.1	20.1±1.7 &	21.4±1.2 &&
	n=8	n=8	n=6	n=6
CA2	14.4±0.7	14.3±0.9	22.7±3.3 *, &	21.8±2.5 *,&
	n=8	n=8	n=6	n=6
CA3	23.0±3.0	24.6±1.4	26.4±1.0	23.1±1.0
	n=8	n=8	n=6	n=6
DG	2.9±0.4	7.8±0.6 &&	5.2±0.4 **,&&	5.0±0.5 **,&&
	n=7	n=8	n=6	n=6
CeA	19.4±2.0	21.9±1.6	30.7±2.0 **,&&	32.8±1.7 **,&&
	n=7	n=8	n=7	n=6
MeA	34.7±4.9	29.3±2.3	43.9±3.2 *	25.3±1.4 ##
	n=8	n=7	n=6	n=6
BLA	31.5±1.8	32.1±2.1	29.4±2.1	31.4±2.9
	n=7	n=8	n=7	n=6
Cg1	29.7±2.2	51.0±3.3 &&	46.4±3.5 &&	51.0±2.9 &&
	n=8	n=8	n=7	n=6
Cg2	41.2±4.4	54.0±3.8	52.2±4.6	53.9±3.0
	n=8	n=8	n=7	n=6
M1	25.4±2.4	32.5±2.0 &&	48.3±2.7 **,&&	42.6±1.7 **,&&
	n=7	n=7	n=7	n=6
M2	28.2±3.6	34.3±5.9	42.1±3.2	40.5±2.4
	n=8	n=7	n=7	n=6
Cpu	28.1±2.1	27.6±3.6	33.1±1.8	30.2±3.0
	n=8	n=7	n=7	n=6
Acb	54.5±3.5	53.3±6.2	65.1±4.3	63.5±5.3
	n=8	n=5	n=6	n=6

The number of immunoreactive neurons per 0.1 mm^2. The data are shown as means±S.E.M. *N* — number of rats. Vehicle 1-ch — rats pretreated chronically with sesame oil only; vehicle 2-ch — sesame oil pretreated rat subjected to contextual fear conditioning; CORT 5-ch — rats given corticosterone at the dose of 5 mg/kg and subjected to contextual fear conditioning; CORT 20-ch — rats given corticosterone at the dose of 20 mg/kg and subjected to contextual fear conditioning. * differs from vehicle 2-ch; & differs from vehicle 1-ch; # differs from CORT 5-ch. *, & $p<0.05$; **, &&, ## $p<0.01$. For more details see experimental procedures.

was found that post-training injection of corticosterone enhanced memory of contextual fear conditioning (Cordero and Sandi, 1998). It seems, that one of the reasons for discrepancies between these and our results may be the higher dose of corticosterone applied in the present study (20 mg/kg), which could induce a much stronger suppression of CRF and the hypothalamo–pituitary–adrenal axis (HPA). The other reason may be the different time intervals between the corticosterone administration and the test procedure. Intraperitoneal corticosterone injection given immediately after training enhanced long-term expression of fear conditioning (Cordero and Sandi, 1998, Hui et al., 2004). On the other hand, pretreatment of animals with the glucocorticoid receptor antagonists 45–60 min before conditioning to the high shock intensity (1.0 mA), failed to influence the extent of fear conditioning (Cordero and Sandi, 1998). It is noteworthy, that in our experiment with similar shock intensity (0.8 mA), corticosterone was given 1.5 h before contextual fear training. These data indicate, that corticosterone may differently influence the acquisition and consolidation phase of fear conditioning.

In the present experiment, chronically corticosterone pretreated rats showed an increase in unconditioned and conditioned fear reactions (neophobia-related decrease of exploration of a novel environment, and increase in conditioned freezing reaction). It is noteworthy, that using somewhat different experimental paradigm and a higher dose of corticosterone (40 mg/kg daily for 21 days), Gregus et al. (2005) could not find significant effects on rat activity levels or fear responses in the open field or social interaction tests, 24 h after steroid hormone administration. It should be pointed out, that in our study the rats repeatedly injected with vehicle explored open field more intensively, than rats receiving an acute injection of saline, revealing habituation to the injection stress. This could be another factor contributing to the fear inducing effects of chronic corticosterone in this test (i.e. higher background exploratory activity could render the rats more vulnerable to the fear evoking effects of the treatment).

It is noteworthy, that in animals repeatedly treated with steroid hormone, the freezing reaction was enhanced, and simultaneously the immediate early gene (c-Fos) reaction to the aversive context stimuli was changed in a structure specific way. Importantly, changes in freezing behavior were not related to differences in the pain threshold or rat motor activity. It is notable, that although pain test was performed 24 h after fear conditioning and last corticosterone injection, it can be assumed that 25 days long and continuous corticosterone administration, should lead to the appearance, and stabilization of long-term changes in the central processes controlling emotions and stimulus perception. The duration of reported effects remains, however, to be established in further studies. In accordance with our results, corticosterone treated rats (2.5 mg/kg, twice a day for five and a half days), 6 days later displayed more fear-conditioned freezing in the retention test (Thompson et al., 2004). Similarly, rats receiving corticosterone (400 μg/ml) in their drinking water for 21 days, 4 days after last exposure to corticosterone also showed enhanced freezing to context in the conditioned fear test, and serum corticosterone levels were negatively correlated with contextual conditioning (Conrad et al., 2004). These data indicate a facilitating effect of repeated administration of corticosterone on the expression of conditioned and unconditioned fear reactions. It was suggested, that facilitation in retention might be due to high levels of corticosterone around the time of acquisition and consolidation, and not the time of testing (Thompson et al., 2004). Such interpretation underlines the role of specific changes in the fear-acquisition related processes. It is also possible, that the decreased levels of corticosterone during retention testing may have enhanced memory retrieval processes, as corticosterone treatment before retention testing was found to impair aversive memory retrieval in a water maze spatial task, and in a one trial inhibitory avoidance task (de Quervain et al., 1998; Pakdel and Rashidy-Pour, 2006). This is, however, indirect evidence and more studies on pre-test administration of glucocorticoids in fear conditioning are necessary.

The central processes involved in this phenomenon are not yet well recognized, however, they undoubtedly play important role in adjusting the behavior of animals to the chronic stressful situation, the biochemical expression of which is shaped by repeated corticosterone administration. As expected, aversive context stimulated c-Fos production in some of the studied brain structures (mPVN, DG, Cg1, M1). These brain areas are well known for their contribution to the processing of emotional inputs (LeDoux, 1993; Heilman and Gilmore, 1998; Davidson, 2002; Steimer, 2002; Richter-Levin, 2004). The most interesting findings show that chronic pretreatment of rats with corticosterone attenuated the effect of aversive context in the mPVN and DG, enhanced it in the M1, and stimulated c-Fos in CA1, CA2 and CeA, in comparison to control animals, not subjected to fear conditioning. Accordingly, higher levels of c-Fos expression in mice exposed to aversive context were observed in the medial parts of the M1, in the caudal and medial parts of the M2, and in the dorsal but not ventral regions of the cingulate cortex (Zvorykina and Anokhin, 2003), thus confirming the present data. It is noteworthy that these brain structures (M1 and Cg1) are parts of the frontal cortex (Diorio et al., 1993; Uylings et al., 2003), a structure that regulates, among others, processes of aversive conditioning (Nitschke et al., 2006; Steimer, 2002; Tang et al., 2005). In another experiment, several brain regions, including the primary motor cortex and the amygdala were significantly activated by re-exposure of mice to a salient versus neutral context one day after aversive training (Zhang et al., 2005). It was also found that subcutaneous implantation of slow-releasing corticosterone pellets (200 mg, 60 day release) produced an elevation of CRF mRNA in the CeA 2 weeks later, whereas CRF mRNA in the PVN was decreased to a large extent in the same rats (Makino et al., 1994). These data closely resemble our results on c-Fos expression in both brain nuclei. Chronically administered glucocorticoids facilitated CRF mRNA expression in the CeA, an essential output for fear behaviors (Anglada-Figueroa and Quirk, 2005), while restrain CRF mRNA expression in the PVN. These findings indicate that primary motor cortex and CeA play an important role in processing of emotional input, and that chronic corticosterone treatment enhances their activity, facilitating the occurrence of fear responses. Interestingly, similarly to animal experiments, functional magnetic resonance imaging demonstrated that facial expressions categorized as fearful activated, among others, also motor cortex and amygdala in Caucasians (Moriguchi et al., 2005).

Chronic corticosterone administration dose-dependently decreased the total endogenous serum corticosterone level in a challenge situation, after presentation of aversive context stimuli, as well as in rats without contextual fear conditioning, 24 h after the last dose of corticosterone. A decrease in blood concentration of corticosterone was accompanied by inhibition of activity of hypothalamic nuclei, mPVN and pPVN, in chronic glucocorticoid pretreated group. The effect in mPVN was more selective as in this nucleus corticosterone diminished also the aversive context-induced c-Fos production. The present data indicate that repeatedly administered corticosterone leads to profound changes in reactivity of the HPA axis to the aversive stimulation. This phenomenon was accompanied by decreased activity of PVN nuclei, and enhanced fear reactions. Thus, the function of HPA axis was down regulated, and less responsive to the aversive stimuli. These data point to the deep, pathological changes in the activity of this important alarm system of the organism. Similar results have been published previously by Umemoto et al. (1997). In control rats, immobilization stress induced c-Fos, fos B, jun B, NGFI-A messenger RNA in the PVN, whereas all of them except NGFI-A were significantly reduced in rats given 200 and 400 mg corticosterone implants. Moreover, chronic corticosterone administration was found to completely block immediate early gene (IEG) and CRF induction in the parvocellular neurosecretory neurons within the PVN nucleus of the hypothalamus (Kovacs and Sawchenko, 1996). As mentioned above, there appeared also a dissociation between the effects of prolonged corticosterone exposure on CRF mRNA in CeA and PVN, with facilitation of CRF mRNA expression in the CeA, and reduction of CRF mRNA in the PVN (Makino et al., 1994). It can be concluded, therefore, that chronic treatment with high doses of corticosterone inhibits the activity of the PVN, and attenuates the expression of CFR mRNA (Makino et al., 1995). This feedback mechanism most probably involves down-regulation of the hippocampal and hypothalamic glucocorticoid receptors (Lowy, 1991; Hugin-Flores et al., 2004). Accordingly, it has been shown that pretreatment of rats with high doses of corticosterone (5 mg/kg/day or 100, 200, 400 mg pellet implantation) induces strong adrenal gland atrophy (Makino et al., 1994; Umemoto et al., 1997). It should be kept in mind, however, that these results were obtained in animals with high levels of circulating corticosterone, after chronic corticosterone pellet implantation, and cannot be directly compared to the presented data (repeated, daily, corticosterone injections).

It is noteworthy, that changes in total corticosterone concentration and c-Fos expression were measured at the time of maximum of biochemical and immunocytochemical responses to the aversive event (10 min vs. 1.5 h, respectively) (Weinstock et al., 1998; Johnstone et al., 2000; Lehner et al., 2004). For this reason, the flinch–jump test, performed 5–10 min before decapitation of animals, could not affect c-Fos expression in a significant way. The short half-life time of corticosterone excludes also the possibility of a direct influence of a final hormone injection, 24 h prior to behavioral (conditioned fear test day), biochemical and immunocytochemical tests, on the results of the present study (the total plasma corticosterone half-life time was found to be about 25 min; Sainio et al., 1988),

Similarly to mPVN, chronic corticosterone treatment significantly attenuated aversive context-induced c-Fos expression also in DG. This finding is of special interest given the assumed role of the hippocampus, and particularly dentate gyrus, in the control of hypothalamic CRF synthesis and release (de Kloet et al., 2005), and can be related to adaptive down-regulation of hippocampal mineralocorticoid and glucocorticoid receptors (Lowy, 1991; Hugin-Flores et al., 2004). Indeed, there are strong arguments indicating that the dorsal hippocampus mediates the feedback of glucocorticoids on the adrenocortical response (Jacobson and Sapolsky, 1991; Feldman and Weidenfeld, 1993). Down-regulation of hippocampal steroid receptors is supposed to be accompanied by decreased neuronal activity, as reflected

by attenuation of local c-Fos expression. It is noteworthy, that monoaminergic innervation of the dentate gyrus is considered to play an essential role in processing of emotional input to the brain (cf. Plaznik et al., 1994). It should be also underlined, that in CA1 and CA2 areas of the hippocampus, aversive context did not significantly induce c-Fos, thus the criterion of the involvement of both brain structures in the control of this aversive reaction was not fulfilled. Corticosterone enhanced c-Fos production in both areas independently of aversive conditioning. The meaning of this phenomenon is not clear, and requires further studies. Finally, it seems that a decrease in neurogenesis and an increase in dendritic atrophy in prefrontal cortex and hippocampus, reported after chronic corticosterone administration (Wellman, 2001; Yu et al., 2004), probably did not play a role in the reported behavioral effects, as the conditioned fear reaction was clearly enhanced.

In sum, the present behavioral, biochemical and immunocytochemical data suggest that chronic corticosterone treatment enhances the activity of primary motor cortex and CeA with subsequent improvement of memory of aversive events, and simultaneously stimulates a negative feedback mechanism operating in PVN with ensuing decrease in blood corticosterone concentration (Fig. 7). These data may help to better understand mechanisms responsible for changes in mood and emotions induced by repeated administration of high doses of glucocorticoids.

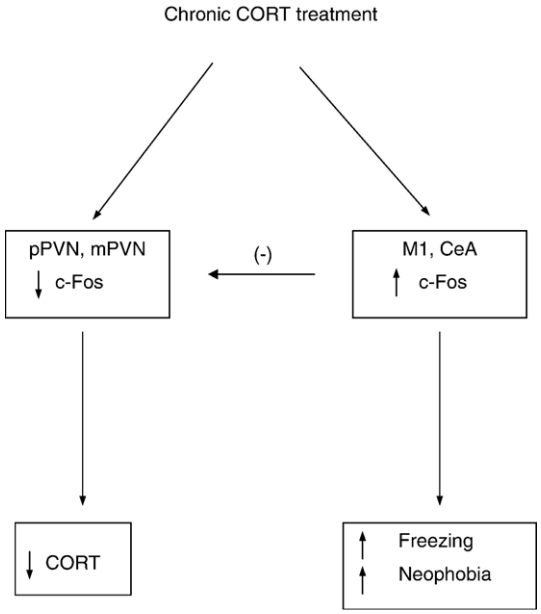

Fig. 7. A schematic representation of neural processes evoked by chronic corticosterone administration. The present study demonstrates that chronic CORT administration decreases plasma CORT concentration, exploratory behavior, and enhances rat freezing response. In addition, repeated administration of corticosterone attenuates c-Fos expression in the pPVN, and mPVN, and stimulates this immunocytochemical reaction in CeA, Cg1 and M1. Imaki et al. (1992) reported that induction or inhibition of c-Fos protein production in PVN reflected changes in CRF mRNA expression. Repeated administration of CORT reduced CRF mRNA expression in the PVN, and facilitated CRF mRNA synthesis in the CeA, in a dose dependent manner (Makino et al., 1994, 2002). Increased CRF levels in the central nucleus of the amygdala were related to freezing, neophobia behavior, and inhibition of locomotor activity (Holsboer, 1999; Koob and Heinrichs, 1999). It can be tentatively hypothesised, that CeA and M1 can control the hypothalamic–pituitary–adrenal axis by enhancing glucocorticoid negative feedback mechanism and reduction in CRF mRNA in the PVN, with resulting decreased plasma CORT concentration. On the other hand, enhancement by chronic corticosterone of activity of primary motor cortex and CeA may lead to improvement of memory of aversive events. CORT — corticosterone; M1 — primary motor cortex; CeA — central nucleus of the amygdala; mPVN — magnocellular hypothalamic nucleus; pPVN — parvocellular hypothalamic nucleus; c-Fos — c-Fos protein, a marker of changes in neuronal activity. ↓decrease; ↑increase; (−) — inhibition.

Acknowledgements

The study was supported by Grant No. 62/2994 from the Institute of Psychiatry and Neurology in Warsaw. The authors thank Mrs. Ala Biegaj for the excellent technical assistance.

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