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# Maternal corticosterone influences behavior, stress response and corticosteroid receptors in the female rat

A. Catalani*, P. Casolini, G. Cigliana, S. Scaccianoce, C. Consoli, C. Cinque, A.R. Zuena, L. Angelucci

*Department of Human Physiology and Pharmacology, University "La Sapienza" P.le Aldo Moro 5, Rome 00185, Italy*

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**Abstract**

In infancy, glucocorticoids have been shown to affect hypothalamus–pituitary–adrenal (HPA) axis activity and behavior. Both the activity of the HPA axis and many aspects of behavior exhibit important gender-dependent differences physiologically. In our previous studies, male offspring of hypercorticosteronemic mothers show long-lasting changes of learning as well as adrenocortical activity. In the light of these findings, this study aims to determine the long-term effects of glucocorticoids in the early stages of life in female rats. Corticosterone (200 μg/ml) was added to the drinking water of the dams. Female offspring exhibited lower adrenocortical secretory response to stress, improvement in learning (water maze at 21, 30 and 90 days; active avoidance at 15 months) and reduced fearfulness in anxiogenic situations (dark–light test at 1 and 15 months; conditioned suppression of drinking at 3 months; plus maze at 15 months) after weaning, from 21 days up to 15 months of age, but not before. No difference in hippocampal adrenocorticoid receptors was observed. These results, together with previous data on male offspring, show that the outcomes of maternal hypercorticosteronemia on hormonal stress response and behavior are similar in males and females, but the effects on some aspects of the HPA axis activity are gender-dependent. Possible explanations for these differences are discussed. 

*Keywords:* Maternal corticosterone; Female offspring; Behavior; Glucocorticoid receptors; Stress response

## 1. Introduction

In animal research, neuroendocrine and behavioral effects of neonatal exposure to glucocorticoids have been studied using two different approaches. Glucocorticoids — directly administered to the pups by injection, gavage or pellet implantation — disrupt learning (Olton et al., 1974; Vicedomini et al., 1986; DeKosky et al., 1982; Golub, 1982; Pavlovska Teglia et al., 1995), alter the hypothalamus–pituitary–adrenal (HPA) axis activity (Turner and Taylor, 1976; Erskine et al., 1979; Nyakas and Endroczi, 1972) and reduce hippocampal adrenocorticoid receptors (Zoli et al., 1990; Felszeghy et al., 1996) in the adult rat. Another experimental approach consists of increasing the plasma concentrations of corticosterone (CORT) in the mothers and, consequently via the milk in the pups, adding the hormone to the drinking water of the mothers. Our previous studies have shown that adult rats, lactated by mothers supplemented with CORT, show good performance in a conditioned learning test, better spatial memory, attenuated fear–behavioral response, lower stress-induced CORT secretion and higher hippocampal adrenocorticoid receptors than controls (Catalani et al., 1993, 2000). The above-mentioned studies have all been conducted using male animals. It should be borne in mind that in many aspects of behavior, as well as HPA activity, marked differences exist between male and female rats. Two types of adrenocorticoid receptors have been identified (de Kloet, 1991). Sexual dimorphism in the distribution of Type II corticosteroid receptors immunoreactivity in the rat hippocampus (Ahima et al., 1992) has been demonstrated. Moreover, a study indicates higher Type I receptors in females over males in various brain areas (McCormick et al., 1992) and sex-dependent differences in the affinity of both Types I and II corticosteroid receptors with reduced affinity in females

* Corresponding author. Tel.: +39-64-991-2513; fax: +39-64-991-2513.

*E-mail address*: assia.catalani@uniroma1.it (A. Catalani).

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(Turner, 1992). The activity of the HPA is enhanced in female rats that show a higher stress-induced CORT secretion compared to males (Kitay, 1961). There is a marked sex difference in the pattern of the adrenocortical function at rest, with higher maximal levels and mean 24-h CORT concentrations in females than in males (Critchlow et al., 1963). Concerning behavioral paradigms, female rats exhibit superior performance in active avoidance tasks (Barrett and Ray, 1970), are more active in an open field test (Valle, 1970) and show lower immobile behavior in the forced swimming test for depression (Alonso et al., 1991). Moreover, exposure to inescapable shocks, before the plus-maze test for anxiety, resulted in a suppression of total motility and rearing and a reduction of time spent in the open anxiogenic arms of the maze, in males but not in females (Steenbergen et al., 1990). It is worthwhile recalling that in humans, sex-related mood disorders, associated with HPA axis activity, are more prevalent in women than in men (Young, 1998). Moreover, several reports have shown the importance of the gender factor in the pathogenesis of some diseases (Duquette and Girard, 1993; Payami et al., 1996; Dohrenwend and Dohrenwend, 1976).

In light of the above-mentioned findings on the effects of an increase of glucocorticoids in infancy in male rats, and considering the sex differences observed in HPA axis activity, we have examined the effects of the maternal increase of CORT on basal and stress-induced CORT secretion, hippocampal adrenocorticoid receptors and different aspects of behavior in the female offspring at different ages from early life to 15 months of age.

## 2. Experimental procedures

### 2.1. Animals

Female COBS Wistar rats (Charles River, Calco, Lecco, Italy), weighing 280–320 g, were housed in a temperature-controlled room (22–25 °C) and maintained on a 12-h light/dark cycle (light on 0800 h); food (Standard Diet Charles River 4RF21) and water were available ad libitum. Females were mated with sexually experienced male rats; one female and one male per cage were left undisturbed for 1 week; after this time, females were housed individually. The day of birth was counted as Day 0, and the next day, litters were culled to eight pups (four males and four females). Some mothers were maintained on tap water, while others had free access to a solution of 200 μg/ml CORT hemisuccinate (Agrar, Rome, Italy). The daily intake of CORT was 13.5±1.5 mg/rat. This dose was selected as previous studies in our laboratory have shown that 200 μg/ml CORT in the drinking water significantly increases the basal plasma levels of the hormone in 10-day-old sucklings from 0.7±0.1 to 1.2±0.2 μg/100 ml in CORT-nursed animals (CORT-nursed) (Catalani et al., 1993) and in the lactating mothers from 4.3±0.5 to 9.5±1.8 μg/100 ml. Sawdust was changed on the 15th day postpartum; otherwise, mothers and pups were left undisturbed until weaning. Moreover, on the 15th day postpartum, the bottles were heightened in order for pups to avoid drinking the CORT solution. Weaning was performed at 21 days of age and animals were housed three per cage. Only female rats were used in this study. To avoid litter effect, each litter contributed one or maximum of two offspring to make a group. We had nine groups of controls and nine of CORT-nursed animals, each of eight animals. Table 1 summarizes the neuroendocrine, biochemical and behavioral parameters studied in different groups of different ages. As shown in the table, some behavioral tests were performed in groups of animals that previously underwent other tests to minimize the number of animals used in this study. All experiments were performed during morning hours. All animal studies were conducted in accordance with the rules laid down by the Italian Ministry of Health.

### 2.2. Plasma levels of CORT in basal and stress conditions

At 11 and 16 days of age, one pup was taken from each litter for the determination of basal plasma levels of CORT and one pup for the determination of plasma levels of CORT after maternal separation stress. Pups were removed from the cage where they lived with their mothers and placed in a different cage for 15 min. They were then killed by decapitation and the blood collected. At 3 months of life, rats were surgically implanted, under pentobarbital (60 mg/kg ip) anesthesia, with a permanent cannula in the right atrium through

Table 1
Parameters studied in controls and CORT-nursed offspring at different ages

| | Age | | | | | |
|---|---|---|---|---|---|---|
| Groups | 11 days | 16 days | 21 days | 30 days | 3 months | 15 months |
| 1 | a.w. b.w. cort. bas/stress | – | – | – | – | – |
| 2 | – | a.w. b.w. cort. bas/stress | – | – | – | – |
| 3 | – | – | – | – | a.w. b.w. cort. bas/stress | – |
| 4 | – | – | – | a.w. b.w. receptors | – | – |
| 5 | – | T maze | – | – | – | – |
| 6 | – | – | Water maze | – | – | Active avoidance |
| 7 | – | – | – | Water maze | Drinking | Plus maze |
| 8 | – | – | – | – | Water maze | Dark–light |
| 9 | – | – | – | Dark–light | – | – |

a.w. = adrenal weight; b.w. = body weight; cort. bas/stress = CORT basal/stress; drinking = water intake in conditioned suppression of drinking test; receptors = Types I and II hippocampal corticosteroid receptors.

the jugular vein. The rats were allowed to recover for 4 days after the operation. The rat was removed from the cage and placed in a Plexiglas restraint device; the tail gate was adjusted to contain the rat firmly without impairing circulation to the limbs. The rat was restrained for 2 min and then immediately returned to its cage. Blood samples for the basal condition were withdrawn before the start of the stress (Time 0); additional samples were obtained 15, 30 and 60 min after stress. The time taken to sample was less than 20 s and the volume for each sample was 600 μl. After sampling, the rats were immediately reinfused with an isovolumetric amount of isotonic saline.

Blood was collected in tubes containing EDTA (2 mg/ml blood) used as an anticoagulant. After centrifugation at $1900 \times g$ at 4 °C for 20 min, plasma was removed, washed with 5 vol of isooctane to remove estrogens and kept frozen at −75 °C for later hormone determination. Plasma CORT concentrations were determined with the method described by Murphy (1969) using plasma from female Wistar rats as the source of the CORT binding globulin. The sensitivity of the assay was 0.4 ng/tube and the inter- and intraassay variations were 7.6% and 3.6%, respectively, at the mean value of 1.3 ng/tube, and 10% and 5.7%, respectively, at the mean value of 4.6 ng/tube.

### 2.3. Types I and II hippocampal corticosteroid receptor binding at 30 days of life

An exchange assay was used for both Types I and II corticosteroid receptors as already described (Casolini et al., 1993, 1997; Henry et al., 1994; Maccari et al., 1995) with some modifications. In particular, for the Types I and II receptor assay, a single saturation concentration (maximum individual binding) of each radioligand was used. Aliquots of cytosol (140 μl) were incubated with titrated CORT (specific activity 72 Ci/mmol; New England Nuclear, Italy) at a concentration of 20 nM, and with a 100-fold excess of unlabeled RU 28362 (Roussel-UCLAF, Paris, France). Unlabeled RU 28362 was used to displace $^{3}$H-CORT from Type II receptors (Moguilewsky and Philibert, 1984). Type II receptor binding was evaluated directly, using pure glucocorticoid $^{3}$H-RU 28362 (specific activity 74.3 Ci/mmol; Roussel-UCLAF) at a concentration of 20 nM. Nonspecific binding for $^{3}$H-CORT was determined in the presence of a 500-fold excess of unlabelled CORT; no specific binding for $^{3}$H-RU 28362 was determined in the presence of a 500-fold excess of unlabelled RU 28362.

### 2.4. Learning tests

#### 2.4.1. Morris water maze

The apparatus consisted of a circular pool (diameter 120 cm, height 60 cm). The training procedure is the same as that described previously (Catalani et al., 1993). Briefly, rats underwent eight trials a day for two consecutive days; rats that did not find the platform were allowed to swim for 60 s and returned to the platform for a further 60 s. The critical measure of the performance was the time elapsed (latency) before the animal located and climbed onto the platform. A session with a visible platform was performed to assess the swimming speed of the two groups of animals.

#### 2.4.2. Aquatic T maze

In this test, rats are trained to escape to a hidden platform located at the end of one arm of the water-filled T maze. To solve this problem, the animal must remember the outcome of a forced run (in which the arm of the maze without the platform is blocked) and use this information to guide its behavior towards a free choice run (in which no arm is blocked and the platform is in the previously unblocked arm). The intertrial interval was 10 s. The criterion is satisfied when the animal makes nine correct choices in 10 consecutive trials. The apparatus and the procedure are similar to those described by Castro et al. (1987).

#### 2.4.3. Conditioned avoidance learning

The apparatus used was a conventional shuttlebox (U. Basile, Italy) divided into two identical compartments by a partition with an opening to allow the rat to move between the compartments. Each compartment was illuminated by a 10-W bulb hanging above the Plexiglas cage lid. Foot shock was delivered through the stainless steel grid floor. Trial sessions were programmed on a schedule of conditioned stimulus (CS = light on), followed 3 s afterwards by an unconditioned stimulus (US = a 4-s, 0.2-mA electric shock). Thus, the rat had 3 s to avoid shock and escape to the adjacent compartment. Responses during CS were considered as avoidances. Four consecutive daily sessions of 20 trials at 1-min intervals were performed: the rat was placed in one compartment and the start of the trial was signaled by the CS followed by delivery of the US. The number of passages from one compartment to the other during the intertrials was recorded.

### 2.5. Tests of anxiety

#### 2.5.1. Elevated plus-maze test

The elevated plus maze consisted of two open arms ($50 \times 10$ cm) and two enclosed arms of the same size with 40-cm-high walls, divided so that one side of the plus maze stood opposite to an identical side (i.e., either open or enclosed) with 10 cm$^2$ square in the middle. The device was made of wood and raised to a height of 50 cm from the floor (Pellow et al., 1985). One-year-old offspring were placed in the middle of the maze, and the time and number of episodes in the open arms as well as total entries in dark and illuminated arms were recorded.

#### 2.5.2. Dark–light test

The plastic test box ($80 \times 40 \times 50$ cm high) was positioned on a bench 1 m above floor level. The box was

open-topped, two-fifths painted black, illuminated by a red light and partitioned from the remainder of the box, which was painted white and brightly illuminated (60-W light source). The compartments were connected by an opening ($6 \times 7$ cm) located at floor level in the middle of the partition. In most experiments, rats were placed in the middle of the white, brightly lit area and the operator withdrawn from the room. Three behaviors were noted: (a) the number of transitions between the two compartments; (b) the time spent in the white and black areas; and (c) the latency of the initial movement from the white to the black area.

#### 2.5.3. Conditioned suppression of drinking

A modified Vogel (Vogel et al., 1971) conflict test procedure was used. The testing chamber consisted of a plastic animal cage ($37 \times 26 \times 13$ cm) with metal floor and top. Protruding from one wall was a metal drinking tube containing a wired stainless steel coil. The tube was attached to a calibrated ($\pm 0.5$-ml units) glass tubing to measure the water intake. The tube was also connected to a shocker and when the rat drunk, it completed a circuit between the floor and the metal drinking tube, which resulted in the delivery of a constant intensity current (210 μA). Water-deprived (48 h without water) offspring in their home cage were moved to the test chamber and allowed to drink without electric shock for 10 min. After 24 h of free access to water in their home cage followed by 48 h of water deprivation, subjects were tested singly in a 10-min session with the shocker connected and water intake was measured. The conflict situation was represented by the fact that the licking of the drinking tube was simultaneously rewarded by water and punished by electric shock.

### 2.6. Statistical methods

Body and adrenal weights were analyzed using a two-way ANOVA (Treatment × Age) followed by comparisons among groups. Maximal individual binding (fmol/mg protein) of corticosteroid receptors was analyzed using the Student's $t$ test. Plasma levels of CORT in basal and stress conditions were analyzed using a three-way ANOVA (Treatment × Condition × Age), followed by a two-way ANOVA (Treatment × Condition) for different ages and by a post hoc Scheffé's test, when appropriate. Paired $t$ test was used to compare stress vs. basal plasma levels of CORT in samples from cumulative rats. Latency values in the Morris water maze were analyzed by a four-factor mixed-design ANOVA with independent factors of Treatment (water or CORT) and Age (21, 30 and 90 days of life) with repeated measures on Day (Days 1 and 2) and in Trials (1–8). The data were further analyzed separately for the different ages at different days using a two-way ANOVA (Treatment × Trial) with repeated measures in trials. Behavioral data in the active avoidance were analyzed both with ANOVA for repeated measures and Student's $t$ test for simple sessions. Dark–light test data were analyzed using two-way ANOVA (Treatment × Age) and post hoc Scheffé's test. Conditioned suppression of drinking and plus-maze data were analyzed using the Student's $t$ test.

## 3. Results

### 3.1. Influence of maternal hypercorticosteronemia on adrenal and body weights

The ANOVA $2 \times 4$ (Treatment × Age) showed a significant interaction between treatment and age [$F(3,56) = 4.18$, $P < .001$]. In order to clarify the nature of interaction, data referring to different ages were analyzed separately. CORT-nursed offspring had lower adrenal weights compared to controls before weaning [$F(1,14) = 33.4$, $P < .001$ at 11 days of age; $F(1,14) = 6.6$, $P < .05$ at 16 days]. This difference was no longer present at 30 and 90 days of age. No difference in body weights was observed between the two experimental groups at 11, 16, 30 and 90 days of age (Table 2).

### 3.2. Influence of maternal hypercorticosteronemia on basal and stress-induced CORT secretion before and after weaning

Before weaning, three-way ANOVA revealed a basal/stress effect [$F(1,56) = 124$, $P < .001$], an age effect [$F(1,56) = 167$, $P < .001$] and interaction between age and basal/stress [$F(1,56) = 71$, $P < .001$]. In order to clarify the nature of interaction, data referring to 11- and 16-day-old pups were analyzed separately. As shown in Fig. 1, 11-day-old CORT-nursed pups showed higher concentrations of CORT in basal conditions [$t(14) = 1.7$, $P < .05$] and stress conditions [$t(14) = 1.5$, $P = .07$] (short of significance), probably due to the transfer of exogenous CORT from mothers to pups via the milk. No difference between controls and CORT-nursed pups was observed at 16 days of life both in basal and stress conditions. A basal/stress effect was present both at 11 and 16 days, as demonstrated

Table 2
Body and adrenal weight of controls and CORT-nursed offspring at different ages

| | Adrenal weight (mg) | | Body weight (g) | |
|---|---|---|---|---|
| Age | Controls | CORT-nursed | Controls | CORT-nursed |
| 11 days | 2.90 ± 0.10 | 2.10 ± 0.11*** | 27.50 ± 2.81 | 28.50 ± 1.85 |
| 16 days | 5.22 ± 0.19 | 4.67 ± 0.14* | 36.33 ± 0.98 | 34.54 ± 0.89 |
| 30 days | 17.90 ± 1.03 | 16.50 ± 0.80 | 101.33 ± 7.58 | 99.57 ± 3.46 |
| 90 days | 54.60 ± 1.80 | 59.26 ± 2.53 | 214.56 ± 2.44 | 220.82 ± 2.97 |

Mean ± S.E.M.; ANOVA.
* $P < .05$ vs. controls ($n = 8$/group).
*** $P < .001$ vs. controls ($n = 8$/group).

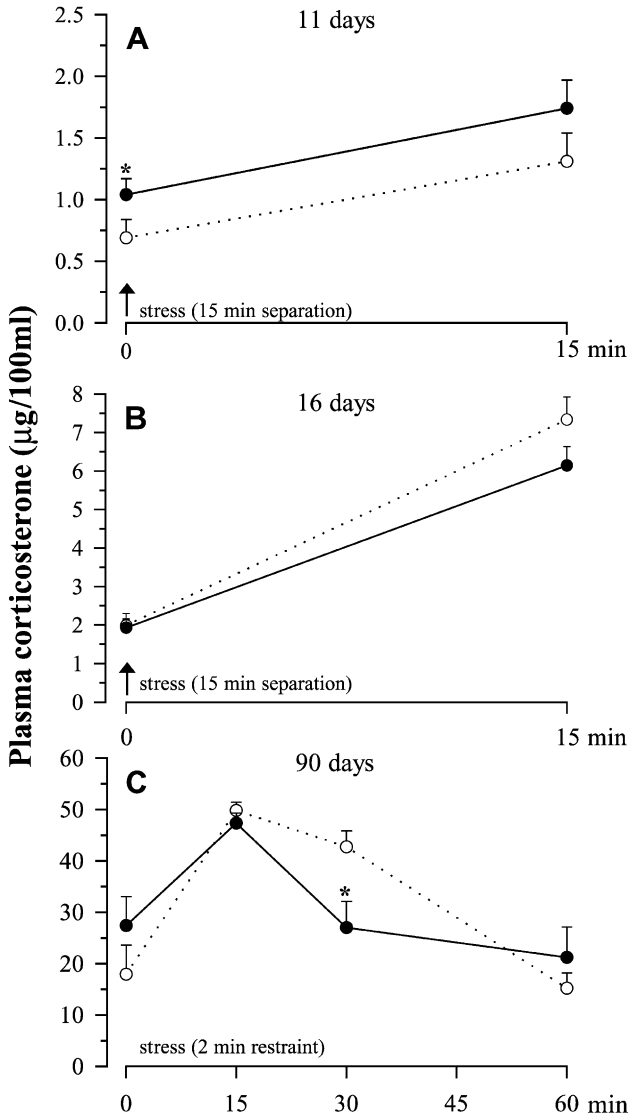


Fig. 1. Plasma CORT concentrations in basal and stress conditions in controls (○- - - - - -○) and CORT-nursed offspring (●——●) at different ages. (A,B) Fifteen minutes of maternal separation at 11 and 16 days, respectively. (C) Two-minute restraint stress at 90 days of age. Mean ± S.E.M. *$P<.05$ vs. controls (Student's $t$ test; $n=8$/group). For statistical details, see the Results section.

by the high increase of CORT secretion after 15 min of separation from the dams [$t(14)=2.6$, $P<.001$ and $t(14)=7.3$, $P<.001$, respectively, in 11- and 16-day-old controls; $t(14)=2.7$, $P<.01$ and $t(14)=7.7$, $P<.001$, respectively, in 11-and 16-day-old CORT-nursed offspring] (Fig. 1).

In the 3-month-old CORT-nursed offspring, the peak (15 min) CORT response to restraint stress was unaffected, while at 30 min, a lower level was observed. At this time, the CORT-nursed offspring, but not the controls, had already returned to resting values [paired $t$ test: $t(14)=0.7$, n.s., for CORT-nursed and $t(14)=3.9$, $P<.01$, for controls]. No difference in basal ($t=0$) values was observed between the two groups. The CORT concentrations at $t=0$, although high, should be viewed as basal values in the female gender, considering the method used for the determination (see Statistical Methods).

### 3.3. Influence of maternal hypercorticosteronemia on corticosteroid receptors

No statistical difference in the $B_{max}$ of Types I and II hippocampal corticosteroid receptors between the two groups was found at 30 days of age (Type I = controls: 130.8 ± 13.4; CORT-nursed: 108.1 ± 12.7; Type II = controls: 379.5 ± 48.4; CORT-nursed: 368.9 ± 20.7 fmol/mg protein; $n=8$/group).

### 3.4. Influence of maternal hypercorticosteronemia on learning/retention performance

#### 3.4.1. Aquatic T maze

In the aquatic T maze, before weaning at 16 days of life, both control and CORT-nursed pups failed to satisfy the criterion of nine correct choices in 10 consecutive trials in 6 days of training (correct choices/totals: controls 7.8 ± 0.7/16 and CORT-nursed 7.7 ± 1.5/16 at the third day of training; data not shown).

#### 3.4.2. Morris water maze

Fig. 2 shows the escape latencies averaged over blocks of two trials in the 2 days of testing in the Morris water maze at 21, 30 and 90 days of life. Overall ANOVA, including all factors, showed that treatment had an effect: offspring of CORT-supplemented mothers learned to locate the hidden platform faster than controls [Treatment: $F(1,252)=37.66$, $P<.001$] and escape latencies improved with training, as indicated by the significant effect of Trials [$F(7,252)=23.3$, $P<.001$] and Days [$F(1,252)=102.1$, $P<.001$]. Furthermore, offspring performed differently depending on Age [$F(2,252)=10.52$, $P<.001$], e.g., at the last trial in the first day of training, young animals of 21 days of age were not able to reach the platform with latencies as short as those attained by older animals (3 months). The results, further analyzed by a two-way ANOVA (Treatment × Trials) for the three ages and for the 2 days separately, provided evidence of a significant effect of treatment at all ages and in both days of training. At the age of 21 days, latencies of CORT-nursed offspring were lower than controls in the second day of training [$F(1,42)=10.1$, $P<.01$]. At 30 and 90 days of life, treated animals performed better both in the first and the second day of training [$F(1,39)=6.6$, $P<.05$ and $F(1,39)=28.9$, $P<.001$ at 30 days of age, in the first and second day of training, respectively; $F(1,48)=7.5$, $P<.01$ and $F(1,48)=7.9$, $P<.01$ at 90 days of age, in the first and second day of training, respectively]. No difference was recorded in the swimming speed as demonstrated by the similarity between the two groups

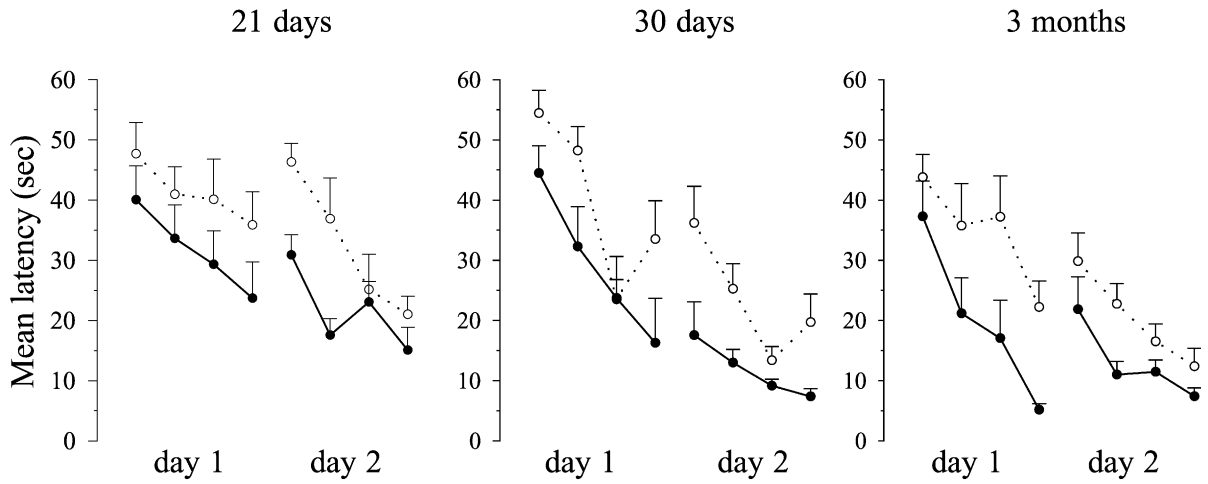


Fig. 2. Mean latencies (±S.E.M.) to escape onto the hidden platform in the water maze of controls (○- - - - - - - -○) and CORT-nursed (●————●) offspring at different ages ($n$=8/group). For statistical details, see the Results section.

in the latency to find the platform when visible (controls: 6.2 ± 1.2 s and CORT-nursed offspring: 6.9 ± 1.3 s at 3 months of age).

*3.4.3. Conditioned avoidance learning*

Table 3 shows the percentage of avoidance responses in the two-way active avoidance test at 15 days of age. The ANOVA for repeated measures revealed better acquisition of CORT-nursed offspring compared to controls [$F(1,34) = 5.2$, $P < .05$]. Student's $t$ test performed on the data of the single days showed a higher number of avoidances in CORT-nursed animals at Day 3 [$t(12) = 2.5$, $P < .01$] and Day 4 [$t(12) = 3.2$, $P < .01$] of training. No difference in the number of passages from one compartment to the other during the intertrial was observed between the two experimental groups (data not shown).

*3.5. Influence of maternal hypercorticosteronemia on behavioral response conflict and anxiogenic situations*

*3.5.1. Plus-maze test*

At 15 months of age, the percentage of time spent in the open arms of the elevated plus maze was higher in CORT-nursed animals compared to controls [controls: 37.3 ± 3.5; CORT-nursed 49.1 ± 5.4; $t(14) = 1.8$, $P < .05$]. No difference was observed in the total motor activity (arm entries), the percent open arm entries and the latency of the initial movement between the two groups (data not shown).

*3.5.2. Dark–light compartment test*

CORT-nursed animals showed a higher percentage of time spent in the light compartment compared to controls, as demonstrated by the effect of treatment [$F(1,28) = 16.6$, $P < .001$] in the two-way ANOVA (Treatment × Age) and by post hoc comparisons ($P < .05$ at 30 days and $P < .01$ at 15 months of age). A two-way ANOVA of the latencies of the initial movement revealed a decrease of this parameter with increasing age, both in controls and in CORT-nursed offspring [$F(1,28) = 8.63$, $P < .001$]. No differences between groups were observed in the total transitions (Table 4).

*3.5.3. Conditioned suppression of drinking*

In the conditioned suppression of drinking test, 3-month-old CORT-nursed offspring assumed a significantly higher amount of water compared to controls [(5.8 ± 0.5 vs. 3.9 ± 0.6 ml; $t(14) = 2.5$, $P < .05$].

Table 3
Learning in the active avoidance test expressed as percent of avoidances in 15-month-old control and CORT-nursed rats

| Days | Controls | CORT-nursed |
|---|---|---|
| 1 | 6.3 ± 3.5 | 4.0 ± 1.3 |
| 2 | 31.7 ± 11.4 | 52.0 ± 10.3 |
| 3 | 50.5 ± 10.5 | 84.0 ± 5.0** |
| 4 | 67.1 ± 6.65 | 88.9 ± 1.9** |

Mean ± S.E.M.; Student's $t$ test.
** $P < .01$ vs. controls ($n$=8/group).

Table 4
Behaviour in "dark–light compartment test"

| | 30 days | | 15 months | |
|---|---|---|---|---|
| Variable | Controls | CORT-nursed | Controls | CORT-nursed |
| Total transitions | 3.6 ± 1.0 | 6.5 ± 1.3 | 5.5 ± 1.1 | 7.9 ± 0.6 |
| Percent light time | 24.9 ± 5.8 | 45.8 ± 6.3* | 25.5 ± 5.4 | 47.3 ± 3.4** |
| Latency of the initial movement | 46.8 ± 18.4 | 77.0 ± 20.8 | 19.0 ± 3.9 | 25.4 ± 4.0 |

Mean ± S.E.M.; ANOVA.
* $P < .05$ vs. controls ($n$ = 8/group).
** $P < .01$ vs. controls ($n$ = 8/group).

## 4. Discussion

This study has investigated the effect of maternal exposure to CORT during lactation on some neuroendocrine and behavioral characteristics of the female progeny from 11 days to 15 months of life. We have found, as summarized in Table 5, that compared to controls, the female offspring of mothers that consumed CORT exhibit: (i) higher plasma concentrations of CORT in basal and stress conditions at 11, but not at 16, days of life, and a faster return to basal values after stress at 3 months; (ii) lower adrenal weights at 11 and 16 days, but not after weaning; (iii) no difference in the individual maximal binding capacity of hippocampal Types I and II corticosteroid receptors at 30 days of life; (iv) better performances in the spatial memory test and conditioned avoidance learning from weaning to 15 months of life, but not in the preweaning period; and (v) attenuated fear-related behavioral response from 1 to 15 months of life.

Concerning the hormonal profile of offspring before weaning, we have shown that, compared to controls, 11-day-old CORT-nursed pups have higher plasma concentration of CORT both in basal and stress conditions. It is reasonable to assume that in CORT-nursed pups, the plasma levels of the hormone were higher because of the maternal contribution through the milk. In 16-day-old pups, no difference was observed in basal and stress CORT secretion between groups. This is not surprising considering that, as pups grow up, dams leave the nest for longer periods and pups partially feed on rat chow as well; when the pups were 16 days old, presumably most mothers were not nursing at the moment of sacrifice; consequently, at that time, the pups did not receive any CORT through maternal milk.

It is important to note that our manipulation did not affect the physiology of the HPA axis during ontogenesis. In fact, the age-dependent increase of CORT was not influenced. This is demonstrated by the fact that the basal plasma concentrations of CORT, both in controls and CORT-nursed offspring, increased progressively from 11 to 16 days. Also, the so-called "hyporesponsive period" (Walker and Vrana, 1993) was not affected, since a lower response to stress was observed in controls and CORT-nursed pups of 11 days compared to those of 16 days of life. Thus, the hormonal alteration we induced in the mother and, through the milk, in the offspring, could be indicated as a good experimental model for the study of the role of CORT in the ontogeny of HPA axis and related behavior, as it respects the normal ontogenetic development of the HPA axis.

Data from HPA axis activity in 3-month-old rats showed that basal plasma concentrations of CORT were unaffected in the offspring of CORT-supplemented mothers, while their hormonal stress response was influenced. In fact, although the 15-min response to restraint stress was similar in controls and CORT-nursed animals, a faster shutoff of the stress response was observed in CORT-nursed offspring. This might indicate that maternal treatment lastingly enhances the efficacy of negative feedback mechanism(s) in the offspring. The hippocampus, the hypothalamus, via the PVN, and the pituitary are the structures most likely involved in this phenomenon and thus possibly affected by maternal hypercorticosteronemia. On the basis of our previous studies, we have focused our attention on the hippocampus where we have measured adrenocorticoid receptor density which, as is known (Reul and de Kloet, 1985), reflects the capability of this structure to convert a hormonal signal into a feedback action. The present data on female rats have shown that there is no difference between controls and CORT-nursed animals in both Types I and II corticosteroid receptors, apparently ruling out the participation of the hippocampus in the differences observed between controls and CORT-nursed offspring with regard to HPA axis activity after stress. Consequently, it can be postulated that increased feedback control originates from the hypothalamus or/and the pituitary. Alternatively, or concomitantly, it may be that maternal hypercorticosteronemia has affected the hypothalamic release of the corticotropin secretagogues needed to maintain ACTH release after stress. In the context of this hypothesis, vasopressin, which is known to control the later (10–20 min), but not the earlier (0–10 min), phases of stress response (Rivier and Vale, 1983), could be the likely factor reduced by maternal hypercorticosteronemia. However, we should also consider that maternal hypercorticosteronemia might have altered the gonadal hormones of the progeny which, in turn, may have affected the corticosteroid receptors in the hypothalamus. In

Table 5
Behavioral and neuroendocrine characteristics of female CORT-nursed offspring at different ages compared to controls

| | Age | | | | | |
|---|---|---|---|---|---|---|
| Parameters | 11 days | 16 days | 21 days | 30 days | 3 months | 15 months |
| Adrenal weight | Reduced | Reduced | | No changes | No changes | |
| Learning in the water maze test | | | Improved learning | Improved learning | Improved learning | |
| Learning in the active avoidance test | | | | | | Improved learning |
| Anxiety in the dark–light test | | | | Reduced fearfulness | | Reduced fearfulness |
| Anxiety in the plus-maze test | | | | | | Reduced fearfulness |
| Anxiety in the drinking test | | | | | Reduced fearfulness | |
| Hippocampal adrenocorticoid receptors | | | | No effect | | |
| Stress-induced CORT plasma concentration | No effect | No effect | | | Reduced | |

The absence of indication indicates that the parameter was not determined.

fact, ovariectomy increases Type II mRNA in the hypothalamus (Peiffer et al., 1991), rather than in the hippocampus, making the hypothalamic negative feedback tone more pronounced. Moreover, since receptors and CORT concentrations have been measured at different times, the observed lack of a relationship between the two could also be explained by a different time course of the changes of both receptors and CORT that, although hypothetical, cannot be excluded. Finally, the idea that the reduced stress response originates from an hypofunction of a peripheral endocrine organ can be discarded. In fact, adrenal weights were reduced during lactation due to the CORT-supplemented maternal milk, but not after weaning.

The effect of the increase of maternal CORT on the learning performance of the progeny was studied throughout their life span (i.e., from 16 days to 15 months of age). At 16 days, as partially expected (Castro et al., 1987), both controls and treated animals failed to solve conditional–spatial discrimination problems in the T maze. Thus, the treatment did not accelerate the development of learning processes. The spatial learning in the Morris water maze was enhanced in CORT-nursed animal at 21, 30 and 90 days of life; motility differences can be excluded, since when probing the animals with the visible platform, the swimming speed was similar in the two experimental groups. Conditioned acquisition learning also was improved at 15 months. The similar number of intertrial passages from one compartment to the other of the two groups rules out the possibility that differences in locomotor activity could have interfered with the interpretation of the results. Thus, the cognitive ability of the progeny is permanently improved by a moderate increase in maternal CORT, possibly indicating that this stress hormone plays a beneficial role. It should be emphasized that cognitive enhancement becomes manifest only after weaning, when the shortening of the hormonal stress response of the offspring is evident. A role for circulating levels of glucocorticoids has been claimed in cognitive processes. It is well known that high elevations of plasma CORT occur in the early phase of the water maze or active avoidance acquisition (Coover et al., 1973). At any rate, performance in some learning tests can be worsened by excessive and/or long-lasting circulating levels of glucocorticoids at the time of testing, although it is impossible to conceive avoidance or learning behavior without arousal. In fact, it has been demonstrated that CORT concentrations are significantly higher in cognitively impaired animals in the Morris water maze (Issa et al., 1990), but, on the other hand, the removal of CORT by adrenalectomy is responsible for water maze impairment (Conrad and Roy, 1995; Islam et al., 1995). We can speculate that the better performance of CORT-nursed rats in our study is ascribed to the fact that, after the initial increase of plasma CORT concentrations in the early stages of the learning test, the return to basal values is faster compared to controls, as evidenced by stress data.

Female rats nursed by hypercorticosteronemic dams show attenuated fear in three different situations: they spent more time in the elevated arms than in the more secure closed arms of the maze and drank more water despite the punishment in the conditioned suppression of drinking. Moreover, in a third conflict procedure, used extensively in the study of anxiety, CORT-nursed animals spend more time exploring an illuminated area than a dark one, where they naturally feel more secure. Longer exploration in the more anxiogenic area observed in procedure-naive animals at 30 days allows us to draw the conclusion that the decrease in fearfulness at 15 months is not influenced by the previous behavioral experience. Moreover, the lack of differences between groups in the total arm entries in the plus maze and total transitions in the dark–light test eliminates the possibility that differences in motor activity might lead to misinterpretation of the results. Thus, faster learning and attenuated fear characterize female rats that during neonatal life had a mother supplemented with CORT. As mentioned earlier, the decreased activity of the HPA axis may have played a role in the enhanced learning ability of CORT-nursed animals, but it is also important to consider the role of fear in learning processes. There is much evidence indicating that performance in many tasks varies as a function of anxiety, with test-anxious individuals showing lower level of performance in some tasks (Eysenck and Calvo, 1992). It may be hypothesized that in female CORT-nursed offspring, their low level of anxiety, shown in the above-mentioned conflict tests, contributes to better performance in the cognitive tests.

The behavioral trait of CORT-nursed female offspring is very similar to that observed previously in males (Catalani et al., 1993, 2000; Casolini et al., 1997). The hormonal response to stress presents some similarities in both female and male offspring. The stress-induced secretion of CORT is lower in both male and female CORT-nursed rats compared to controls, with the peculiarity that in females, the shutoff of the endocrine response to stress occurs earlier than in males. The outcome of maternal treatment is a progeny of both sexes with improved learning abilities and low level of anxiety in conflict paradigms.

It is interesting to note that there are similarities between our findings, in females and males, and the effects induced by neonatal handling. Meaney et al. (1988, 1991) have demonstrated that handling early in life results in an attenuation of spatial cognitive dysfunction associated with aging, a lower secretion of CORT following the termination of stress, a reduction of emotionality and an acceleration of two-way active avoidance learning (Nunez et al., 1995) in adult and aged rats. It is temping to suppose that the outcomes of both early handling and a moderate maternal hypercorticosteronemia, may, through the rise in CORT concentrations, induce an increase of maternal care, initiating a chain of events resulting in the observed effects. This hypothesis is supported by the demonstration that maternal care actively contributes to the development of neural systems that mediate cognitive development (Liu et al., 2000). Finally, the increased concentrations of CORT, via

milk, in the pups could have "directly" induced profound and long-lasting changes in the HPA and in other systems during a period of life in which the CNS undergoes dramatic anatomical and physiological changes and rapid maturation cannot be excluded.

In summary, this study corroborates our previous observations indicative of the beneficial action of a moderate increase of maternal glucocorticoid hormone during lactation. However, it is very important to clearly define "moderate," given the fact that a supramoderate increase reverses the effect from beneficial to detrimental. Today, the term cannot be defined accurately, but we can only suggest that it applies more likely to physiological rather than pharmacological conditions.

## References


Ahima RS, Lawson AN, Osei SY, Harlan RE. Sexual dimorphism in regulation of type II corticosteroid receptor immunoreactivity in the rat hippocampus. Endocrinology 1992;131:1409–16.

Alonso SJ, Castellano MA, Afonso D, Rodriguez M. Sex differences in behavioral despair: relationships between behavioral despair and open field activity. Physiol Behav 1991;49:69–72.

Barrett RS, Ray OS. Behavior in the open-field, Lashley III maze, shuttlebox and Sidman avoidance as a function of strain, sex and age. Dev Psychol 1970;3:73–7.

Casolini P, Piazza PV, Kabbaj M, Leprat F, Angelucci L, Simon H, Le Moal M, Maccari S. The mesolimbic dopaminergic system exerts an inhibitory influence on brain corticosteroid receptor affinities. Neuroscience 1993;55:429–34.

Casolini P, Cigliana G, Alemà GS, Ruggieri V, Angelucci L, Catalani A. Effect of increased maternal corticosterone during lactation on hippocampal corticosteroid receptors, stress response and learning in offspring in the early stages on life. Neuroscience 1997;79:1005–12.

Castro CA, Paylor R, Rudy JW. A developmental analysis of the learning and short-term-memory processes mediating performance in conditional–spatial discrimination problems. Psychobiology 1987;15:308–16.

Catalani A, Marinelli M-, Scaccianoce S, Nicolai R, Muscolo LAA, Porcu A, Korányi L, Piazza PV, Angelucci L. Progeny of mothers drinking corticosterone during lactation has lower stress-induced corticosterone secretion and better cognitive performance. Brain Res 1993;624:209–15.

Catalani A, Casolini P, Scaccianoce S, Patacchioli FR, Spinozzi P, Angelucci L. Maternal corticosterone during lactation permanently affects brain corticosteroid receptors, stress response and behaviour in rat progeny. Neuroscience 2000;100:319–25 (in process citation).

Conrad CD, Roy EJ. Dentate gyrus destruction and spatial learning impairment after corticosteroid removal in young and middle-aged rats. Hippocampus 1995;5:1–15.

Coover GD, Ursin H, Levine S. Plasma-corticosterone levels during active-avoidance learning in rats. J Comp Physiol Psychol 1973;82:170–4.

Critchlow V, Liebelt A, Bar-Sela M, Mountcastle W, Lipscomb HS. Sex difference in resting pituitary–adrenal function in the rat. Am J Physiol 1963;205:807–15.

de Kloet ER. Brain corticosteroid receptor balance and homeostatic control. Front Neuroendocrinol 1991;12:95–164.

DeKosky ST, Nonneman AJ, Scheff SW. Morphologic and behavioral effects of perinatal glucocorticoid administration. Physiol Behav 1982;29:895–900.

Dohrenwend BP, Dohrenwend BS. Sex differences and psychiatric disorders. Am J Sci 1976;81:1447–54.

Duquette P, Girard M. Hormonal factors in susceptibility to multiple sclerosis. Curr Opin Neurol Neurosurg 1993;6:195–201.

Erskine MS, Geller E, Yuwiler A. Effects of neonatal hydrocortisone treatment on pituitary and adrenocortical response to stress in young rats. Neuroendocrinology 1979;29:191–9.

Eysenck MW, Calvo MG. Anxiety and performance: the processing efficiency theory. Cognit Emotion 1992;6:409–34.

Felszeghy K, Gaspar E, Nyakas C. Long-term selective down-regulation of brain glucocorticoid receptors after neonatal dexamethasone treatment in rats. J Neuroendocrinol 1996;8:493–9.

Golub MS. Maze exploration in juvenile rats treated with corticosteroids during development. Pharmacol, Biochem Behav 1982;17:473–9.

Henry C, Kabbaj M, Simon H, Le Moal M, Maccari S. Prenatal stress increases the hypothalamo-pituitary–adrenal axis response in young and adult rats. J Neuroendocrinol 1994;6:341–5.

Islam A, Henriksson B, Mohammed A, Winblad B, Adem A. Behavioural deficits in adult rats following long-term adrenalectomy. Neurosci Lett 1995;194:49–52.

Issa AM, Rowe W, Gauthier S, Meaney MJ. Hypothalamic–pituitary–adrenal activity in aged, cognitively impaired and cognitively unimpaired rats. J Neurosci 1990;10:3247–54.

Kitay JI. Sex differences in adrenal cortical secretion in the rat. Endocrinology 1961;68:818–23.

Liu D, Diorio J, Day JC, Francis DD, Meaney MJ. Maternal care, hippocampal synaptogenesis and cognitive development in rats. Nat Neurosci 2000;3:799–806.

Maccari S, Piazza PV, Kabbaj M, Barbazanges A, Simon H, Le Moal M. Adoption reverses the long-term impairment in glucocorticoid feedback induced by prenatal stress. J Neurosci 1995;15:110–6.

McCormick CM, Beers D, Smythe JW. Sex differences in type I corticosteroid receptor binding in selective brain areas of rats. Ann NY Acad Sci 1992;431–3.

Meaney MJ, Aitken DH, van Berkel C, Bhatnagar S, Sapolsky RM. Effect of neonatal handling on age-related impairments associated with the hippocampus. Science 1988;239:766–8.

Meaney MJ, Aitken DH, Bhatnagar S, Sapolsky RM. Postnatal handling attenuates certain neuroendocrine, anatomical, and cognitive dysfunctions associated with aging in female rats. Neurobiol Aging 1991;12:31–8.

Moguilewsky M, Philibert D. RU 38486: potent antiglucocorticoid activity correlated with strong binding to the cytosolic glucocorticoid receptor followed by an impaired activation. J Steroid Biochem 1984;20:271–6.

Murphy BEP. Some studies of the protein-binding of steroids and their application to the routine micro and ultramicro measurement of various steroids in body fluids by competitive protein-binding radioassay. J Clin Endocrinol Metab 1969;27:464–8.

Nunez JF, Ferré P, Garcia E, Escorihuela RM, Fernandez Teruel A, Tobena A. Postnatal handling reduces emotionality ratings and accelerates two-way active avoidance in female rats. Physiol Behav 1995;57:831–5.

Nyakas C, Endroczi E. Effect of neonatal corticosterone administration on behavioural and pituitary–adrenocortical responses in the rat. Acta Physiol Acad Sci Hung 1972;42:231–41.

Olton DS, Johnson CT, Howard E. Impairment of conditioned active avoidance in adult rats given corticosterone in infancy. Dev Psychobiol 1974;8:55–61.

Payami H, Zareparsi S, Montee KR, Sexton GJ, Kaye JA, Bird TD, Yu CE, Wijsman EM, Heston LL, Litt M, Schellenberg GD. Gender difference in apolipoprotein E-associated risk for familial Alzheimer disease: a possible clue to the higher incidence of Alzheimer disease in women. Am J Hum Genet 1996;58:803–11.

Pavlovska Teglia G, Stodulski G, Svendsen L, Dalton K, Hau J. Effect of oral corticosterone administration on locomotor development of neonatal and juvenile rats. Exp Physiol 1995;80:469–75.

Peiffer A, Lapointe B, Barden N. Hormonal regulation of type II glucocorticoid receptor messenger ribonucleic acid in rat brain. Endocrinology 1991;129:2166–74.

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.

Reul JM, de Kloet ER. Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. Endocrinology 1985;117: 2505–11.

Rivier C, Vale W. Modulation of stress-induced ACTH release by corticotropin-releasing factor, catecholamines and vasopressin. Nature 1983; 305:325–7.

Steenbergen HL, Heinsbroek RP, van Hest A, van de Poll NE. Sex-dependent effects of inescapable shock administration on shuttlebox-escape performance and elevated plus-maze behavior. Physiol Behav 1990;48: 571–6.

Turner BB. Sex differences in the binding of type I and type II corticosteroid receptors in rat hippocampus. Brain Res 1992;581:229–36.

Turner BB, Taylor AN. Persistent alteration of pituitary–adrenal function in the rat by prepuberal corticosterone treatment. Endocrinology 1976; 98:1.

Valle FP. Effects of strain, sex, and illumination on open-field behavior of rats. Am J Psychol 1970;83:103–11.

Vicedomini JP, Nonneman AJ, DeKosky ST, Scheff SW. Perinatal glucocorticoids disrupt learning: a sexually dimorphic response. Physiol Behav 1986;36:145–9.

Vogel JR, Beer B, Clody DE. A simple and reliable conflict procedure for testing anti-anxiety agents. Psychopharmacologia 1971;21:1–7.

Walker SJ, Vrana KE. Pituitary corticotropin function during the stress hyporesponsive period in neonatal rats. Neuroendocrinology 1993;57: 1003–10.

Young EA. Sex differences and the HPA axis: implications for psychiatric disease. J Gender-Specific Med 1998;1:21–7.

Zoli M, Agnati LF, Fuxe K, Ferraguti F, Biagini G, Cintra A, Gustafsson JA. Long-lasting reduction of glucocorticoid receptor immunoreactivity in the hippocampal field CA1 but not in the dentate gyrus after neonatal treatment with corticosterone in the rat. Acta Physiol Scand 1990;138: 577–9.