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Serotonin, cortisol, and stress-related psychopathology

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Document Version

Publisher's PDF, also known as Version of record

Publication date:

2009

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Tanke, M. A. C. (2009). *Serotonin, cortisol, and stress-related psychopathology: from bench to bed*. s.n.

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Chapter 5

Sustained release of corticosterone in rats does not affect habituation to immobilization and acoustic startle

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Life Sciences, 2008 Jul 18;83(3-4):135-141.

ABSTRACT

Depression is often preceded by stressful life-events and accompanied with elevated cortisol levels and glucocorticoid resistance. It has been suggested that a major depressive disorder may result from impaired coping with and adaptation to stress. The question is whether or not hypothalamus-pituitary-adrenal (HPA)-axis dysfunction influences the process of adaptation. We examined the effect of a dysregulated HPA-axis on the adaptation to acoustic stimuli in rats with or without preceding restraint stress. HPA-axis function was altered via slow release of corticosterone (CORT, 90 mg) from subcutaneously implanted pellets for 7 or 14 days. The rate of body temperature increases during restraint (10 minutes) and the response to acoustic stimuli (of 80 + 120 dB) were used to quantify daily stress reactivity. Rats habituated to either stress regardless of CORT treatment. CORT treatment combined with restraint decreased the initial reactivity and the variability in response, but the rate of habituation was not influenced. These results show that suppressing normal HPA-axis function by chronic exposure to CORT does affect the course of habituation, but not habituation per se. This implies that altered HPA-axis function in depressed patients may not be causally related to stress coping, but instead may influence the course of the disorder.

INTRODUCTION

Exposure to physical or emotional aversive stimuli activates multiple stress systems, such as the adrenergic sympathetic adrenal medulla system and the HPA-axis (1;249), regulating glucocorticoid function. Activation of these stress systems results in behavioral and physiological changes which allow the organism to cope with the stressor (249). Normally, repeated exposures to the same stressor will lead to diminished arousal and stress responses (250). Such habituation indicates the capability of an organism to adapt to stress (15;16;18;132). Habituation may be a very early form of learning (132) and the involvement of the HPA-axis in learning and memory processes in animals and humans is well documented (251;252).

Recurrent stress without habituation of the stress responses has been implicated in many psychiatric disorders. For example, major aversive life events or continuous daily hassles have been associated with both the development and the relapse of affective disorders (6-8). Some major depressive disorders are associated with an altered HPA-axis activity (27). Relevant changes include increased cortisol levels in plasma, urine and cerebrospinal fluid, enlarged pituitary and adrenal glands (28-31), an attenuated circadian rhythm of cortisol secretion and impaired glucocorticoid feedback regulation (glucocorticoid resistance) (34;35). Resistance and attenuated circadian variations may be the result of chronically elevated cortisol levels (28;36;37). The increased HPA-axis activity seen in affective disorders may be a secondary effect of persistent attempts to adapt to stress or, conversely it impairs coping to stress and causes then depression. The latter idea is supported by the increased frequency of psychiatric co-morbidity in somatic diseases such as Cushing's disease (253), that are characterized by elevated plasma levels of cortisol. The question remains, whether behavioral habituation to a repeatedly applied stressor depends on stress induced release of the corticoids or that both are independent processes. This issue was explored in the here presented experiments.

We tested whether continuous release of corticosterone (CORT) in rats with intact adrenals affects physiological responses and habituation to immobilization and acoustic stress. The hormone (80 mg over 2 weeks) was released from subcutaneously implanted CORT pellets, following the design of Meyer et al.(254). Accordingly, the nearly constant levels of corticosterone were obtained (that were about half maximal between normal and stress induced levels in normal rats), the daily variations were virtually absent and all the HPA-responsiveness to stress was suppressed. Other consequences of the continuously elevated levels of corticosterone were the decreased body weight, adrenal weight and thymus weight (255-258). Using the well documented designs (254-256), together with the effects on the adrenals and thymus we could avoid additional stress as caused by frequent blood sampling to control the corticosterone levels during the experiments. A stress-design such as used here appears to be highly sensitive to the cumulative effects of more than one type of stress.

Adaptive capacity and reactivity were initially assessed with an unconditional response to acoustic stimuli in rats. Acoustic stimuli are widely used to test reactivity and habituation and this design is very sensitive to arousal and the perception of stress (118;123;182). The first experiment showed that corticosterone pellet implantation alone did not alter reactivity to the acoustic stimuli and that also habituation remained unaffected. Therefore a stressor was added, consisting of ten minutes of immobilization daily. In the naïve rat such brief daily exposures to immobilization are sufficient to produce marked behavioral changes and elevation in HPA-axis activity (118-122). In the second experiment, the following stress-responses were measured: Reactivity and habituation to the acoustic stimuli (76;123), changes in rectal temperature during immobilization (stress-induced hyperthermia) (259;260), food intake, and immediate early gene expression in the paraventricular nucleus of the hypothalamus (PVN), basolateral amygdala (BLA), central amygdala (CeA), locus coeruleus, dentate gyrus, and nucleus accumbens, all regions involved in the stress response (158;159).

MATERIAL & METHODS

The experimental procedures were approved by the Animal Ethics Committee of the University of Groningen. Every reasonable effort was made to minimize the numbers of animals used and their discomfort. We performed two experiments. The general procedures are described first followed by a description of the experimental design.

Animals

Male Wistar rats (Harlan, the Netherlands) weighing 200-274 gram at the start of the experiment, were housed individually cages of 45 cm x 28 cm x 20 cm in a temperature controlled environment (21-23 °C). The animals had ad libitum access to water and food. Animals were kept on a 12h reverse light/dark cycle with lights on from 1900h to 0700h. The experiments were conducted during the dark period. All animals were handled daily to minimize handling stress during the experiment. Food intake and body weights were measured daily.

Pellet implantation

On day 0, after one week of acclimatization, blood samples (baseline levels) of approximately 0.5-0.75 ml were collected from a small tail wound (165). Thereafter, the animals received either a cholesterol pellet (chol, 90 ± 5 mg) or CORT pellet (91 ± 4 mg) as treatment, which was implanted subcutaneously conforming the literature (254;255). Surgery was performed under general anesthesia (70% O₂ / 27% NO₂ / 3% Isoflurane mixture). After surgery, rats received a single finadyne injection (2.5 mg/kg i.p.) to suppress postoperative pain.

CORT pellets are known to alter normal HPA-axis function. A 100 mg CORT pellet released

about 3 mg CORT per day and induced stable high levels of corticosterone persisting for at least 2 weeks in adrenalectomized rats (254). In intact rats, a pellet containing 80 mg CORT increased average daily plasma CORT levels and inhibited all responsiveness in the HPA-axis to stress and in addition decreased in body weight, adrenal weight, and thymus weight (255). We therefore used the decreases in those the body and organ weights to evaluate the effectiveness of CORT treatment. Thus the painful and stressful of blood sampling that might intervene with habituation to the stressor could be avoided.

Acoustic stimulus testing

Behavioral testing was done as described before (182). Briefly, the rats were taken to a separate room and received acoustic stimulus sessions (163), using a Startle Response Measuring System (TSE GmbH, Bad Homburg, Germany). The rats were subjected to the same conditional program every day; consisting of a five-minute acclimatization period to the startle chamber, and 10 sets of four trials with an inter-stimulus interval of 10 seconds. One set contained successively one trial of nothing, one trial of a stimulus alone (20 ms 80dB 5000 Hz), followed by two trials with a paired stimulus, consisting of a 20 ms 80dB 5000 Hz pulse followed 100 ms later a 40 ms 120 dB 5000 Hz pulse. During the acclimatization period and the actual experiment, a constant background noise of 70 dB was present. Only the responses to the first paired stimulus of each set were used and hereafter reported. The response was measured with a movement sensor (grams). Percent motor responses for day x were determined as $(\text{average response day } x / \text{average response day } 1) * 100$. Habituation was measured as percent reduction in motor response to the first conditioned stimulus of each day.

Immobilization procedure

The animals were taken to a separate room and were immobilized manually by placing them on their backs for 10 minutes four hours before the acoustic stimuli testing. Basal rectal temperatures and rectal temperatures during restraint were recorded automatically every five seconds. Temperatures were determined to an accuracy of 0.001 °C using an YSI 4610 precision thermometer (Yellow Springs, Ohio, USA), with the thermistor probe (YSI 4612 small flexible general-purpose probe) inserted 40 mm into the rectum. Maximal increase in temperature and initial slope during the first minute of restraint were taken as indicators of responsiveness of the animals.

Termination of the experiment

Rats were terminated two hours after the last immobilization stress, when brain fos levels are maximal (161). The rats were anaesthetized, using Isoflurane, and an intra-cardial blood was taken for the determination of plasma corticosterone. Subsequently, the rats were transcardially perfused for two minutes with 50 ml heparinized saline (0.9%) and for 20 minutes with 300 ml of a 4% paraformaldehyde solution 0.1 M sodium phosphate buffer

(pH = 7.4). Thymus and adrenal glands were removed and weighed and reported as permillage of body weight. The brains of the animals in experiment 2 were removed and post-fixed in the latter solution overnight at 4°C. Fos positive cells in the paraventricular nucleus of the hypothalamus (PVN), basolateral amygdala (BLA), central amygdala (CeA), locus coeruleus, dentate gyrus, and nucleus accumbens, were quantified as described before (182).

Experimental design

Experiment 1

To evaluate the effect of relation between corticosterone treatment on reactivity and habituation to acoustic stimuli, six CORT treated and six chol animals were subjected daily for 5 days to acoustic stimulus sessions starting on day 5.

Experiment 2

To evaluate the effect of additional stress to the design of experiment 1, the rats were subjected to 10 minutes of manual immobilization four hours prior to the acoustic testing. Restricted time for food consumption (1100h – 1800h) was introduced to avoid rat-droppings disturbing the temperature measurements, when available; food was still supplied ad libitum.

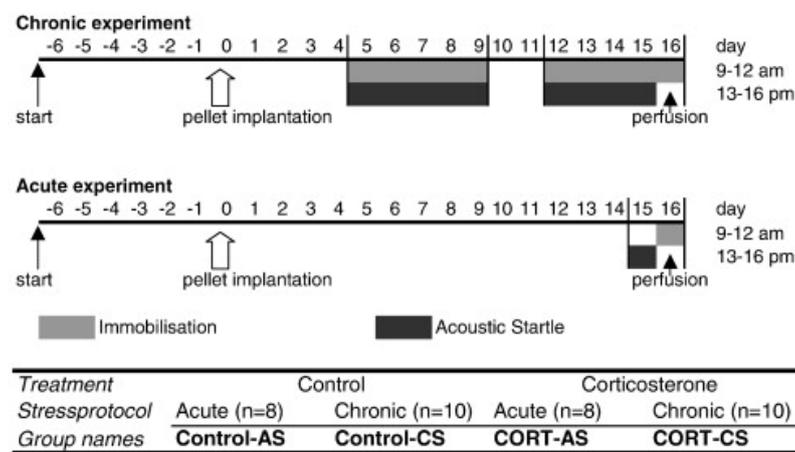


Figure 1: Schematic overview of experiment 2 and group names

An overview of experiment 2 is given in figure 1. Twenty animals (10 CORT and 10 chol) were used in the chronic stress (CS) group (CORT-CS and Control-CS). In addition, an acute stress (AS) group (8 CORT-AS and 8 Control-AS) was added to be able to distinguish between habituation effects and treatment effects. The CS animals were subjected to daily immobilization stress and acoustic stimulus sessions for 10 days starting at day five. The AS rats received both procedures only once: the acoustic stimulus session on the day before termination and immobilization stress on the day of termination.

Statistical analysis

Statistical analyses were done with SPSS (version 12.0); $P < 0.05$ was considered significant. Data are presented as mean \pm S.E.M. Plasma CORT levels, organ weight (corrected for body weight), and c-fos data were analyzed using a one-way ANOVA. Weight gain, food intake, response to acoustic stimuli, and rectal temperature during immobilization periods were analyzed with repeated measures ANOVA. Because of difficulties of the temperature procedure, and therefore missing data, one period consisted of two or three consecutive days of testing. A mixed analysis on differences in variance in startle data showed different variances for the different treatment groups (CORT or Control). No differences in absolute values (group mean) were seen. Therefore, post hoc group differences in variability in startle response were tested by using the absolute deviance in response from their own group mean (CORT-CS or Control-CS) as a parameter for variability. Statistical analysis was done with a repeated measures MANOVA using days and trials as within subject factors and treatment as between subject factor. Greenhouse-Geisser corrections were used, when the assumption of sphericity was not met.

RESULTS

Experiment 1

At the start of the experiment, body weight did not differ between the two groups ($250 \text{ g} \pm 5 \text{ gram}$). While the Control animals continued to grow after pellet implantation, the CORT animals stopped growing or even lost some weight (interaction effect of day*treatment, $F = 40.268$, $P < 0.001$). At the end of the experiments, the Control animals weighted $289 \pm 9 \text{ gram}$, while the weight of the CORT animals was $246 \pm 8 \text{ gram}$ ($F = 13.426$, $P = 0.005$). No effect of treatment was seen on food intake. Plasma CORT levels determined before implantation of the pellet did not differ between the two treatment groups (Control $578 \pm 99 \text{ nmol/l}$, CORT $656 \pm 150 \text{ nmol/l}$). Plasma CORT levels from blood samples taken at termination showed a trend towards higher CORT levels in the CORT group (Control $241 \pm 46 \text{ nmol/l}$, CORT $458 \pm 84 \text{ nmol/l}$, $P = 0.063$). CORT pellet implantation had a negative effect on relative adrenal weight (Control $0.17 \pm 0.005 \%$; CORT $0.09 \pm 0.007\%$, $F = 77.845$, $P < 0.001$) and relative thymus weight (Control $1.94 \pm 0.07 \%$; CORT $0.5 \pm 0.12\%$, $F = 81.615$, $p < 0.001$).

Repeated exposure to the acoustic stimuli resulted in a decrease in response in both CORT and Control animals, both within one session (short-term habituation; main effect of trial $F = 6.213$, $P = 0.005$) and across experimental days (long-term habituation; main effect of day $F = 5.802$ $P = 0.01$). There was no difference between CORT treated and Control animals in reactivity to the acoustic stimuli or in the habituation.

Experiment 1 demonstrated that CORT treatment did not affect the reactivity of the animal, nor the habituation to the acoustic stimulus sessions.

Experiment 2

Body weights, food intake, and organ weights

At day 0, body weight did not differ between CORT and Control animals in the CS (273 ± 2 g), or the AS experiment (253 ± 10 g). After pellet implantation the CORT animals showed significantly less weight gain, or even weight loss, compared to the Control animals (interaction effect day*treatment; $F = 42.827$, $P < 0.001$). Additionally, a significant interaction effect of experimental paradigm, CS or AS, on weight gain (effect of stress*day; $F = 3.653$, $P = 0.027$) was seen (figure 2).

CORT treatment also altered food intake: CORT-CS animals ate significantly less than Control-CS in the week before stress and the first week of stress ($F = 16.96$, $P = 0.001$; and $F = 6.849$, $P = 0.017$). This effect disappeared in CS animals during the second week of the stress procedure. In AS animals, overall food intake was significantly lower in CORT animals compared to Controls ($F = 6.107$, $P = 0.027$). Plasma CORT levels at the day of termination were not significantly different in the CORT treated groups compared to Control (table 1). CORT treatment resulted in a significant decrease in relative adrenal weight (CS; $F = 33.773$, $P < 0.001$. AS; $F = 21.513$, $P < 0.001$) and thymus weight (CS; $F = 47.436$, $P < 0.001$. AS; $F = 16.207$, $P = 0.001$) compared to control animals (table 1). Adrenal weight and thymus weight were negatively correlated with the plasma CORT levels (adrenals CS; Pearson correlation -0.808 , $P < 0.001$. AS; Pearson correlation -0.778 , $P < 0.001$. Thymus CS; Pearson correlation -0.7851 , $P < 0.001$. AS; Pearson correlation -0.732 , $P = 0.001$).

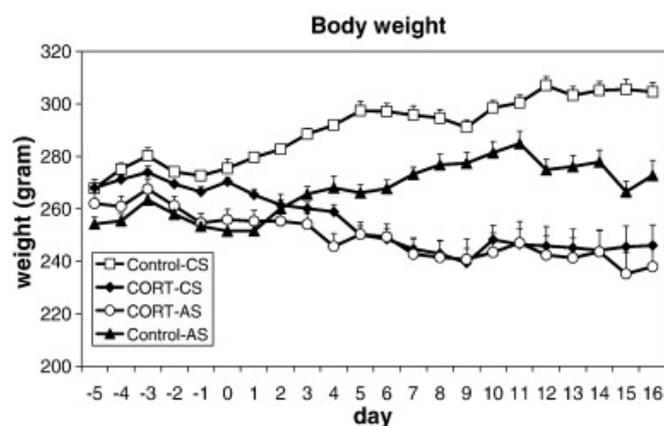


Figure 2: Body weight during experiment 2 in chronically stressed rats compared with acute stressed rats. CORT = corticosterone treated rat, Control = control animal CS = chronic stress, AS = Acute stress

Body temperature

CS experiment: Average basal body temperature did not differ between CORT-CS (37.33 ± 0.09 °C) and Control-CS (37.34 ± 0.03 °C). Immobilization increased body temperature within seconds in both Control-CS and CORT-CS animals. An interaction effect of treatment and period was found in maximal increase in body temperature during restraint stress (period*treatment; $F = 3.845$, $P = 0.041$) (figure 3a). The slopes of the temperature responses were steepest in the first period, and weakened over the treatment periods ($F = 12.631$, $P < 0.001$), with no difference between CORT-CS and Control-CS (figure 3b).

In the AS experiment, basal rectal temperature of CORT-AS (37.6 °C \pm 0.25 °C) did not differ from the Control-AS (37.7 °C \pm 0.24 °C). Acute restraint stress led to an increase in body temperature of 0.75 °C \pm 0.28 °C in CORT-AS and 0.82 °C \pm 0.36 °C in Control-AS. There was no significant difference in maximal increase in temperature or slope of the temperature curve between CORT-AS and Control-AS (figure 3). Comparison of the temperature curves of the AS group with the temperature curves of the first day of stress of the CS group showed no difference in basal rectal temperature or maximum increase in temperature. However CORT-AS animals showed significantly lower slopes than the CORT-CS animals ($F = 9.577$, $P = 0.009$) on the first day of stress. This difference was not observed in the Control group. When comparing these parameters with the responses of the last day of the CORT-CS rats, there was no difference between CORT-CS and CORT-AS rats. In view of the habituation observed in our CS group, we must conclude that both repeated exposure and CORT treatment may have contribute to this reduction.

Table 1: HPA-axis function parameters experiment 2

	Control		Corticosterone treated	
	CS (n=10)	AS (n=8)	CS (n=10)	AS (n=8)
C-fos (PVN)	213 \pm 18	254 \pm 18	154 \pm 14*	161 \pm 14***
Adrenals	0.19 \pm 0.01	0.19 \pm 0.01	0.11 \pm 0.01***	0.11 \pm 0.02***
Thymus	1.75 \pm 0.12	1.60 \pm 0.13	0.64 \pm 0.11***	0.70 \pm 0.18***
CORT day 0	387 \pm 72	414 \pm 80	382 \pm 81	321 \pm 70
CORT day 16	313 \pm 48	249 \pm 40	423 \pm 62	294 \pm 32

Average values of c-fos count in the PVN (per 0.1 mm²), relative adrenal and thymus weight (mg / gr body weight) and plasma corticosterone (CORT) concentrations before pellet implantation (day 0) and two hours after the last test session(day 16)(nmol / l) \pm S.E.M. in control or corticosterone treated animals after chronic stress (CS) or acute stress (AS). Treatment effect of corticosterone vs control * $p \leq 0.05$, *** $p \leq 0.001$

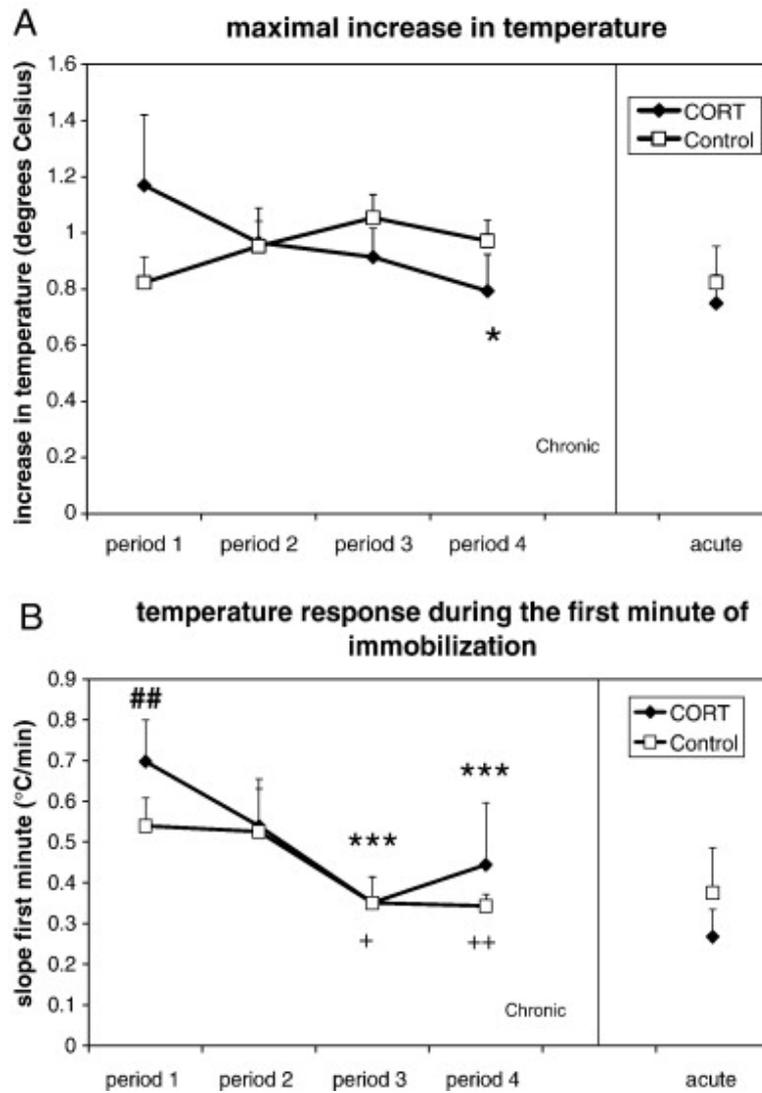


Figure 3: Maximal increase in temperature (a) and initial temperature response (b) during restraint stress in chronically stressed rats compared with acute stressed rats. CORT = corticosterone treated rat, Control = control animal. The results are expressed as group mean \pm S.E.M. One period is the average response on 2 or 3 successive days. CORT rats, unlike Controls, show adaptation in increase in temperature during restraint. Both CORT and Control show decelerated initial temperature response, but corticosterone treatment also led to a slow initial response of the temperature in the acutely stressed rats compared to the first day of the chronically stressed corticosterone treated rats. (a+b) Comparison of the response with the response of day 1: CORT: * $p \leq 0.05$, *** $p \leq 0.001$; Control: + $p \leq 0.05$, ** $p \leq 0.01$ (b) CORT-AS vs CORT-CS ## $p \leq 0.01$.

Acoustic stimulus testing

Repeated exposure to acoustic startle resulted in a decrease in startle motor response, both within one test session (short-term habituation, main effect of trial CS; $F = 10.829$, $P < 0.001$. AS; $F = 4.672$, $P = 0.009$) and over the days (long-term habituation, main effect of day; $F = 14.693$, $P < 0.001$: figure 4a). Repeated measures analysis revealed an interaction effect of treatment on startle motor response in the first four days of stress in the CS experiment (day*trial*treatment; $F = 2.169$, $P = 0.043$. trial*treatment; $F = 2.332$, $P = 0.025$). Post hoc analysis showed that in Control-CS, the startle responses on the 1st, 2nd, and 3rd day of stress were significantly higher than on day 4, while in CORT-CS only day 1 differed significantly from day 4 (figure 4a). This interaction effect seems to be the result of the lower naïve startle response of the CORT-CS animals ($Z = -2.873$, $P = 0.003$). When considering habituation as a percent decrease in startle motor response, no difference was found between CORT-CS and Control-CS animals (figure 4b). Thus, CORT treatment does not influence the occurrence of habituation itself, but influences the magnitude of the response. Control-CS animals showed higher within group variability in response to acoustic startle compared to CORT-CS during the whole experiment (effect of trial*treatment; $F = 5.373$, $P = 0.007$, day*treatment; $F = 3.045$, $P = 0.045$), which was most pronounced during the first four days (day*treatment; $F = 4.003$, $P = 0.027$, figure 4c). This variability in response also showed adaptation, since it decreased over time (main effect of day; $F = 20.854$, $P < 0.001$; main effect of trial $F = 6.211$, $P = 0.004$). In the AS experiment, no treatment effect was observed. Differences in startle response could not be explained by differences in body weight or food intake, since no correlations were found between body weight or food intake and startle response.

No difference in startle response was observed between AS and CS animals on their first day of stress. Comparing the first pulse of day 16 (CS) with the naïve pulse of the AS animals shows that AS animals have significantly larger responses ($Z = -2.914$, $P = 0.003$). These results demonstrate habituation to the startle procedure, independent of CORT treatment. Comparing the variability of the AS group with the first day of startle of the CS group did not show any differences. Comparing the variability of the AS rats with the variability of the rats on the last day of startle of the CS experiment, only revealed significantly lower variability in Control-CS rats compared to Control-AS rats ($F = 19.100$, $P < 0.001$)

C-fos immunohistochemistry

CORT treatment was associated with significantly decreased c-fos expression in the PVN, both in the CS group ($F = 6.835$, $P < 0.001$) and in the AS animals ($F = 22.092$, $P = 0.001$, table 1). There was no treatment effect in the other brain regions, including nucleus accumbens, amygdala, and locus coeruleus (results not shown).

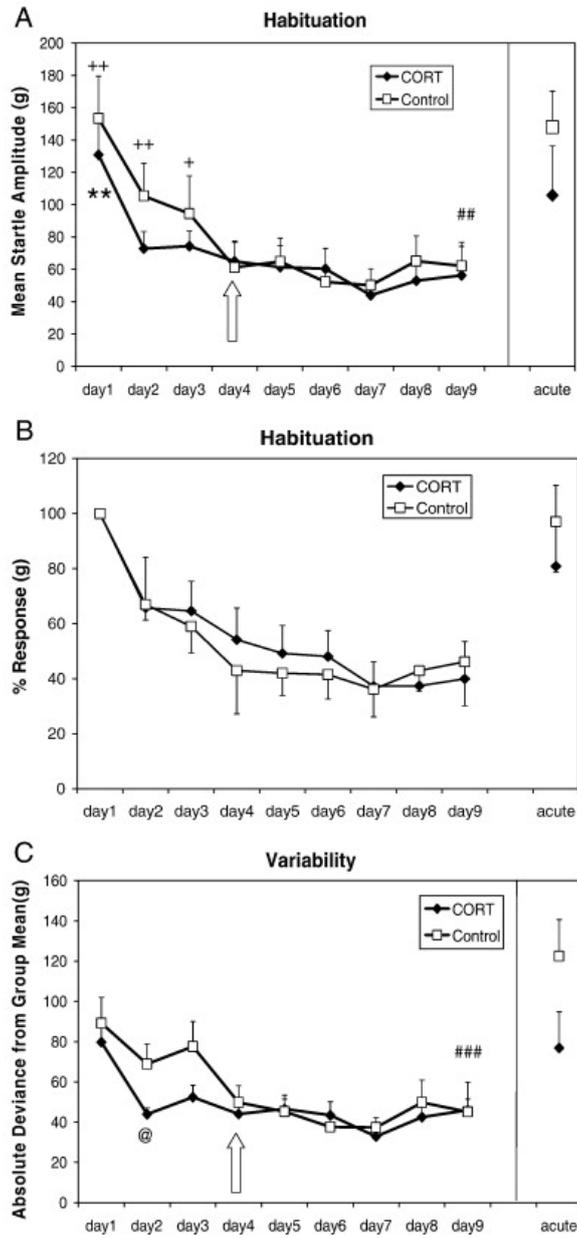


Figure 4: Habituation (a+b) and variability (c) of the startle response of chronically and acutely stressed rats. CORT = corticosterone treated, Control = control animal. (b) startle response on the first day of stress of chronically stressed animals is set to 100%. The results are expressed as group mean \pm S.E.M. CORT rats show faster habituation but also less variability than control animals. (a) Comparison of the response with the response of day 4 (arrow): Control: + p <0.05, ++ p <0.01; CORT: ** p <0.01. Stress effect: AS vs CS ### p <0.01, (c) Control-CS vs Control-AS ### p <0.001. Treatment effect: (c) CORT-CS vs Control CS @ p <0.05

Discussion

The present study shows that continuous exposure to exogenous CORT does not prevent habituation to stress, but decreases the response to both the immobilization and acoustic stress. Moreover, the variability of the responses was less in the CORT group. Apparently, a stress-reactive HPA-axis is not essential for habituation to the stress, as applied in this study, but may influence the reactivity to and so the impact of a stressful event.

The interpretation of the present results hinges heavily on the subcutaneously implanted CORT pellets to disrupting normal HPA-axis regulation. The sustained release of CORT will lead to an attenuated circadian rhythm and an uncoupling of HPA-axis activity to stress (255). Implantation, a one-time procedure done before the behavioral observations, is a relatively non-stressful procedure, and therefore less stressful than for example, daily injections (254). Implantation provides a more accurate and continuous hormone dosage than administration via food or drinking water. A disadvantage of pellet implantation method is a less well controlled release of the hormone and thus a risk of over- or under-dosage. To minimize these risks we copied the design of previous studies where the levels of CORT were monitored (Meyer etc see also the introduction). The decreased adrenal, thymus and body weights, as well as the decreased c-fos expression in the PVN in the CORT exposed rats, observed here, indicate sustained effects on the endocrine regulation, which is still present at the time of termination (17;45;255-257).

Previous studies in both humans and rats also showed that CORT manipulation did not prevent habituation (43;45;261;262). However, CORT administration in adrenalectomized rats did accelerate the adaptation of autonomic system responses, such as heartbeat and core temperature, and the expression of immediate-early genes in the brain to repeated restraint stress (45). Also (261) conclude that glucocorticoids itself play a role in the habituation of HPA axis responsiveness to immobilization stress. In adrenalectomized mice with or without CORT treatment no significant effect on startle habituation within one startle session was seen, although CORT treatment reduced baseline startle response (43;262). In humans, a single dose of hydrocortisone altered the magnitude of the acoustic startle response, but not the habituation (263). These findings support the view that the rate of habituation and the responsiveness to stress may be affected by CORT treatment, thus enabling the animals to better cope to the stressor, rather than affecting habituation per se. We observed a decreased inter-individual variability in the response to the acoustic stimuli in CORT-CS animals, mainly during the first three days of the experiment in which the actual habituation takes place. These observations are in line with reports that CORT facilitates interpretation and storage of new information by the extinction of behavior that is no longer relevant (46).

Differences in results between our study and the above mentioned studies may be due to several factors. First, the experimental design to dysregulate the HPA-axis differs. In the present experiment, the adrenals were left intact to enable many of the hormonal regulatory

processes, including catecholamine release during stress and to allow a physiological normal control group. The release of adrenaline may mediate motor response during stress (264;265). In addition we preferred to use the slope of temperature response within the first minutes of immobilization and not the maximal body temperature to assess the responsiveness to stress, because we had the impression that the maximal temperature response was not always attained within the recording period. Moreover, the maximum temperature does not necessarily reflect the reactivity of the rat to stress because glucocorticoids have an intrinsic inhibitory effect on stress-induced fever and so the body temperature (266). This CORT effect was also observed in our CORT-AS experiment, and thus body temperature might not have been the best parameter of measuring reactivity, nor the best method of measuring habituation. Although the acoustic stress does not suffer from such possible ambiguities, the results with this approach led to strikingly similar conclusions concerning stress responsiveness and habituation.

The present study questioned whether an intact HPA-axis function is essential in the process of habituation. Evidently, a functional HPA-axis is not required to adapt to a stressor, although the constant exposure to corticosteroids alters both the rate and the extent of the stress responses. From this point of view, the alterations in habituation, seen in HPA-axis dysregulation, are not necessarily the results of a dysfunctional HPA-axis, but could rather be the result of indirect influences of corticosteroids in the brain. One of the possible mechanisms might be an altered corticotrophin releasing hormone (CRH) excretion. Corticosterone treatment significantly decreases CRH expression after long-term (up to 7 days) of in rats (267). Our results in combination with the observations that intra-cerebrally injection of CRH in rats results in increased responses to acoustic startle, without effects on habituation (268;269), suggests that alterations in behavior may be mediated by decreased CRH levels in the brain, independently of its effect on the HPA-axis (270).

A clinically important issue is whether hypercortisolism and glucocorticoid resistance are either directly or indirectly related to the depressive state. The present study favors the idea that an altered HPA-axis function is not necessarily causally related to impaired stress coping. This conclusion does not exclude the possibility that long-term exposure to high and constant levels of corticosteroids may interfere with coping mechanisms, thereby influencing the course of the disorders. For instance, our observation that high and persistent corticosterone levels reduced inter-individual variability might be related to the reduced fluctuations in mood as seen in depressed patients. If so, than variations in corticosteroids may point to a more general capacity of an organism to express a variety of affective states.

ACKNOWLEDGEMENTS

We would like to thank K. Venema and P. Bakker for their technical assistance and E. Alseda, K. Goeman, J. Gorter, and P. van der Most for their help with gathering the data.