Defensive emotional reactions and stress
Mesanvi, Kokoe

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CHAPTER 4

A SINGLE CORTICOSTERONE ADMINISTRATION INDUCES A DELAYED REDUCTION IN ANXIETY-LIKE BEHAVIOR:
ROLE OF NORADRENERGIC ACTIVITY

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ABSTRACT

There is strong evidence that administration of cortisol could have beneficial effects in posttraumatic stress disorder. This hormone is thought to reduce the experience of traumatic memories by impairing retrieval and ultimately promoting the extinction of such memories. However, an alternative explanation is that the beneficial effects of glucocorticoids derive from interaction with neural substrates regulating anxiety behavior. Recent evidence showed that corticosterone administration could reverse the effects of stress on anxiety-like behavior and amygdala neuron morphology, suggesting that this hormone also directly can affect anxiety levels in addition to its known effects on memory. To better understand the effect of glucocorticoids on anxiety, we investigated the mechanisms and the time course of an acute administration of corticosterone on anxiety-like behavior and the activity of the HPA axis in naïve male Wistar rats. Our findings indicate that corticosterone can cause a delayed and transient anxiolytic effect that is dependent on the noradrenergic system. Indeed, corticosterone-treated animals displayed a significantly increased exploration of the open-arm in the elevated plus maze 10 days after the treatment, but not 1 or 21 days after corticosterone administration. Importantly, blockade of the β-adrenoceptors by propranolol abolished the anxiolytic effect observed at 10 days. We also demonstrated that corticosterone administration could induce a temporary suppression of the HPA axis resulting in lower plasma corticosterone levels on the day following the treatment. However, homeostasis was soon reinstalled, since baseline levels were back to normal at 10 and 21 days. Taken together, the present findings illustrate that corticosterone, interacts with the noradrenergic system to induce a temporary modification of the HPA axis activity and anxiety-like behavior. These results provide additional information about the mechanism underlying the therapeutic properties of glucocorticoids in anxiety disorder such as PTSD.
INTRODUCTION

Anxiety has been defined as an anticipatory emotional state in which an individual prepares himself to cope with an upcoming potentially negative event (Barlow, 2000; Grillon, 2008; Kalin and Shelton, 1989). This defensive emotion is highly adaptive and conserved throughout evolution (Robinson et al., 2013). However excessive expression of anxiety is maladaptive in normal conditions and can evolve into a pathological state. Anxiety disorders are among the most common psychiatric disorder nowadays, and considerable effort has been made to try to understand the neurobiological mechanisms of normal anxiety and its pathological forms.

Post-traumatic stress disorder (PTSD) is a common anxiety disorder that can develop after a traumatic event. It is characterized by long-lasting symptoms, specifically the re-experiencing of the original trauma, avoidance behavior and hyperarousal (DSM-III). At the neurobiological and physiological levels, there is an increased activity in areas of the limbic system, such as the amygdala (Liberzon et al., 1999; Rabinak et al., 2011; Rauch et al., 2000); and increased noradrenergic activity and often low circulating cortisol levels are observed (Mason et al., 1986; Yehuda et al., 1995 and 1998). In addition, the sensitivity of the negative-feedback of the hypothalamic-pituitary-adrenal axis (HPA axis) is increased in PTSD patients (Goenjian et al., 1996; Yehuda et al., 1993). Only a minority of individuals experiencing severe trauma will actually develop the disorder, indicating that individual differences in the capacity to properly regulate and terminate the stress response during and after the traumatic experience, play a major role in the development of the disorder (Fletcher et al., 2010; Perkonigg et al., 2000; Yehuda, 2002; Yehuda and LeDoux, 2007).

The suspected failure of the regulation of the HPA axis during traumatic events makes the glucocorticoids hormones, as the endpoint hormone of this endocrine cascade, an important component of the pathophysiology of PTSD (Yehuda et al., 2004). PTSD patients generally have a low baseline circulating cortisol concentration (Yehuda, 2002). Recent clinical reports have suggested that the level of circulating cortisol at the moment of the traumatic experience may be an important parameter for the outcome on the emotional state (Delahanty et al., 2000; McFarlane et al., 2011). Thereafter it was shown that reversal of a low cortisol level by administration of exogenous cortisol would likely protect against PTSD (Schelling et al., 2001). Patients treated in intensive care units (ICU) have a high risk of developing PTSD (Jones et al., 2001; Schelling et al., 1998). A study by Schelling and colleagues showed that administration of hydrocortisol during septic shock in ICU patients indeed could reduce the risk of developing PTSD, confirming that low cortisol levels at the time of the trauma are a risk factor for the development of this psychiatric disorder (Schelling et al., 1999). The protective effects of cortisol against PTSD were confirmed by subsequent studies in intensive care units patients in whom cortisol levels were increased (Schelling et al., 2001, 2004 and 2006; Weiss et al., 2006).

In addition, cortisol administration can also reduce the retrieval of traumatic memories in PTSD patients (Aerni et al. 2004; de Quervain and Margraf, 2008). This is consistent with animal and human studies demonstrating the impairing effects of
glucocorticoids on memory retrieval (de Quervain et al., 1998 and 2000; Kuhlmann et al., 2005a&b). Similarly to memory consolidation, these effects of glucocorticoids on memory retrieval are dependent on emotional arousal-induced noradrenergic activity and the integrity of the amygdala (de Quervain et al., 2007; Kuhlmann and Wolf, 2006; Roozendaal et al., 2003 and 2004a&b). In the case of PTSD, cortisol is believed to weaken traces of aversive memories by impairing their retrieval and consequently promoting eventually the extinction (Brewin, 2001; de Quervain et al., 2009).

However an alternative explanation is that beneficial actions of glucocorticoids in PTSD are due to direct effects on the neural substrates regulating anxiety behavior. A recent study investigating the protective effects of glucocorticoids in an animal model of anxiety brought some new insights about the mechanisms involved in the processes (Rao et al., 2012). Using a paradigm in which immobilization stress applied to male rats during 2 hours leads to an increase of anxiety-like behavior and of spine density of BLA principal neurons 10 days later, the authors could demonstrate that administration of corticosterone prior to stress exposure suppressed the delayed enhancement of amygdala connectivity and anxiety-like behavior (Rao et al., 2012). This suggests that glucocorticoid-induced modification of amygdala morphology, as well as anxiety state could also play a role in therapeutic effects of cortisol in treatment of PTSD.

To better understand the mechanisms by which glucocorticoids can decrease pathological anxiety, it is necessary to determine the mechanisms of action of these hormones in non-stressful and/or non-pathological conditions. For this purpose, we investigated the effects of corticosterone administration on anxiety-like behavior and HPA axis reactivity. We also studied the temporal dynamics of these effects. Male Wistar rats received a single injection of corticosterone and thereafter we assessed changes in the anxiety-like behavior of these animals, and measured the concentration of plasma corticosterone at several time points. In addition, in view of the extensive evidence demonstrating the implication of the noradrenergic system in corticosteroid-induced effect on memory (Roozendaal et al., 2009), we have blocked β-adrenergic activity with propranolol to determine whether the potential anxiolytic effects of corticosterone are also dependent on the activity of the noradrenergic system.

MATERIALS AND METHODS

Animals
Adult male Wistar rats (350-400 g at the time of drug treatment) from Charles River Breeding Laboratories (Germany) were group-housed (2 or 3 rats per cage) in an air-conditioned colony room (21 ± 1 °C) and maintained on a 12-h/12-h light/dark cycle (lights on: 07:00-19:00 h). Food and tap water were available ad libitum. Each cage only contained animals that underwent the same drug treatment. Rats were adapted to the vivarium for at least 1 week after arrival and were handled for 1 min on four consecutive days before drug injection. Drug injection and behavioral testing were performed during the light phase of the light/dark cycle between 10:00 and 14:00 h, at
the rat nadir of the diurnal rhythm of corticosterone. All procedures involving animal care and treatments were in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and approved by the Institutional Animal Care and Use Committee of the University of Groningen, The Netherlands.

Drug Treatment
Corticosterone (3 or 10 mg/kg; Sigma-Aldrich) either alone or together with the β-adrenoceptor antagonist propranolol (3 mg/kg; Sigma-Aldrich) was administered subcutaneously in a volume of 2 ml/kg. Corticosterone and propranolol were first dissolved in 100% ethanol and then diluted in peanut oil (Sigma-Aldrich) to reach its appropriate concentration. The final concentration of ethanol was 5%. The vehicle solution contained 5% ethanol in peanut oil only. All drug solutions were freshly prepared before each experiment. The rats were left undisturbed in their cages until testing on the elevated plus maze at 1, 10 or 21 days after drug treatment. Those different time points were chosen based on previous work investigating the effects of stress and corticosterone on anxiety-like behavior (Mitra et al., 2005; Mitra and Sapolsky, 2008; Rao et al., Vyas et al., 2002).

Elevated plus maze
The wooden-made apparatus consisted of two opposite closed arms (50 x 10 cm, surrounded by a 50-cm-high black wall) and two open arms (50 x 10 cm, surrounded by a 2-cm-high rim) elevated 50 cm above the floor. The protocol was adapted from Pellow and File (1986). The animal was placed in the center of the apparatus, facing an open arm and was allowed to explore the maze for 5 min. The maze was cleaned with 10% (vol/vol) ethanol after each trial. All the trials were video-recorded and the analysis was done off-line using The Observer software (Noldus, Wageningen, The Netherlands). The number of entries and the time spent in open arms were measured and used as parameters to assess the lack of anxiety. In addition, the number of entries in closed arms was used as a measure of locomotor activity (Mitra and Sapolsky, 2008). Relative open-arm exploration was measured by normalizing open-arm exploration (entries and time spent) to total exploration of the closed and open arms (entries and time spent), exploration of the center of the maze was excluded.
Different groups of animals were tested in the elevated plus maze at the three different time points chosen (1, 10 and 21 days after corticosterone administration).

Plasma corticosterone levels measurement
For each time point (1, 10 and 21 days post-treatment), immediately after testing on the elevated plus maze, the animals were anesthetized with pentobarbital (100 mg/kg) and decapitated 90 s later. Trunk blood was collected in tubes containing 0.5 M EDTA and samples were centrifuged at 1,900xg at 4°C for 10 min. Plasma was stored at -20°C and analyzed for corticosterone using radioimmunoassay, according to a
previously described protocol (Lin et al., 2008).

**Statistics**
Data are expressed as mean ± SEM. Data were analyzed with one- or two-way analysis of variance (ANOVA), and Fisher’s LSD post hoc test was used for multiple comparisons with significance levels set at $p < 0.05$.

**RESULTS**

**Effect of corticosterone administration on anxiety-like behavior 10 days later**
The effects of corticosterone alone, or with the β-adrenoceptors antagonist propranolol, were measured in the elevated plus maze 10 days after the treatment.
To examine any potential effect of the injection itself on the anxiety-like behavior and locomotor activity after 10 days, we first compared the home cage non-injected rats with the vehicle-treated rats. An independent-samples t-test revealed no significant difference between home cage and vehicle-treated animals for the open arm entries [30.4 ± 3.9 and 21.7 ± 4.2 respectively], the time spent in the open-arm [34.5 ± 4.5 and 23.4 ± 5.7 respectively] nor for the closed-arm entries [11.7 ± 0.7 and 11.9 ± 0.7 respectively] as shown in Fig. 1A. These data indicate that the injection itself did not influence either anxiety-like behavior or locomotor activity 10 days later. Therefore the results were expressed as percentage of home cage control.
As shown in Fig. 1B, two-way ANOVA for entries in the open-arm, indicated a significant corticosterone effect [F(2, 94) = 4.4; $p < 0.05$] and a significant propranolol effect [F(1, 94) = 11.4; $p < 0.01$], but no significant interaction effect between these two parameters [F(2, 94) = 2.1; $p = 0.13$] as shown in Fig. 1B. Fisher’s LSD post-hoc analysis revealed that corticosterone-treated rats displayed a higher percentage of entries in the open arms of the elevated plus maze compared to the vehicle-treated rats and also compared to the rats that received a co-administration of propranolol with vehicle or either of the two doses of corticosterone (both doses $p < 0.05$).
Similarly, two-way ANOVA analysis for the time spent in open-arm revealed a significant corticosterone effect [F(2, 94) = 3.9; $p < 0.05$, but no significant propranolol effect [F(1, 94) = 3.2; $p = 0.08$] and no interaction effect between these two parameters [F(2, 94) = 1.2; $p = 0.3$] as shown in Fig. 1C. Fisher’s LSD post-hoc analysis revealed that corticosterone-treated rats spent more time in the open arms of the elevated plus maze compared to the vehicle-treated rats (both dose $p < 0.05$).
These data demonstrate that a single injection of corticosterone could increase the open-arm exploration in the elevated plus maze, indicating a reduced anxiety-like behavior in the treated animals. Co-administration of the β-blocker propranolol abolished the corticosterone-induced effect on anxiety.
Two-way ANOVA for the number of entries in enclosed arms showed no significant corticosterone effect [F(2, 94) = 1.4; $p = 0.25$], nor a propranolol [F(1, 94) = 0.3; $p
CORTICOSTERONE EFFECTS ON ANXIETY-LIKE BEHAVIOR

= 0.57] or interaction effect [F(2, 94) = 1.3; p = 0.28], indicating that the locomotor activity was not modified by corticosterone or propranolol administration.

In conclusion, the present findings demonstrate that corticosterone, in concert with the noradrenergic system, exerts an anxiolytic effect that is visible in open field behavior 10 days after a single administration.

**Figure 1.** Corticosterone causes an anxiolytic effect that is dependent on the noradrenergic system. Anxiety-like behavior was measured in the elevated plus maze (EPM) 10 days after the acute treatment. Open-arm exploration was measured by the percentages of entries and time spent in the open arms (mean ± SEM). The locomotor activity was measured by the number of entries in the enclosed arms (mean ± SEM). (A) Anxiety-like behavior and locomotor activity in the elevated plus maze of the home-cage animals (n = 23) and the vehicle animals (n = 14) 10 days after the injection. (B) Percentage of entries in the open arms of the elevated plus maze 10 days after the injection. VEHICLE animals received either an injection of vehicle (n = 14), 3 mg/kg of corticosterone (n = 14) or 10 mg/kg of corticosterone (n = 13). PROPRANOLOL animals received 3 mg/kg of the β-blocker propranolol together with vehicle (n = 19), 3 mg/kg of corticosterone (n = 20) or 10 mg/kg of corticosterone (n = 20). ** p < 0.05 as compared with all the other groups. (C) Percentage of time spent in the open arms of the elevated plus maze by the above-mentioned groups of animals 10 days after the injection. * p < 0.05 as compared with the VEHICLE-vehicle group. (D) Locomotor activity in the elevated plus maze of the above-mentioned groups of animals 10 days after the injection.

**Effect of corticosterone administration on anxiety-like behavior**

To determine the temporal dynamics of the corticosterone effects on the reduction of anxiety-like behavior, animals were assigned to the 4 treatment groups described above and their anxiety-like behavior was assessed in the elevated plus maze at two different time points.

We first tested the rats on the day following the treatment. In agreement with the prior experiment, the injection itself did not influence the anxiety-like behavior of
the animals. An independent-samples t-test revealed no significant difference between the home cage group and the vehicle group for the open-arm entries [30.5 ± 4.5 and 25.6 ± 4.2 respectively] and for the time spent in the open arms [34.± 6.3 and 23.4 ± 4.3 respectively] (Fig 2A left panel). However, there was a difference in locomotor activity between the two groups (p = 0.03) (Fig. 2A, right panel). These data indicate that the injection did not significantly modify the state of anxiety of the animals but affected locomotion. The findings were, as previously, expressed as percentage of the home cage control group. As shown in Fig 2B, corticosterone did neither alter the number of open-arm entries [F(2,34) = 0.13; p = 0.99] nor the time spent in the open-arms [F(2,34) = 0.16; p = 0.85] when tested one day after the injection. These data indicate that the effects of corticosterone on anxiety-like behavior are not yet visible the day following the treatment. Fig. 2B shows also that the locomotor activity was almost similar for all groups [F(2,34) = 0.71; p = 0.50]. These results indicated that the anxiolytic effect provoked by a single corticosterone injection is not visible one day after the treatment and, since it was demonstrated on day 10 in the previous experiment, we conclude that it is a delayed effect of the corticosterone administration.

We subsequently investigated whether the corticosterone-induced anxiolytic effect was permanent or would disappear after some time. A previous study, also in male Wistar rats, has demonstrated that chronic restraint stress results in an alteration of the anxiety-like behavior that was still present 21 days after the cessation of the stress procedure. Therefore, we tested the behavior of our animals 21 days after they had received the treatment.

Also in this experiment, the injection did not result in a significantly altered state of anxiety in the home cage group compared to the vehicle group [open-arm entries: 33.2 ± 4.5 versus 26.1 ± 4.3; time spent in open-arm: 38.8 ± 5.8 versus 29.0 ± 5.5; locomotor activity: 10.2 ± 0.6 versus 10.5 ± 0.8; Fig. 2C]. A comparison between the treatment groups (Fig. 2D) revealed no significant drug effect for both the open-arm entries [F(2,45) = 0.05; p = 0.95] and the time spent in open-arm [F(2,45) = 0.01; p = 0.99], indicating that 21 days after corticosterone treatment there was no visible effect on anxiety-like behavior. Besides the locomotor activity was the same [F(2,45) = 0.17; p = 0.84] for all groups as is also shown on Fig. 2D.

Taken together, these experiments demonstrate that the noradrenaline-dependent corticosterone reduction of anxiety-like behavior observed ten days after the treatment is a delayed and reversible phenomenon.
Figure 2. Time course of corticosterone effect on anxiety-like behavior.
(A) One day after the injection: comparison of the anxiety-like behavior and the locomotor activity in the elevated plus maze between the home-cage animals (n = 15) and the vehicle animals (n = 11) the day following the injection. * p < 0.05 compared to home-cage group (B) Open-arm exploration and locomotor activity of the vehicle animals (n = 11) and the animals treated with either 3 mg/kg of corticosterone (n = 13) or 10 mg/kg of corticosterone (n = 13) one day after the injection. (C) Twenty-one days after the injection: comparison of the anxiety-like behavior and the locomotor activity in the elevated plus maze between the home-cage animals (n = 18) and the vehicle animals (n = 17) the day following the injection. (D) Twenty-one days after the injection: anxiety-like behavior and locomotor activity of the vehicle-treated animals (n = 17) and the corticosterone-treated animals, 3 mg/kg (n = 18) and 10 mg/kg (n = 13).
Corticosterone injection induces an early and temporary down-regulation of the HPA axis reactivity

To examine the effects of the corticosterone treatment on the HPA axis reactivity and determine a potential link with the anxiety-like behavior, seven to ten animals were sacrificed immediately after testing on the elevated plus maze. Their blood was collected and the concentrations of corticosterone in the plasma were measured as shown in table 1.

For the animals tested on day 1 post-treatment, one-way ANOVA revealed a significant effect of drug treatment on the plasma corticosterone concentration \( [F(3,33) = 6.87; p < 0.001] \). Post-hoc analysis showed that injection of the higher dose of corticosterone (10 mg/kg) induced a significant decrease of circulating corticosterone concentration the day after. This suggests that the HPA axis is down-regulated one day after the corticosterone treatment.

For the animals tested 10 days after the injection, one-way ANOVA did not reveal any drug treatment effect on plasma corticosterone concentration \( [F(3,36) = 0.87; p = 0.46] \). These findings indicate that at day 10 post-injection, the corticosterone-induced alterations of the HPA axis reactivity have disappeared, but most importantly they suggest that there is no direct link between the anxiety-like behavior and the plasma level of corticosterone.

We then examined the plasma level of corticosterone at the latest time point, 21 days. For this time point, no significant difference in the level of corticosterone among the different treatment groups \( [F(3,29) = 1.98; p = 0.14] \) was found, confirming that the activity of the HPA axis was not altered anymore.

Taken all together, the present findings suggest that an acute treatment of a high dose of corticosterone causes an early and temporary down-regulation of the HPA axis reactivity, and that this reactivity of the HPA axis is not directly responsible for the behavior observed after 10 days.

Table 1. Acute corticosterone treatment causes a temporary down-regulation of the HPA axis.

<table>
<thead>
<tr>
<th></th>
<th>Home cage</th>
<th>Vehicle</th>
<th>CORT 3</th>
<th>CORT 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>460 ± 51</td>
<td>566 ± 84</td>
<td>409 ± 53</td>
<td>221 ± 32 *</td>
</tr>
<tr>
<td>Day 10</td>
<td>571 ± 43</td>
<td>611 ± 22</td>
<td>667 ± 50</td>
<td>581 ± 60</td>
</tr>
<tr>
<td>Day 21</td>
<td>460 ± 53</td>
<td>586 ± 61</td>
<td>490 ± 44</td>
<td>612 ± 46</td>
</tr>
</tbody>
</table>

The plasma level of corticosterone (nM) was measured by radio-immunoassay in the different experimental groups at the three time points studied. The day following the treatment, the highest corticosterone resulted in a significant decrease in the plasma concentration of corticosterone. * \( p < 0.05 \). At day 10, the level of plasma corticosterone was similar in all the groups. At day 21, the level of corticosterone was also equivalent between the different groups.
DISCUSSION

The present study examined the effects of the administration of exogenous corticosterone on anxiety-like behavior and the HPA axis reactivity, and also the temporal dynamic of those effects in naïve male Wistar rats. Our findings show that a single systemic injection of corticosterone can induce a delayed and transient anxiolytic effect. Moreover, corticosterone actions on anxiety behavior are dependent on the noradrenergic system, since blockade of the β-adrenoceptors by propranolol abolished the anxiolytic effect. We also found that corticosterone could induce a temporary down-regulation of the HPA axis resulting in lower plasma corticosterone response the day following the treatment. However this alteration of the HPA axis activity does not seem to be directly the cause of the behavior, since at the time point when circulating levels of corticosterone are lower on day 1, the animals showed a normal anxiety behavior on the elevated plus maze. In contrast, when the anxiolytic effect of corticosterone is observed on the behavioral level on day 10, its plasma concentration is found to be within the physiological range. These results provide further insights about the mechanisms underlying glucocorticoid modulation of anxiety behavior. They are of interest to better understand the protective and therapeutic effects of glucocorticoids against PTSD.

There is growing evidence that administration of exogenous cortisol can have beneficial effects in PTSD (Aerni et al., 2004; de Quervain and Margraf, 2008; Schelling et al., 1999 and 2001; Weis et al., 2006). These clinical studies have shown that exogenous cortisol could prevent the development of PTSD when it was administered at the time of the traumatic experience, or decrease symptoms when it was given to PTSD patients. In line with this evidence, it has been demonstrated that acute administration of corticosteroids can reduce anxiety behavior in stress-sensitive individuals or in stressful conditions (Het et al., 2012; Putman and Roelofs, 2011; Rao et al., 2012; Soravia et al., 2006). Our present findings demonstrate that even in non-stressed animals, corticosterone can reduce anxiety-like behavior. Few studies have investigated the effects of acute glucocorticoid administration in non-stressful or non-pathological conditions and they provide conflicting results. While some have found no effect of cortisol on anxiety-like behavior (Buchanan et al., 2001), others demonstrated an anxiogenic effect with very high doses of cortisol (60 mg) (Grillon et al., 2011). Importantly, in agreement with the present results some studies also showed an anxiolytic effect of corticosteroids on non-stressed individuals or in a neutral situation (Andreatini and Leite, 1994; File et al., 1979; Reuter, 2002). Together with these previous studies, our findings suggest that glucocorticoids can have a suppressive effect on anxiety behavior by themselves and we confirm a complex and non-linear relation between glucocorticoids levels and emotional state. However it is important to mention that the times of observation in the mentioned studies are different from ours. In the mentioned studies, the anxiety behavior was assessed on the same day as the treatment, while in our present experiments we observed alteration of anxiety-like behavior 10 days after the treatment. A previous study using a similar...
time point as ours (12 days post injection) and the same dose of corticosterone found an increase of anxiety behavior after a single administration of corticosterone together with morphological changes in the amygdala; an increase in BLA principal neuron dendritic branches (Mitra and Sapolsky, 2008). This could suggest that in our experimental conditions, the animals had a high basal level of stress, which was reversed by the corticosterone treatment. We have used controls that allow us to rule out this hypothesis. Firstly, using a control group of rats that received no injection and a control group that received a vehicle injection, we showed that the injection itself had no significant effect on the parameters measured in the EPM. This suggests that the injection did not induce any serious stress that could have been reversed by the exogenous corticosterone as already demonstrated (Rao et al., 2012). Additionally to rule out any possible interference of the state of arousal at the moment of the test, we habituated the animals to the testing room during the 3 days preceding the test. It resulted in an increased open-arm exploration for all the groups; however the trend of an anxiolytic effect in the corticosterone-treated groups was conserved (data not shown). These results indicate that in our experimental conditions, the level of arousal was not excessively high and that it did not interfere with the injection or the test. However the question of how elevation of plasma corticosterone can have anxiolytic effects in certain experimental conditions while it has anxiogenic effects in others, remains. The behavioral response could be related to the state of activity of the BLA at the moment of the injection. The BLA is an important region for the expression of anxiety behavior (Tye et al., 2011; Wang et al., 2011). There is also evidence that the level of anxiety is tightly correlated with the complexity of BLA principal neurons dendritic arbors (Adamec et al., 2012; Rao et al., 2012; Vyas et al., 2003). It is possible that corticosterone induces two opposite behavioral outcomes depending on the basal intrinsic activity of the BLA (Alfárez et al., 2008; Karst et al., 2010). In the present study we did not assess the morphology of these BLA neurons, but we expect that in our corticosterone-treated animals, BLA principal neurons have less dendritic branching than the controls. We can further speculate that in our experimental conditions, the actions of corticosterone would have resulted in reduced dendritic branching or spine density, leading to a reduced response of amygdalar neurons in the stressful situation. However without morphological data of the BLA, it is difficult to define a hypothesis about the mechanisms underlying this phenomenon. Future experiments will provide information about changes in dendrite morphology in the BLA of these animals. Another important finding of this study is the implication of the noradrenergic system in the effects of corticosterone on anxiety. We found that blockade of the β-adrenoceptors with propranolol suppressed the anxiolytic effects induced by corticosterone. The implication of norepinephrine in anxiety-like behavior has already been demonstrated, and it is thought that stress-induced release of norepinephrine can promote anxiety-like behaviors (Cecchi et al., 2002; Charney et al., 1987; Morilak et al., 2005; Lapiz et al., 2001). Our data indicate that similarly to what happens during the processing of emotional memories, glucocorticoids and norepinephrine act in concert to modulate the state of anxiety. This provides further evidence that anxiety-like behavior and memory consolidation of emotional information may share common mechanisms.
engaged by the two main stress mediators (Adamec et al., 2007). It is possible that those mechanisms take place in the amygdala, particularly in the BLA. Further experiments will determine whether norepinephrine also mediates corticosterone-induced alteration of the morphology of BLA principal neurons.

By observing the animals’ behavior in the elevated plus maze at three different time points, we were able to obtain a dynamic picture of the effects of corticosterone on the state of anxiety. We found that the effects of corticosterone on anxiety were built up gradually. Consistent with our findings, other studies investigating the effects of acute stress or corticosterone on anxiety, have demonstrated that these changes were not visible one day after treatment or stress (Dickinson et al., 1985; Lim et al., 2012; Mitra et al., 2005; Mitra and Sapolsky, 2008). On the other hand, changes in the behavior were observed 10 days after the corticosterone injection. It is already known that a single exposure to severe stress or high dose of stress hormones can induce long-lasting changes on emotional states (Adamec et al., 1999 and 2004; Adamec and Shallow, 1993; Mitra et al., 2005; Mitra and Sapolsky, 2008). However, the reasons why these modifications take several days to appear remain unclear. Finally we found that the effects of corticosterone on anxiety were transient. We chose to look 21 days after the treatment in reference to a study in which animals have been allowed to recover 21 days after termination of a chronic stress of 10 days. The authors demonstrated that although the level of anxiety was still high after this period, it was not as high as at the termination of the stress treatment. Similarly, the stress-induced alterations of BLA principal neurons morphology were persistent after the recovery period, although there were less strong than just after the stress period (Vyas et al., 2004). Here we find that, 21 days after the injection, the effects of corticosterone have completely dissipated. In our experiments, the total recovery can be explained by the relative mildness of our treatment compared to the chronic stress of Vyas and colleagues. A single corticosterone injection does not induce as much changes as 10 days of chronic stress; therefore the alterations induced by the single corticosterone dose are likely to disappear more rapidly.

Our findings further indicate that corticosterone caused a transient reduction of the HPA axis reactivity. Five-min exposure to the elevated plus-maze is sufficient to induce a significant increase in plasma corticosterone (Rodgers et al., 1999). Therefore the measures from the samples collected immediately after the tests reflect HPA axis response to this aversive test. We found that on the day following the injection, the concentration of plasma corticosterone was significantly lower in the group of animals that received the highest dose of corticosterone (10 mg/kg) compared to the home cage and vehicle groups. The injection of 3 mg/kg of corticosterone also resulted in a decrease of plasma corticosterone levels, although it did not reach significance. It has been shown that administration of exogenous corticosterone results in an increase of circulating levels of corticosterone (Sandi and Rose, 1997). This high concentration of corticosterone exerts a negative feedback on the HPA axis resulting in a subsequent decreased release of glucocorticoids by the adrenal glands (Ginsberg et al., 2003; Hodges and Sadow, 1967). Our results indicate that the amount of hormone injected was sufficient to induce a shutdown of the HPA axis that was still visible the following
day but not 10 days later. Indeed the data show that HPA axis response was within the physiological range 10 and 21 days after the corticosterone administration. These results indicate that HPA axis reactivity is not directly linked to the anxiety-like behavior observed in the elevated plus maze. Indeed the time course of changes in anxiety-like behavior and corticosterone plasma concentration did not match. This indicates that glucocorticoids induce distinct modifications of physiological and behavioral responses, possibly by acting on different neural substrates.

In conclusion, the present study demonstrates that in basal condition, corticosterone in interaction with the noradrenergic system can induce a delayed and transient anxiolytic effect. Corticosterone also induces a transient reduction of the HPA reactivity that has a different time frame than the effects on anxiety-like behavior. The present findings provide further understanding of the mechanisms that underlie glucocorticoids-induced modulation of anxiety behavior and could be relevant for from a therapeutical perspective in the case of PTSD.
REFERENCES


