Anxiogenic-Like Effect of Chronic Corticosterone in the Light–Dark Emergence Task in Mice

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Chronic hypercortisolemia is a hallmark of neuroendocrine and psychiatric disorders, such as Cushing’s disease and depression. Whether cortisol directly contributes to the altered mood and anxiety symptoms seen in these diseases remains unclear. To address this, the authors have modeled hypercortisolemia by administering corticosterone in the drinking water of female Swiss Webster mice for 17 or 18 days (13 mg/kg). Light–dark emergence, startle habituation, and startle reactivity were measured. Chronic but not acute treatment with corticosterone increased the latency to emerge into the light compartment, an anxiogenic-like effect. Chronic corticosterone treatment did not affect startle habituation, but did reduce startle reactivity. This study suggests that chronic hypercortisolemia may contribute to anxiety-related behavior in patients with Cushing’s disease and depression.

Keywords: depression, cortisol, anxiety

Prolonged hypersecretion of hypothalamic–pituitary–adrenal (HPA) axis effectors, such as corticotropin-releasing hormone (CRH) and cortisol, in individuals with depression is one of the most highly replicated findings in biological psychiatry (Nemeroff & Evans, 1984; Sachar et al., 1985; Varghese & Brown, 2001). However, despite intensive investigation, it is not yet clear whether there is a causal relationship between HPA axis dysfunction and psychiatric liability (Gillespie & Nemeroff, 2005; Sapolsky, 2000). Understanding whether HPA axis effectors can induce some of the symptoms of depression is critical not only for understanding the etiology of the disease, but also for identifying novel therapeutic targets for treating depression. Therefore, determining the precise relationship between elevated HPA axis effectors and psychiatric illness is of great importance.

At least four lines of evidence suggest that corticosteroids may actually cause the mood and behavior changes in depression and, specifically, the anxiety-like features that are frequently comorbid with depression. First, more than 50% of individuals with Cushing’s disease, characterized by high blood cortisol levels, present with symptoms of depression and anxiety (Fava, Sonino, & Morphy, 1987; Kelly, 1996; Sablowski, Pawlik, Ludecke, & Herrmann, 1986). Second, the anxious-retarded subtype of depression, characterized by high anxiety levels and psychomotor retardation, is commonly associated with disruption of the HPA axis (de Winter et al., 2003). Third, individuals receiving glucocorticoid therapy for inflammatory and other disorders have long been known to have an increase in mood-related side effects, including anxiety and depression (Kayani & Shannon, 2002; Soliday, Grey, & Lande, 1999). Finally, elevated glucocorticoid levels for chronic periods are associated with increased activity in anxiety-related brain regions, such as the amygdala, in both rodents and humans (Drevets et al., 2002; Erickson, Drevets, & Schulkin, 2003; Makino, Gold, & Schulkin, 1994a, 1994b; Schulkin, Gold, & McEwen, 1998). On the basis of this evidence, we hypothesized that prolonged but not acute glucocorticoid treatment would increase anxiety-like behavior in the light–dark emergence task. Because the startle response is another unconditioned behavior that can be modulated by affective states (Grillon & Baas, 2003), we also tested the effects of acute and chronic corticosterone on habituation of the startle response and overall startle reactivity.

The idea that chronic but not acute glucocorticoid treatment may selectively affect anxiety-like behavior fits with the large literature suggesting that acute and transient stress system activation is adaptive and helps to maintain homeostasis (Munck, Guyre, & Holbrook, 1984). This is in contrast to the effects of chronic stress system activation or chronic exposure to HPA axis effectors, which may be maladaptive or increase vulnerability to psychiatric disease (McEwen, 2003; Schulkin, McEwen, & Gold, 1994). We report here that chronic but not acute administration of corticosterone to mice by using a noninvasive treatment method resulted in an anxiogenic-like effect in the mouse light–dark box (Crawley, 1981; Malmberg-Aiello, Jpponi, Bartolini, & Schunack, 2002). Chronic corticosterone, however, did not affect startle habituation but did reduce startle reactivity.

Materials and Method

Subjects

Adult (30–35 g) female Swiss Webster mice (Taconic Farms, German Town, NY) were group housed (5 per cage) in plastic cages in a temperature- and light-controlled room. Mice had access to food and water ad libitum. Mice were habituated to the facility for at least 1 week before...
testing. All procedures were conducted in accordance with McLean Hos-

pital institutional animal care and use committee-approved protocols.

**Drugs**

A well-established, noninvasive corticosterone treatment method (Fair-

child, Leitch, & Ingram, 2003; Magarinos, Orchinik, & McEwen, 1998; 

Nacher, Gomez-Climent, & McEwen, 2004) was used so as to avoid the 

stress associated with chronic intraperitoneal injections. Corticosterone 

(Sigma, St. Louis, MO) was dissolved in ethanol, diluted, and administered 

during the drinking water at a final concentration of 0 or 35 ng/ml in 0.3% 

ethanol. This concentration resulted in the ingestion of approximately 13 

mg/kg per day of corticosterone. Mice in the chronic treatment group were 

for 17 or 18 days with corticosterone or vehicle containing 0.3% 

ethanol in the drinking water. On Day 17, mice were tested for anxiety-like 

behavior in the light–dark emergence task. On Day 18, a different set of 

mice, also receiving corticosterone, were tested for startle behavior. Mice 

in the acute treatment group received a 24-hr exposure to corticosterone in 

the drinking water. Pentobarbital (15 mg/kg) was dissolved in 0.9% saline 

and was injected intraperitoneally 15 min before light–dark testing.

**Light–Dark Emergence**

The light–dark box test makes use of rodents’ natural aversion to bright 

areas compared with darker ones. In the two-compartment light–dark box, 

rodents prefer the smaller dark area and hesitate to enter the brightly lit, 

open area. Although there have been numerous adaptations of the light– 

dark box (Crawley, 1985; Imaizumi, Miyazaki, & Onodera, 1994; Onaivi 

& Martin, 1989; Stratton et al., 1993), the basic premise always remains the 

conflict between exploration of a large, bright, and open area and the safety 

of the smaller, enclosed area. In our preliminary studies, we found that 

when started in the light side of the compartment, mice given anxiogenic 

treatments did not explore rapidly enough to find and enter the dark 

compartment; instead, they tended to freeze and remain immobile for a 

majority of the test session (Ardayfio & Kim, 2004). This resulted in 

a number of false positives because increased latencies to enter the dark 

compartment are generally thought to reflect anxiety-like effects. In 

stead, we found the emergence latency to leave the dark compartment and 

enter the light compartment to be the most reliable indicator of anxiety-like 

behavior sensitive to both anxiogenic and anxiolytic treatments. The emer-

gence task was carried out in a plastic apparatus (56 cm long × 33 cm wide 

× 30 cm deep), which was divided into two compartments by a vertical 

sliding door that remained open (8 cm). The larger exploration compart-

ment comprising two thirds of the apparatus was transparent, open, and 

illuminated by a 60-W lamp placed above the compartment. The smaller 

start compartment was black and had a lid that was closed during testing. 

At the beginning of each session, the mouse was placed in a far corner of 

the dark box, facing the light compartment. The latency to enter the light 

compartment with all four paws was recorded. Mice that failed to enter 

the lit compartment within 10 min were removed and given a maximum 

latency score of 10 min. The floor of each box was cleaned with 70% 

ethanol between sessions, and mice were tested in a counterbalanced order 

in regard to treatment.

**Home-Cage Return Latency**

We developed a novel home-cage return task as a control measure to ensure 

that long latencies to emerge from the dark box did not reflect a 

general inability to locomote, but instead reflected anxiety-related behav-

ior. The apparatus used was a Plexiglas runway (47 cm long × 5 cm wide 

× 21 cm deep) that was open at one end. The home cage of test animals 

was turned on the side and placed at the open end of the runway such that 

mice could ambulate from the runway directly into the cage. Mice were 

individually placed at the closed end of the runway and allowed to traverse 

the runway into the home cage. The time required to traverse the runway 

and place all four paws in the home cage was recorded as the latency.

**Startle Habituation**

Startle reflexes were measured by using the San Diego Instruments (San 

Diego, CA) SR-Lab system, consisting of a nonrestrictive Plexiglas cyl-

inder (4-cm inner diameter, 13-cm length) mounted on a Plexiglas platform 

and placed in a ventilated, sound-attenuated chamber. Cylinder movements 

were detected and measured by a piezoelectric element mounted under 

each cylinder. Chambers were calibrated before use to ensure similar 

sensitivity across chambers. Startle stimuli were presented through a high 

frequency speaker inside the startle chambers. Background noise was 65 

dB. Startle magnitudes were sampled each millisecond during a period of 

200 ms beginning at the onset of the startle stimulus. For startle testing, 

the subject was placed into the startle chamber and allowed to acclimatize 

for 5 min. Mice were presented with 80 startle stimuli of 112 dB, with a 

duration of 40 ms and a fixed interstimulus interval of 15 s.

**Statistics**

**Light–dark box and home-cage return.** Control experiments with pen-

tobarbital and home-cage return latency were analyzed by using an un-

paired t test. Corticosterone experiments in the light–dark box were ana-

lyzed with a Mann–Whitney test.

**Startle habituation and reactivity.** Startle habituation was tested by 

using a repeated measures two-way analysis of variance with block and 

treatment as factors. For startle reactivity, the first 10 startle stimuli in each 

group were compared by using a Mann–Whitney test. An alpha level of .05 

was used for all tests.

**Results**

In previous studies, clinically effective anxiolytics have been 

shown to reduce the latency to emerge from the small, dark 

compartment into a large, brightly lit, and open area, whereas 

stress and anxiogenic treatments increase emergence latency and time spent in the dark (Crawley, 1985; Onaivi, Todd, & Martin, 1989; Shimada et al., 1995). To validate our test and ensure that our paradigm was indeed sensitive to changes in anxiety, we 

demonstrated that pentobarbital exerted anxiolytic effects at a dose 

(15 mg/kg) found to be anxiolytic in other paradigms in mice 

(FIGURE 1A). Unlike the anxiolytic effect of pentobarbital in the light–dark box, treatment of mice with 13 mg/kg corticosterone for 

17 days significantly increased the latency to emerge from the dark 

side of the box (p < .05, U = 34.0; FIGURE 1C). In contrast, acute 

corticosterone treatment failed to influence emergence latency 

(p > .05; FIGURE 1B). Therefore, our results are consistent with the 

interpretation that chronic but not acute corticosterone treatment has 

anxiety-provoking properties. One issue that must always be 

given consideration in chronic drug studies is whether toxicity is 

occurring and influencing behavior. In such a scenario, mice 

treated chronically with corticosterone perhaps showed increased 

latencies to enter the light as a result of sickness behavior, rather 

than an increase in anxiety-like behavior. We verified that after 

acute and chronic treatment with corticosterone, mice were still 

able to ambulate normally as shown by the lack of difference in the 

latency to traverse a runway to get to the home cage (p > .05; 

FIGURE 2).

As described by Grillon (Grillon, 2002; Grillon & Baas, 2003), 

the startle reflex is a ubiquitous cross-species response to abrupt 

and intense stimulation. The startle response can serve as a probe
for affective states and has been shown to be modulated by anxiety- and stress-related stimuli. Furthermore, some anxiety-inducing stimuli have been shown to disrupt habituation of the startle reflex. We tested whether corticosterone treatment would affect habituation of the startle reflex. After acute treatment both corticosterone- and vehicle-treated mice showed a normal habituation of the startle reflex as indicated by a significant main effect of block, $F(7, 91) = 6.67, p < .0001$, but no Treatment $\times$ Block interaction, $F(7, 91) = 0.85, p = .5459$ (Figure 3A). Similarly, chronic corticosterone treatment had no effect on habituation as the effect of block was significant, $F(7, 56) = 4.84, p < .0001$, but there was no Treatment $\times$ Block interaction, $F(7, 56) = 1.99, p = .0722$ (Figure 3B). In contrast to the lack of effect of corticosterone on startle habituation, startle reactivity, which was measured as the response to the first 10 startle stimuli in the habituation session, was significantly reduced by chronic ($p < .05$, $U = 2.0$; Figure 4B) but not acute corticosterone treatment (Figure 4A).

**Discussion**

We set out to investigate the effect of acute and chronic glucocorticoid treatment on anxiety-like behavior, with the hopes of establishing a link between the observation of high corticosteroid levels and changes in mood. Although several recent studies have demonstrated that chronic corticosterone can enhance conditioned fear (Conrad et al., 2004; Thompson, Erickson, Schulkin, & Rosen, 2004), the effects of chronic corticosterone on unconditioned fear or anxiety remain unclear. We report that in the light–dark emergence task, chronic but not acute corticosterone treatment at a dose of 13 mg/kg increased the emergence latency. To our knowledge, this is the first report of such an effect after chronic corticosterone treatment in this paradigm, and we are unaware of any studies that have compared the effects of both chronic and acute glucocorticoid treatment on anxiety-like behavior, including the previously mentioned studies on conditioned fear.

There are several novel aspects of our study that must be noted. First, because depression is twice as common in women as men, we used female mice in our study. An additional advantage of this was a reduction in the dominance hierarchies that typically develop in group-housed males, which can also strongly influence anxiety-like behavior (Ferrari, Palanza, Parmigiani, & Rodgers, 1998). To deliver corticosterone, we used a noninvasive drinking water method that has been well established and shown to effectively elevate serum corticosterone (Fairchild et al., 2003; Magarinos et al., 1998; Nacher et al., 2004). This was done to avoid stress and neural changes associated with repeated intraperitoneal injec-
tions (Ryabinin, Wang, & Finn, 1999). In these studies, we used a dose that results in stress-relevant increases in serum corticosterone, although our dose was considerably lower than the 40 mg/kg dose that causes changes in fear conditioning (Conrad et al., 2004; Corodimas, LeDoux, Gold, & Schulkin, 1994), hippocampal neurogenesis (Huang & Herbert, 2005), or dendritic organization and morphology (Wellman, 2001; Woolley, Gould, & McEwen, 1990).

Finally, most studies that have investigated the effects of chronic corticosterone on behavior have not simultaneously determined the effects of acute treatment, making it difficult to attribute effects to the chronicity of the treatment.

Our results suggesting an anxiogenic-like effect of chronic corticosterone are unlikely to be caused by gross differences in motor activity due to toxicity, as chronic corticosterone treatment had no effect on the latency to traverse a runway and return to the home cage. Most revealing are the data showing that both vehicle- and acute and chronic corticosterone-treated mice had mean latencies to traverse the runway of under 15 s (see Figure 2), which is well below the mean latency of 382 s that the chronic corticosterone-treated mice took to enter the light compartment. As we clearly showed that corticosterone-treated mice could easily locomote when highly motivated to do so, we conclude that the differences we observe in latency to enter the light compartment are due to an increase in anxiety-like behavior produced by chronic but not acute exposure to corticosterone.

Other exploratory paradigms have been used to address whether chronic glucocorticoids affect anxiety-like behavior. Corticosterone administered directly into the amygdala enhanced anxiety-like behavior in the elevated-plus maze in rats (Shepard, Barron, & Myers, 2000). Although systemic corticosterone given chronically to rats was reported to have no effect in the elevated-plus maze (Andreatini & Leite, 1994), the synthetic glucocorticoid prednisone increased anxiety-like behavior in this task (Gonzalez-Perez, Ramos-Remus, Garcia-Estrada, & Luquin, 2001). Similarly, chronic corticosterone-treated rats increased a subset of defensive behaviors (Gregus, Wintink, Davis, & Kalynchuk, 2005; Kalyn-

![Figure 2](image2.png)

**Figure 2.** Home-cage return latency after acute (A: n = 7 per group) and chronic (B: n = 5 per group) corticosterone treatment.

![Figure 3](image3.png)

**Figure 3.** Startle habituation in mice treated acutely (A: vehicle, n = 7; corticosterone, n = 8) or chronically (B: n = 5 per group) with corticosterone (cort). Each block represents the mean (± SEM) response to 10 startle stimuli.
on startle reactivity, with low doses elevating and high doses attenuating baseline startle reactivity (Buchanan, Brechtel, Sollers, & Lovallo, 2001). Second, low depression has been shown to enhance baseline startle, whereas high depression is associated with an attenuated startle response relative to low depression (Kaviani et al., 2004). Finally, other indirect evidence that glucocorticoids may modulate startle is the observation that individuals with post-traumatic stress disorder often have high baseline startle and frequently present with low glucocorticoid levels (Marshall & Garakani, 2002; Yehuda, McFarlane, & Shalev, 1998). Likewise, low glucocorticoid levels in humans, nonhuman primates, and rodents are associated with high baseline startle levels (Milde, Sundberg, Roseth, & Murison, 2003; Sanchez et al., 2005). This preclinical and clinical literature, together with our data, suggests that glucocorticoids may be important modulators of startle reactivity as well as anxiety-like behavior.

Given the results observed from other reports comparing both startle and exploratory models of anxiety (Bannon et al., 2000; Desousa, Wunderlich, De Cabo, & Vaccarino, 1998), our differing results in the light–dark task and startle reflex are not surprising and suggest that these tasks may be sensitive to different types of anxiety or affective states. Numerous studies have found either opposing or different effects between exploratory anxiety tasks, such as the light–dark box and startle reactivity (Jonkman, Henry, Semenova, & Markou, 2005; Paterson, Whiting, Gray, Flint, & Dawson, 2001; Podhorna & Didriksen, 2004; Roskoden, Hanke, Yilmazer-Hanke, & Schwegler, 2005; Yilmazer-Hanke, Wigger, Linke, Landgraf, & Schwegler, 2004), suggesting little correlation between the two tasks. In support of this, the clinically anxiogenic compounds FG-7142 and yohimbine show anxiogenic effects in the light–dark box (Bilkei-Gorzo, Gyertyan, & Levay, 1998; Fernandez, Misilmeri, Felger, & Devine, 2004), although they have no effect on startle reactivity (Risbrough & Geyer, 2005). This underscores that anxiety is not a unitary construct but a complex behavior both in humans (Kendler et al., 1996; Mannuzza et al., 1989) and animals (Belzung & Griebel, 2001; Flint, 2001; Rodgers, 1997).

Our results further strengthen the view that acute exposure to glucocorticoids may be adaptive, whereas chronic exposure has...
detrimental effects to brain and behavior. Although other behavioral studies have not generally compared acute and chronic glucocorticoid treatment, several studies have found emotion-related neural changes that occur selectively with chronic but not acute glucocorticoid exposure. For example, in rats, chronic but not acute corticosterone given through the drinking water attenuates 5-HT (1A) autoreceptor function in the dorsal raphe nucleus (Fairchild et al., 2003). Also, acute versus chronic corticosterone treatment in rats produced opposite effects on neural cell adhesion molecule expression in the frontal cortex (Sandi & Loscortales, 1999). Likewise, chronic but not acute corticosterone treatment increased homovanillic acid and 5-hydroxyindoleacetic acid levels in the prefrontal cortex of rats (Inoue & Koyama, 1996). Another potential mechanism that may mediate the anxiety-like effect of chronic but not acute corticosterone treatment is the increase in amygdalar CRH induced by chronic corticosterone treatment, which has been reported by several investigators (Makino et al., 1994a, 1994b; Shepard et al., 2000; Swanson & Simmons, 1989; Thompson et al., 2004). However, because these studies did not assess the effects of acute corticosterone exposure, the differences we found between acute and chronic corticosterone on behavior cannot be directly attributed to the increase in CRH caused by chronic corticosterone treatment.

In experiments described in this article, we sought to explore the relationship between elevated glucocorticoid levels and depression-related behavior. Establishing clear links between pathological changes in physiology and subsequent changes in behavior is key to designing novel psychiatric drugs that address the causal or etiological factors involved in disease rather than those drugs that may be attenuating only epiphenomena. Thus, on the basis of these studies and others, one might expect that antagonists against HPA axis-related systems might be particularly useful against some types of anxiety symptoms in depression. Consistent with this, several preclinical studies have found an anxiolytic-like effect of glucocorticoid blockade in rodents as measured by the elevated-plus maze (Schullkin et al., 1998). Other treatments that attenuate the HPA axis have also been found to attenuate anxiety-like behavior (Seymour, Schmidt, & Schulz, 2003).

If indeed, as our light–dark study suggests, long-term exposure to elevated circulating glucocorticoid provokes anxiety-like responses in ambiguous situations, then this finding has important implications for depression, in which a significant population of affected individuals are hypercortisolemia. Thus, hypercortisol-emia may in fact contribute to the symptom profile of depression, rather than being a consequence. Such a model is consistent with the observation of high comorbidity of anxiety and depression (Cloninger, 1990), the high rates of anxiety in Cushing’s syndrome and Cushing’s disease (Fava, 1994; Loosen, Chambliss, DeBold, Shelton, & Orth, 1992; van Aken et al., 2005), the altered HPA axis associated with the anxious-retarded subtype of depression (de Winter et al., 2003), and the increased amygdala activity seen in depressed patients that are hypercortisolemic (Drevets et al., 2002). In summary, we have found that chronic but not acute corticosterone treatment in mice attenuated exploratory behavior in the light–dark task, attenuated startle reactivity, and had no effect on habituation of the startle reflex. Future studies should investigate the neural mechanisms involved in glucocorticoid attenuation of startle reactivity and exploratory behavior.

References


Roskoden, T., Hanke, J., Yilmazer-Hanke, D., & Schweger, H. (2005). Reduced number of CRF-containing neurons in the central amygdala correlated with enhanced locomotor activity following early postnatal


Sapolsky, R. M. (2000). Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. *Archives of General Psychiatry*, 57, 925–935.

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