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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, Ipponi, 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

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testing. All procedures were conducted in accordance with Mclean Hospital institutional animal care and use committee-approved protocols.

Drugs

A well-established, noninvasive corticosterone treatment method (Fairchild, 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 via the drinking water at a final concentration of 0 or 35 μg/μl 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 treated 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 anxiolytic-like effects. Instead, 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 emergence task was carried out in a plastic apparatus (56 cm long $\times$ 33 cm wide $\times$ 30 cm deep), which was divided into two compartments by a vertical sliding door that remained open (8 cm). The larger exploration compartment 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 behavior. The apparatus used was a Plexiglas runway (47 cm long $\times$ 5 cm wide $\times$ 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 cylinder (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 pentobarbital and home-cage return latency were analyzed by using an unpaired *t* test. Corticosterone experiments in the light–dark box were analyzed 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

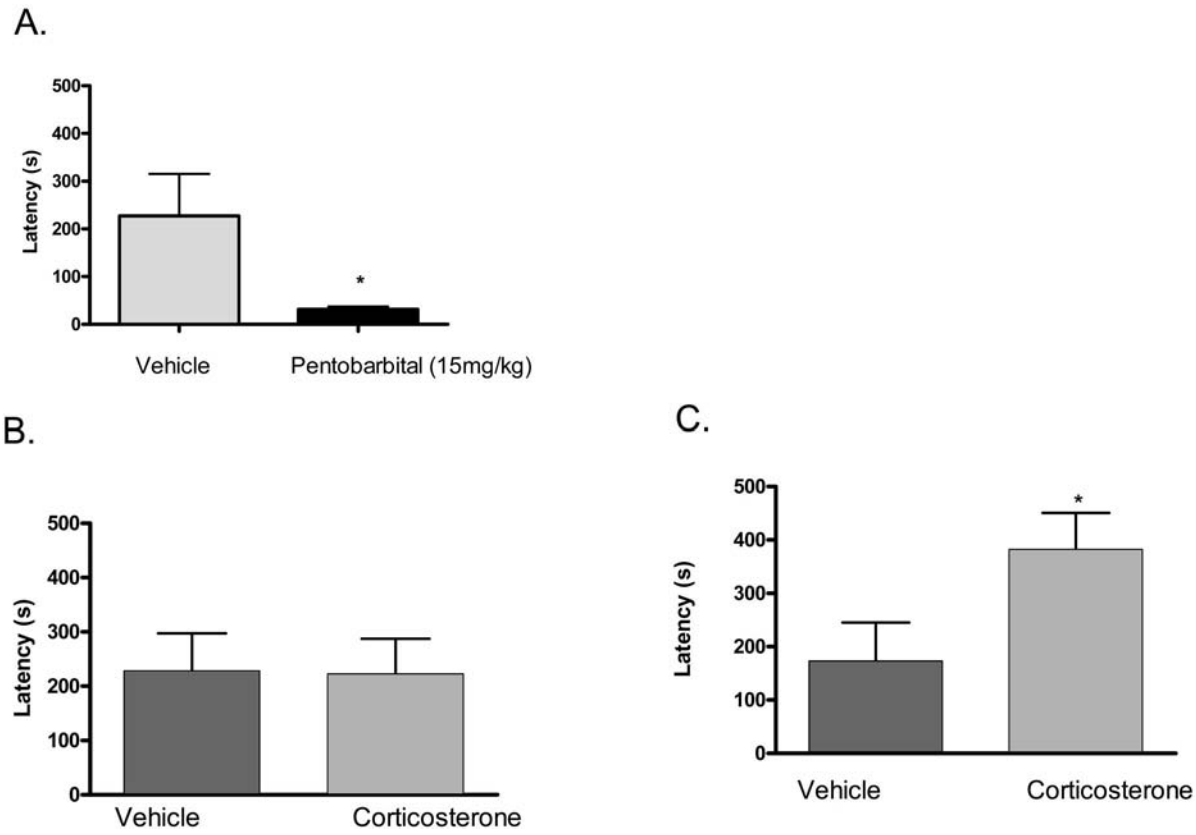

Figure 1. Effects of the well-validated anxiolytic drug pentobarbital (A: vehicle, $n = 9$; pentobarbital, $n = 6$) and acute (B: vehicle, $n = 10$; corticosterone, $n = 8$) and chronic (C: vehicle, $n = 12$; corticosterone, $n = 13$) corticosterone treatment on emergence latency in the light–dark box. $^*p < .05$, compared with vehicle-treated mice.

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 × 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 × 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-

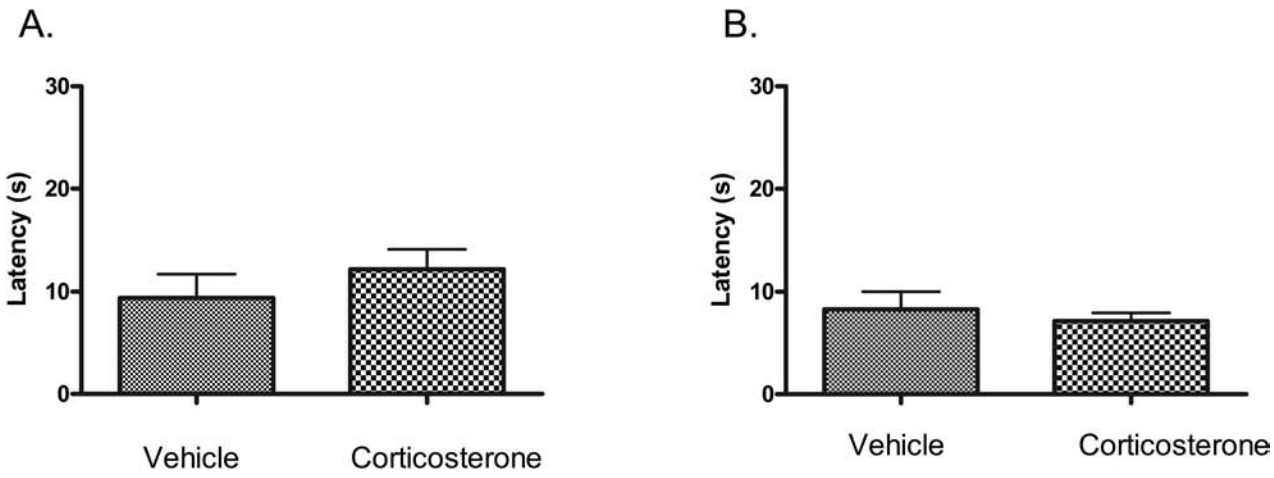

Figure 2. Home-cage return latency after acute (A: $n = 7$ per group) and chronic (B: $n = 5$ per group) corticosterone treatment.

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-

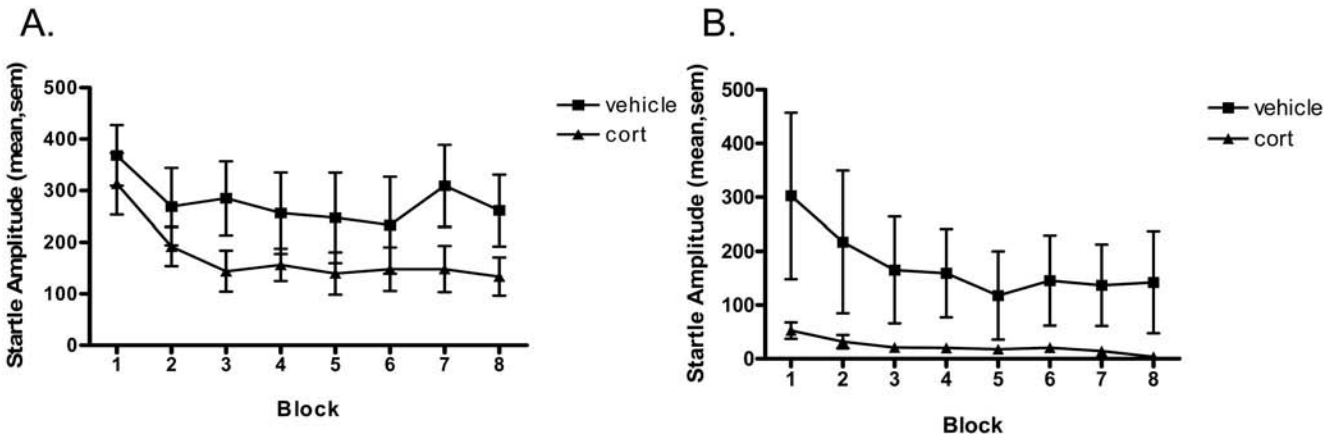

Figure 3. Startle habituation in mice treated acutely (A: vehicle, $n = 7$; corticosterone, $n = 8$) or chronically (B: $n = 5$ per group) with corticosterone (cort). Each block represents the mean ($\pm$ *SEM*) response to 10 startle stimuli.

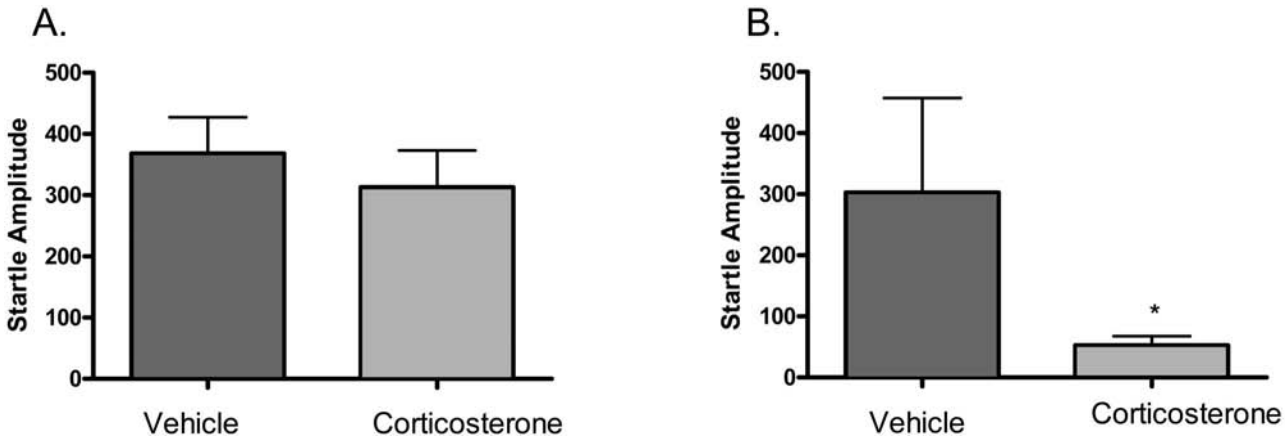

Figure 4. Startle reactivity in mice treated acutely (A: vehicle, $n = 7$; corticosterone, $n = 8$) or chronically (B: $n = 5$ per group) with vehicle or corticosterone. Means ($\pm SEM$) of the first 10 startle stimuli in the habituation session are shown. $^*p < .05$, compared with vehicle-treated mice.

chuk, Gregus, Boudreau, & Perrot-Sinal, 2004) but not open-field behavior. Finally, numerous studies have found that acute and chronic corticosterone increase anxiety-like behavior in conditioned fear paradigms (Conrad et al., 2004; Corodimas et al., 1994; Hui et al., 2004; Thompson et al., 2004); however, when using conditioned fear paradigms, it is difficult to dissect whether changes are due to enhanced learning and memory, increased anxiety, or both.

In contrast to our findings with light–dark emergence, chronic corticosterone treatment did not affect startle habituation (see Figure 3), but reduced startle reactivity as measured by the response to the first 10 startle stimuli (see Figure 4). Similarly, we found that chronic corticosterone treatment significantly reduced the overall startle reactivity when all 80 startle stimuli were pooled (data not shown). Our observation of reduced startle reactivity in mice receiving chronic corticosterone is, to our knowledge, the first replication of the findings of Stevens, Bullock, and Collins (2001), who examined the effects of chronic corticosterone on prepulse inhibition and also reported reduced startle reactivity in mice receiving chronic corticosterone treatment. In addition, our findings are consistent with several lines of preclinical and clinical evidence that suggest that HPA axis hyperactivity may act to attenuate the startle response.

First, Glowa, Geyer, Gold, and Sternberg (1992) have suggested that an inverse relationship exists between HPA axis activity and startle reactivity, based on studies of startle in Lewis and Fisher rats. Second, mice overexpressing CRH that mimic many aspects of the neuroendocrine abnormalities in depression, including dexamethasone resistance and elevated basal corticosterone levels, also show reduced startle reactivity (Dirks et al., 2002). The findings in the CRH-overexpressing mice are similar to our data showing reduced startle reactivity in mice receiving chronic corticosterone and suggest that chronically increased basal corticosterone may be the mechanism by which CRH-overexpressing mice show an attenuated startle response. Consistent with these animal studies, studies in humans also suggest that glucocorticoids can modulate startle reactivity. First, cortisol was found to have a biphasic effect 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 & Loscertales, 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 (Schulkin 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 hypercortisolemic. Thus, hypercortisolemia 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.

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