Published in final edited form as:

Physiol Behav. 2012 February 28; 105(4): 958–965. doi:10.1016/j.physbeh.2011.10.032.

Lack of elevations in glucocorticoids correlates with dysphoria-like behavior after repeated social defeat

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Abstract

Activity of the hypothalamic–pituitary–adrenocortical (HPA) axis is often abnormal in depression and could hold clues for better treatment of this debilitating disease. However, it has been difficult to use HPA activity as a depression biomarker because both HPA hyperactivity and HPA hypoactivity have been reported in depression. Melancholic depression has typically been associated with HPA hyperactivity, while atypical depression has been linked with HPA hypoactivity. Many animal models of chronic stress recapitulate behavioral aberrations and elevated HPA activity that could represent a model for melancholic depression. However, there are no animal models that could be used to elucidate the etiology or treatment of atypical depression. We have used repeated social defeat in mice to test the hypothesis that this chronic stress would induce dysphoria-like behavior associated with HPA hypoactivity in a subset of subjects. Intruder mice were placed in the home cage of an aggressive resident mouse for 5 min/d for 30 days. The majority of intruder mice had elevated basal plasma corticosterone (High Morning Corticosterone, or HMC) and adrenal 11 β hydroxylase mRNA levels relative to control mice that were handled daily. However, a subset of intruder mice (Low Morning Corticosterone; LMC) exhibited basal plasma corticosterone and 11 β hydroxylase mRNA levels that were indistinguishable from control levels. Significant changes in emotional behavior only occurred in LMC mice, which exhibited anxiety-like increases in activity and defecation during tail suspension and anhedonia-like decreases in sucrose preference. Relative to HMC mice, LMC mice also showed increases in gene expression of mineralocorticoid receptor in CA2 hippocampus, consistent with the possibility that HPA activity in this group is constrained by increased sensitivity to glucocorticoid negative feedback. LMC mice also exhibited increased c-fos gene expression compared to HMC mice in the paraventricular hypothalamus and lateral septum suggesting that central pathways fail to habituate to chronic stress even though adrenocortical activity is not stimulated. We conclude that LMC mice showed adrenocortical hyporesponsiveness, which in combination with the behavioral abnormalities in this group may represent a model for the HPA hypoactivity associated with atypical depression.

Keywords

Depression; Post-traumatic stress disorder; Glucocorticoid; Glucocorticoid receptor; Mineralocorticoid receptor; Habituation

1. Introduction

Depression is a significant health problem that affects millions of people, for many of whom depression will be a recurring and debilitating problem throughout life. Depression disrupts a person's sleep cycles, appetite, energy level, and motivation, ultimately affecting his work and social life [1]. The main difficulties in treating depression include the wide range of symptoms and variable responsiveness of patients to pharmacological interventions. In addition, psychoactive drugs can cause significant side effects, including weight gain and sexual dysfunction [2]. In a meta-analysis using 182 trials from 1980 to 2007 with a total of approximately 36,000 patients, little more than half of patients being treated with major depressive disorder achieved remission after a single treatment [3]. Even for patients who do experience remission, roughly half will continue to experience residual symptoms of depression such as sleep disturbances, apathy, and guilt and other negative thoughts [3].

The biological features of depression symptoms include alterations in neuroendocrine function, of which changes in hypothalamic–pituitary–adrenocortical (HPA) activity have received some of the most interest [1,4]. During HPA activation, the hypothalamus releases corticotropin releasing hormone (CRH), vasopressin, and/or oxytocin, which subsequently act on the anterior pituitary gland triggering the release of adrenocorticotrophic hormone (ACTH). ACTH in turn acts on the adrenal cortex to stimulate the secretion of glucocorticoids (cortisol in humans and corticosterone in rodents). Glucocorticoid feedback inhibition also controls HPA activity by inhibiting both hypothalamic secretion of ACTH-releasing factors and pituitary secretion of ACTH [5].

HPA activity is regulated by stress, a circadian rhythm, and glucocorticoid negative feedback [5], each of which may be abnormal in depression [6–8]. HPA dysfunction in depressed patients is typically evident as increased secretion of cortisol and/or reduced sensitivity to feedback inhibition by glucocorticoids such as dexamethasone [4]. Impaired feedback inhibition in depression has been proposed to be due to reduced expression or function of glucocorticoid receptors [9]. Antidepressants have been shown to increase glucocorticoid receptor (GR) expression in animals, leading to the suggestion that antidepressant-induced increases in GR expression might compensate for glucocorticoid receptor dysfunction in depression [9]. Aberrant cortisol levels or dexamethasone non-suppression can be normalized by successful treatment, whereas HPA function that does not normalize is a strong predictor of relapse [4]. Consequently, HPA activity has been of interest as a biological marker to help predict and track antidepressant response.

While 50 to 80% of patients with depression present with HPA axis hyperactivity, a significant portion shows either normal or reduced HPA axis activity [9–11]. These differences in HPA activity are exemplified by the differences between atypical and melancholic depression. Melancholic depression is defined by mood non-reactivity (inability to respond positively to pleasurable experiences), as well as three additional symptoms that may include weight loss, inordinate guilt, early morning insomnia, or agitation [12]. Melancholic depression is typically associated with HPA hyperactivity, which is thought to be caused by impaired feedback inhibition due to GR dysfunction [9].

In contrast, the symptoms of atypical depression can be seen as a reversal of the vegetative symptoms present in melancholic depression. Atypical depression is defined by mood reactivity (ability to experience improved mood in response to pleasurable events) and two additional symptoms, which may include leaden paralysis (subjective difficulty in moving), increased appetite, increased sleep, and rejection sensitivity [12]. Also in contrast to melancholic depression, atypical depression exhibits evidence of HPA hypoactivity and increased HPA sensitivity to glucocorticoid feedback. Atypical depressed patients more

often exhibit low or normal rather than elevated levels basal cortisol [13]. Instead of being resistant to suppression by the synthetic glucocorticoid dexamethasone (as has been found in “typical” or melancholic depression), cortisol levels in atypical depression appear to be more readily inhibited, being suppressed to a greater extent by both standard and low doses of dexamethasone [13–15]. Although HPA hypoactivity in atypical depression has also been attributed to insufficient production of CRH from the hypothalamus [1] it cannot be excluded that low CRH could also result from greater sensitivity to glucocorticoid feedback. Atypical depression and melancholic depression can thus be separated not only by psychiatric symptoms but also by differences in HPA basal activity and sensitivity to glucocorticoid inhibition.

Atypical and melancholic depression have also been distinguished by antidepressant efficacy. Before the introduction of SSRIs (selective serotonin reuptake inhibitors), patients with melancholic depression were found to respond to either tricyclic antidepressant (TCAs) or monoamine oxidase inhibitors (MAOIs) [16]. Atypical depression, particularly if patients experience early onset and chronic depression, appeared to respond most robustly to MAOIs with limited responsiveness to TCAs [16]. Thus, atypical and melancholic depression are characterized not only by opposing psychiatric symptoms and HPA abnormalities but also by differential responses to antidepressants.

Despite the dearth of robust evidence for the efficacy of newer antidepressants in atypical depression [17], MAOIs are currently little used because of their potentially severe side effects and required dietary restrictions. Developing an animal model of depression with suppressed HPA activity might aid in identifying antidepressants to replace MAOIs for treatment of atypical depression and could also reveal if HPA hypoactivity predicts responsiveness to specific antidepressants. HPA hypo-responsiveness has been reported in a subset of socially subordinate male rats housed in a naturalistic visible burrow system [18]. These nonresponsive subordinates demonstrated HPA hypoactivity, attributed to hypothalamic dysfunction, in response to restraint stress [19]. Such HPA hypo-responsiveness could be a useful model of the HPA hypoactivity reported in atypical depression.

An additional model for chronic social stress, social defeat, uses repeated exposure of an intruder male to the territory of a resident male who defends his home cage by dominating the intruder in physical combat [18]. Repeated social defeat has also been proposed to induce a depression-like state [20]. We hypothesized that repeated social defeat mimics the social stress effects of the visible burrow system and would induce HPA hypoactivity in a subset of intruder mice. We also hypothesized that mice with HPA hypoactivity would also show a depressive-like phenotype in classic behavioral tests of depression-like behavior. Supporting our hypothesis, our results suggest that a subgroup of mice with low morning corticosterone levels indeed does show several rodent correlates of dysphoric behavior such as anhedonia-like loss of sucrose preference and abnormal, anxiety-like activity and autonomic reactivity during tail suspension. This model may be useful for elucidating the etiology and treatment of atypical depression.

2. Experimental design

2.1. Animals

The Institutional Animal Care and Use Committee of Albany Medical College approved all animal procedures. Male mice on a C57BL/6 background (2–5 months of age) were housed either individually (intruder group) or with a female (resident group) that was removed before resident–intruder encounters. Mice were housed on a 12:12 light cycle (lights-on, 7 am).

2.2. Social defeat stress

Mice assigned to the intruder group were placed in the home cage of a resident male mouse, whereupon the resident attacked and chased the intruder. Encounters were limited to 5 min per day to avoid harm to the intruders. Intruders were rotated among different resident males to avoid any reduction in aggressiveness or habituation of resident–intruder behavior. Resident–intruder encounters took place every day for 30 days. A group of control mice was briefly handled each day but otherwise left undisturbed.

2.3. Behavioral assays

Depression-like behavior was scored in intruder and control mice by tail suspension and sucrose preference tests. Despair-like immobility was scored as the time spent hanging immobile in a 5 minute tail suspension test on day 17, 1 h after resident–intruder encounters. Sucrose preference was measured on days 18 and 19 as the percentage of total fluid intake in a two-bottle choice test between 1% sucrose and water. Mice were not food-deprived before sucrose preference testing. All behavioral data were collected and scored by trained individuals blind to the group assignments of the mice.

2.4. Tissue collection

Blood was collected in intruder and control mice on day 13 by submandibular venipuncture within an hour of lights-on. On day 30, intruder and control mice were subjected to a 5 min resident–intruder encounter and killed by decapitation 30 min later. Trunk blood was collected for assay of plasma ACTH and corticosterone. Brain and adrenals were fresh frozen in OCT embedding medium (Sakura Finetek, Torrance CA) and stored at -80°C for subsequent in situ hybridization analysis.

2.5. Hormone assays

Plasma ACTH and corticosterone were assayed with radioimmunoassay assay kits from MPBiomedicals (Solon, OH), using all reagents and samples at half-volume, as previously described [11].

2.6. In situ hybridization

Coronal brain or adrenal sections ($10\text{ }\mu\text{m}$) were thaw-mounted on to SuperFrost Plus slides (Fisher Scientific, Pittsburgh, PA) and stored at -80°C until use. Sections were fixed for 20 min in 4% phosphate-buffered paraformaldehyde, rinsed in standard saline citrate buffer, acetylated in 0.25% acetic anhydride/0.1 M triethanolamine, dehydrated in increasing ethanol concentrations, and stored at -80°C until hybridization. Corticotropin-releasing hormone (CRH) mRNA was detected with an antisense ^{35}S -labeled cRNA probe complementary to the 606 bp Sty I–Msc I fragment of the rat CRH cDNA [21]. Vasopressin primary transcript was detected with a probe complementary to the 800 bp Kpn I–Xba I fragment of intron 1 of the mouse vasopressin gene [22]. Brain c-fos gene expression was analyzed with a ^{35}S -cRNA probe complementary to the 1.5 kb Pst I fragment of the mouse c-fos DNA (pGEMfos3; [23]), which was generously provided by Dr. Michael Greenberg (Children's Hospital, Boston, MA). In situ hybridization of adrenal 11β hydroxylase mRNA was performed with a ^{35}S -labeled cRNA probe complimentary to the 600 bp Spe I/Bam HI fragment of the plasmid 11BOHpCMV5 [24], corresponding to the 3' untranslated region of the mouse 11β -hydroxylase. This fragment was sub-cloned into the Spe I and Xba I sites of pBluescript II SK + (Stratagene, La Jolla, CA) for use as a template for probe synthesis. Glucocorticoid and mineralocorticoid receptor gene expression was analyzed with ^{35}S -cRNA probes synthesized from clones generously provided by Gunther Schütz, as previously described [25]. Hybridization was carried out for 16–20 h at 60 (CRH, c-fos), 55 (vasopressin primary transcript) or 50°C (11β -hydroxylase). In situ hybridization for orexin

was performed as previously described [26], using a probe derived from a clone provided by Dr. Teresa Reyes (University of Pennsylvania, Philadelphia, PA).

Following hybridization, slides were treated with 20 µg/ml RNAase A, then washed several times in 1X and 0.5X sodium citrate buffer at 25 °C, followed by a wash in 0.5% sodium citrate buffer at hybridization temperature, and dehydrated with increasing concentrations of ethanol. Hybridized slides were exposed simultaneously to phosphorimager screens (GE Life Sciences, Niskayuna, NY), with slides from control and intruder groups on each screen. C-14 autoradiography standards (146B, American Radiolabeled Chemicals, St. Louis, MO) were also exposed on each screen to confirm that exposures were within the linear range and to provide a basis for comparison between screens. Screens were scanned with a Typhoon 9210 phosphorimager (GE Healthcare, Niskayuna, NY) at 50 µm resolution.

Densitometric readings were collected from phosphorimager images using a hand-drawn template for each brain region. Background signal was measured in a non-expressing area of the same tissue section. Variations in area among templates for a given region were corrected by scaling the optical density of a region by the largest area for all readings in this region. Readings were corrected for background and then normalized to ¹⁴C standards. The highest two normalized readings were averaged to give a single value for each mouse prior to statistical analysis.

2.7. Data analysis

Data were analyzed by one-way ANOVA with by post-hoc testing by Student's *t*-test with Bonferroni correction. Group *N*s occasionally differ for different endpoints because insufficient sections were available through a given region for in situ hybridization analysis. Data are reported as mean ± SEM; significance was set at *P*<0.05.

3. Results

We first tested if the resident–intruder social defeat model had differential effects on HPA activity in intruder mice (Fig. 1). Using a cut-off of 2 standard deviations above the mean of control mice, we found that 6 intruder mice had elevated plasma corticosterone relative to control (Fig. 1B) at the circadian nadir after 13 days of social defeat (High Morning Corticosterone, or HMC), whereas 3 intruder mice had corticosterone levels that did not differ significantly from those in control mice (Low Morning Corticosterone, or LMC). These group designations were used for all subsequent data analysis, although data were collected in a blinded manner. ACTH did not differ among experimental groups (Fig. 1A).

We performed several behavioral tests to determine if mice subjected to social defeat demonstrated common behavioral phenotypes of affective dysfunction. Tail suspension immobility, a behavioral correlate of the despair or apathy of depression [27], was unchanged in HMC but significantly lower in LMC intruders compared to controls (Fig. 2A). Defecation, a measure of stress-related autonomic reactivity [28], was significantly higher compared to controls during tail suspension in LMC intruders but not HMC intruders (Fig. 2B). Sucrose preference, a measure of depression-like anhedonia [29], decreased between the two days of sucrose access in LMC intruders but not HMC intruders compared to controls (Fig. 2C).

To determine which levels of the HPA axis exhibited differential activity between HMC and LMC intruders, and whether LMC and HMC mice might adapt differentially to the stress of repeated social defeat, we analyzed plasma hormones and hypothalamic and adrenal gene expression 30 min after a 5-minute encounter with a resident male in all mice, representing the thirtieth encounter for intruder mice and the first encounter for control mice. Neither

hypothalamic corticotropin-releasing hormone mRNA levels nor vasopressin heteronuclear RNA levels differed among groups (Fig. 3A and B). Although there was a trend for decreased plasma ACTH across intruder groups, particularly in LMC intruders, plasma ACTH and corticosterone also did not differ among groups 30 min after the beginning of the resident–intruder encounter (Fig. 3C and D). However, adrenal gene expression of 11 β -hydroxylase, the final enzyme for corticosterone biosynthesis, was significantly higher in HMC intruders, mirroring the differences in circadian nadir corticosterone observed on day 13 (Fig. 3E).

Because corticosteroid feedback regulation of HPA activity occurs primarily at the brain [30], we used in situ hybridization to test if differences in adrenocortical axis activity among groups were associated with differences in glucocorticoid (GR) or mineralocorticoid receptor (MR) mRNA levels in the hippocampal formation, prefrontal cortex (PFC), paraventricular hypothalamus, locus coeruleus, and dorsal raphe nucleus (Fig. 4). We and others have found antidepressant regulation of corticosteroid receptor expression in these regions ([22,25,31], and references therein), suggesting that these structures might be involved in glucocorticoid-relevant effects on emotional behavior. GR and MR expression patterns throughout the brain (not shown) were similar to those we have previously reported [25,31]. Because the ventral and dorsal regions of the medial prefrontal cortex have been functionally distinguished with regard to their effects on HPA activity [32], we analyzed GR and MR expression separately in the ventromedial (Fig. 4E and M) and dorsomedial (not shown) prefrontal cortex. GR gene expression (Fig. 4A–H) did not differ significantly among groups in any region except the paraventricular nucleus of the hypothalamus (PVN), where GR was significantly higher than controls in HMC but not LMC intruders (Fig. 4F).

No differences in MR mRNA (Fig. 4I–M) were observed among groups except in the CA2 subfield of the hippocampus and in the ventromedial prefrontal cortex. MR mRNA in CA2 hippocampus was significantly lower in HMC vs. control mice (Fig. 4J). LMC mice had significantly higher levels of CA2 MR gene expression than HMC mice, but did not differ from controls (Fig. 4J). Ventromedial prefrontal cortex MR gene expression was significantly lower relative to control levels in both HMC and LMC intruders, but LMC intruders tended, although not significantly ($P = 0.091$), to have higher levels than HMC intruders (Fig. 4M). Dorsomedial prefrontal cortex MR gene expression did not differ among groups (not shown).

We also examined expression of the neuronal activity marker c-fos to determine if activity of any brain regions correlated with differences in adrenocortical axis activity of the different groups. We observed marked induction of c-fos gene expression after social defeat in a number of brain regions, including the paraventricular hypothalamus, lateral septum, ventromedial prefrontal cortex, and paraventricular nucleus of the thalamus (Fig. 5). In both the paraventricular hypothalamus and the lateral septum, induction of c-fos gene expression was significantly lower in HMC vs. control mice; however, c-fos gene expression did not differ between LMC and control mice (Fig. 5A and B). In the ventromedial prefrontal cortex (Fig. 5C), which has been linked with habituation of HPA responses to repeated stress [33], there was a similar but nonsignificant trend in c-fos gene ($F_{2,9} = 3.69$; $P = 0.0675$; $N = 4, 5$, and 3 for control, HMC, and LMC groups, respectively). The posterior paraventricular thalamic nucleus has also been shown to play a role in changes in HPA activity after repeated stress [34]. Levels of c-fos mRNA in the posterior paraventricular thalamus tended to be reduced in both HMC and LMC mice relative to control mice, but these differences were not significant (Fig. 5D; $F_{2,11} = 3.213$; $P = 0.0796$; $N = 5, 6$, and 3 for control, HMC, and LMC groups, respectively). Several other brain regions were analyzed for c-fos gene expression, including the dorsomedial prefrontal cortex, the anterior paraventricular

thalamic nucleus, the dorsomedial hypothalamus, and the bed nucleus of the stria terminalis, none of which exhibited any significant differences among groups (data not shown).

We further analyzed gene expression of prepro-orexin in the lateral hypothalamus because this neuropeptide has been implicated in the regulation of HPA activity, social dominance, and depression-like symptoms [20,26,35]. There was a trend toward lower preproorexin mRNA levels in both intruder groups, with LMC intruders having the lowest levels. However, these differences were not significant (Fig. 6).

4. Discussion

We have found evidence of differential HPA adaptation in mice exposed to repeated social defeat. One subset of repeatedly defeated intruders exhibited evidence of the elevated basal HPA activity that has often been found after chronic stress [18]. However, another subset of intruder “low morning corticosterone,” or LMC, mice failed to exhibit this increase in basal HPA activity. Although their failure to elevate morning corticosterone suggested resiliency to the stress of chronic social defeat, the consistently exhibited abnormal performance in tests of emotional behavior revealed that other responses to stress, namely behavioral coping, were impaired in this second subset of mice. This second subset may represent a rodent model for affective disorders such as atypical depression, in which HPA activity is not elevated or may even be suppressed.

Within a group of similarly-treated intruder mice, we found two distinct groups that either elevated or failed to elevate adrenocortical activity after repeated social defeat. In basal conditions, the HMC intruders showed elevations in basal plasma corticosterone that were consistent with those reported in a number of chronic stress models [18,36]. This increase in basal corticosterone was also consistent with increase in mRNA levels of 11 β -hydroxylase, the final step in glucocorticoid synthesis, in HMC intruders. LMC intruders were distinguished by their lack of elevations in basal plasma corticosterone after repeated social defeat, and also did not exhibit any increase in 11 β -hydroxylase gene expression. Our findings are in agreement with evidence of a HPA hyporesponsiveness in a subset of submissive rodents [18]. In the latter study, HPA hypoactivity was reported for a subset of subordinate rats that failed to show significant increases in corticosterone after exposure to novel, acute restraint stress and exhibited reduced corticotropin-releasing hormone (CRH) gene expression in the paraventricular nucleus of the hypothalamus (PVN). We did not observe differences in stress-induced hormone secretion or hypothalamic ACTH-releasing factor gene expression among groups, possibly because the timing of our single samples missed critical times at which these endpoints did diverge. However, although LMC mice did not show absolute HPA hypoactivity relative to control mice, the lack of increases in both basal plasma corticosterone and 11 β hydroxylase mRNA in LMC intruders does suggest a reduced HPA responsiveness to the chronic stress of daily defeat.

Although their hormone and 11 β -hydroxylase expression levels might suggest that the LMC mice were unaffected by resident–intruder social stress, the behavioral data suggested otherwise. We observed behavioral correlates of affective disorders only in LMC mice. The sucrose preference test, a behavioral correlate of anhedonia [29], showed that LMC mice did not maintain preferential consumption of sucrose, but instead decreased consumption after the initial presentation. This decline in sucrose intake in LMC mice is consistent with the loss of interest in pleasurable activities that is a classic symptom of depression [29]. Immobility during tail suspension test is conventionally interpreted as depression-like helplessness or despair behavior [27]. While the greater mobility of LMC mice during tail suspension might be interpreted as a more resilient, less depression-like state, it is more likely that this higher activity reflects an anxiety-like response. Decreased immobility in

tests of depression-like behavior after stress has been interpreted as an anxiety response [37–39]. Open field testing has also shown evidence of increased anxiety after social defeat in mice [40]. Anxiety-like behavior in LMC mice would be consistent with their increased defecation, an index of autonomic hyperresponsiveness associated with anxiety-like responses [28,39,41].

Overall, the phenotype exhibited by LMC mice comprised a mix of depression-like and anxiety-like behaviors. Notably, all significant differences in emotion-related behaviors occurred only in LMC mice, which did not exhibit any increases in HPA activity indicative of stress. Since orexin has been reported to stimulate HPA activity and to correlate positively with social dominance, the lower orexin gene expression in LMC intruders would be consistent both with their lower HPA reactivity to repeated social defeat stress and with their social subordination [26,35]. It is possible that orexin differences in our study did not reach the level of significance reported by other investigators because we used a less sensitive method of detection (in situ hybridization vs. qPCR), because daily contact between the resident and intruder was less prolonged [20], or because the LMC group was small.

Our results are highly consistent with those reported by Krishnan et al. [42] in that increases in social avoidance and anhedonia-like behaviors in repeatedly defeated mice correlated with lower plasma glucocorticoid levels. However, Krishnan et al. did not observe any differences in plasma corticosterone until 4 weeks after the end of a 10-day period of daily defeat [42]. Krishnan et al. also found only increases in anxiety-like behavior, regardless of whether defeated mice exhibited avoidance and anhedonia-like behaviors [42]. Our results indicate that significant differences in HPA responsiveness can be detected during repeated defeat within 2 weeks and are associated with greater anxiety- as well as anhedonia-like behavior. Further experiments are necessary to determine if HPA changes emerge first, and thus might be predictive of behavioral responses to the chronic stress of social defeat.

Our study also provides information on neuronal corticosteroid receptors and activity that may help elucidate links between altered HPA regulation and behavioral abnormalities. GR and MR gene expression showed a differential response to chronic defeat stress in our HMC and LMC intruders. CA2 MR gene expression was significantly decreased in HMC intruders compared to control and significantly higher in LMC mice relative to HMC intruders. A similar pattern occurred in the ventromedial prefrontal cortex, where MR was significantly decreased in the HMC and LMC intruders compared to control but tended to be higher ($P = 0.091$) in LMC vs. HMC intruders. Decreases in MR in our HMC intruders would be consistent with down-regulation by the higher levels of glucocorticoids in this group, while the higher levels of MR gene expression in LMC intruders could reflect their lower plasma levels of corticosterone compared to HMC intruders. Corticosteroid receptor down-regulation is typical after chronic stress [43], and reductions in hippocampal MR have been reported in subordinate animals in the visible burrow system, another model of chronic stress [18]. Alternatively, since corticosteroid receptor levels influence sensitivity to glucocorticoid feedback and the high affinity MR is sensitive to lower levels of glucocorticoids [5], the relative differences in MR gene expression might account for the differential HPA responsiveness of HMC and LMC mice to repeated social defeat. The increase in PVN GR gene expression in HMC intruders is not readily explained, although it might be speculated that there is a localized block in glucocorticoid access to PVN neurons that allows PVN GR upregulation and increased adrenocortical axis activity.

HMC and LMC mice also exhibited differential induction of the neuronal activity marker c-fos after repeated social defeat. Gene expression of c-fos was significantly lower in HMC vs. control mice in the paraventricular hypothalamus and septum. Previous research has

confirmed reductions in c-fos in the PVN and the intermediate subdivision of the lateral septum after repeated social defeat stress [44]. Reduced responsiveness to a repeated stimulus is a hallmark of habituation [33,45,46] and would be expected in intruder mice subjected to repeated defeat relative to controls subjected to defeat for the first time. In contrast, however, LMC mice did not exhibit the same degree of attenuation of neuronal activity, and did not exhibit significant differences from controls in their PVN and septal levels c-fos gene expression. Similar respective patterns in c-fos induction occurred in the ventromedial prefrontal cortex, but did not reach statistical significance. The similar trends in activation of the PVN and ventromedial prefrontal cortex are consistent with the stimulatory influence of the ventromedial prefrontal cortex on PVN neuroendocrine neuron activity [32].

Counterintuitively, despite their failure to habituate PVN activity, LMC mice tended to exhibit lower plasma ACTH levels relative to control and HMC mice in response to the thirtieth defeat. This tendency suggests that there could be reduced pituitary responsiveness to hypothalamic stimulation. Alternatively, PVN activation could reflect other processes such as autonomic drive [47] that are not solely dedicated to HPA control.

Some possible limitations of our study include our small sample size for the LMC intruders. The small size of this group could have confounded our interpretation of differences between LMC mice and either control or HMC mice in orexin, c-fos, or corticosteroid receptor gene expression. Nevertheless, the low proportion of LMC mice is consistent with the lower percentage of depressed patients with atypical depression [48]. Despite this small group size, significant effects were still detectable in LMC intruders in behavioral tests.

Depression is frequently associated with HPA axis dysregulation. Melancholic depression is typically associated with HPA hyperactivity [9–11], which has been attributed to impaired function or expression of receptors for glucocorticoids [9]. Our HMC mice showed evidence of HPA hyperactivity as measured by significant increases, compared to control, in basal morning corticosterone and in gene expression of the glucocorticoid synthesizing enzyme, 11 β -hydroxylase. HMC mice did not exhibit decreases in GR expression, but their lower CA2 MR gene expression was consistent with the reduced corticosteroid receptor function postulated to occur in melancholic depression [9]. Interestingly, however, we did not detect increases in behavioral correlates of depression in HMC mice.

Atypical depression has been linked with HPA axis hypoactivity that may result from increased glucocorticoid feedback inhibition. The greater sensitivity of cortisol to dexamethasone suppression supports the likelihood that the frequently low basal cortisol levels in these patients result from an increased HPA axis sensitivity to glucocorticoid negative feedback [13–15]. While HPA measures were not significantly lower in LMC vs. control mice, LMC mice did exhibit evidence of HPA under-reactivity to repeated social defeat, indicated by their lack of increases in basal morning corticosterone and adrenocortical 11 β -hydroxylase mRNA relative to HMC mice. Although HPA measures might suggest that the LMC mice were resilient to resident–intruder stress, the behavioral data suggest that LMC mice were actually the most prone to developing depression- and anxiety-like behaviors. This combination of HPA hypo-reactivity with depression and anxiety symptoms mimics some symptoms of atypical depression, in which there is a higher incidence of anxiety disorders than in nonatypical depression [48].

An alternative interpretation for our experiments is that LMC mice may instead be a model for symptoms found in post-traumatic stress disorder (PTSD; [18]). PTSD is also often characterized by low cortisol levels [49], and failure to increase glucocorticoids has been linked with the development of PTSD after a traumatic event [49]. Treatment with low-dose

cortisol in patients with PTSD has also been found to reduce the intrusion of the traumatic memories [50]. LMC mice recapitulate some of the symptoms of PTSD in exhibiting less HPA and more behavioral and autonomic reactivity to repeated social defeat stress, and also in failing to suppress neural activation to this repeated stress.

Precise classification of the affective disorder modeled by LMC mice may be less critical because there appears to be significant overlap between atypical depression and PTSD. Patients with atypical depression have a higher comorbidity for panic disorder, a common feature of PTSD, than did those with nonatypical depression [51]. A high rate of comorbidity (approximately 30%) of PTSD has been found in patients diagnosed with atypical depression [28], and a history of childhood sexual abuse or neglect is significantly more likely in atypical vs. nonatypical depression [51]. The relatively low proportion of people (10–20%) who develop PTSD after traumatic experience [52] is also consistent with the low proportion of LMC mice that we found. Given the concordance between atypical depression and PTSD, the combination of HPA hypo-reactivity and anxiety- and depression-like behavior in LMC mice could be a valuable model for elucidating the etiology and treatment of both of these diseases.

Acknowledgments

We are grateful to Rebecca Rokow-Kittell for expert assistance with these studies and to Dr. Susan Akana for generously assaying plasma ACTH. Supported in part by a Young Investigator Award from the National Alliance on Schizophrenia and Depression and by MH080394 to LJ.

References

1. Gold PW, Gabry KE, Yasuda M, Chrousos GP. Divergent endocrine abnormalities in melancholic and atypical depression: clinical and pathophysiological implications. *Endocrinol Metab Clin North Am.* 2002; 31:37–62. [PubMed: 12055990]
2. Rapaport M. Translating the evidence on atypical depression into clinical practice. *J Clin Psychiatry.* 2007; 68(Suppl. 3):31–36.
3. Trivedi MH, Hollander E, Nutt D, Blier P. Clinical evidence and potential neurobiological underpinnings of unresolved symptoms of depression. *J Clin Psychiatry.* 2008; 61:9–15.
4. Ising M, Horstmann S, Kloiber S, Lucae S, Binder EB, Kern N, et al. Combined dexamethasone/corticotropin releasing hormone test predicts treatment response in major depression — a potential biomarker? *Biol Psychiatry.* 2007; 62:47–54. [PubMed: 17123470]
5. Jacobson L. Hypothalamic–pituitary–adrenocortical axis regulation. *Endocrinol Metab Clin North Am.* 2005; 34:271–292. [PubMed: 15850842]
6. Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, et al. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science.* 2003; 301:386–389. [PubMed: 12869766]
7. Balbo M, Leproult R, Cauter EV. Impact of sleep and its disturbances on hypothalamo–pituitary–adrenal axis activity. *Int J Endocrinol.* 2010 Article ID 759234.
8. Rush AJ, Giles DE, Schlessner MA, Orsulak PJ, Parker CR Jr, Weissenburger JE, et al. The dexamethasone suppression test in patients with mood disorders. *J Clin Psychiatry.* 1996; 57:470–484. [PubMed: 8909334]
9. Anacker C, Zunszain PA, Carvalho LA, Pariante CM. The glucocorticoid receptor: pivot of depression and of antidepressant treatment? *Psychoneuroendocrinology.* 2011; 36:415–425. [PubMed: 20399565]
10. Bouwer C, Claassen J, Dinan TG, Nemeroff CB. Prednisone augmentation in treatment-resistant depression with fatigue and hypocortisolemia: a case series. *Depress Anxiety.* 2000; 12:44–50. [PubMed: 10999245]

11. Kier A, Han J, Jacobson L. Chronic treatment with the monoamine oxidase inhibitor phenelzine increases hypothalamic–pituitary–adrenocortical activity in male C57BL/6 mice: relevance to atypical depression. *Endocrinology*. 2005; 146:1338–1347. [PubMed: 15564336]
12. Stewart JW, McGrath PJ, Quitkin FM, Klein DF. DSM-IV depression with atypical features: is it valid? *Neuropsychopharmacology*. 2009; 34:2625–2632. [PubMed: 19727067]
13. Davidson JRT. A history of the concept of atypical depression. *J Clin Psychiatry*. 2007; 38(Suppl. 3):10–15. [PubMed: 17348762]
14. Stewart JW, Quitkin FM, McGrath PJ, Klein DF. Defining the boundaries of atypical depression: evidence from the HPA axis supports course of illness distinctions. *J Affective Disord*. 2005; 86:161–167.
15. Levitan RD, Vaccarino FJ, Brown GM, Kennedy SH. Low-dose dexamethasone challenge in women with atypical major depression: pilot study. *J Psychiatry Neurosci*. 2002; 27:47–51. [PubMed: 11836976]
16. Stewart JW, McGrath PJ, Quitkin FM. Do age of onset and course of illness predict different treatment outcome among DSM IV depressive disorders with atypical features? *Neuropsychopharmacology*. 2002; 26:237–245. [PubMed: 11790519]
17. Stewart JW. Treating depression with atypical features. *J Clin Psychiatry*. 2007; 68(Suppl. 3):25–29. [PubMed: 17348764]
18. Tamashiro KKK, Nguyen MMN, Sakai RR. Social stress: from rodents to primates. *Front Neuroendocrinology*. 2005; 26:27–40.
19. Albeck DA, McKittrick CR, Blanchard DC, Blanchard RJ, Nikulina J, McEwen BS, et al. Chronic social stress alters levels of corticotropin-releasing factor and arginine vasopressin mRNA in rat brain. *J Neurosci*. 1997; 17:4895–4903. [PubMed: 9169547]
20. Lutter M, Krishnan V, Russo SJ, Jung S, McClung CA, Nestler EJ. Orexin signaling mediates the antidepressant-like effect of calorie restriction. *J Neurosci*. 2008; 28:3071–3075. [PubMed: 18354010]
21. Jacobson L. CRH and NPY are not decreased in hypophagic, protein-deprived rats. *Peptides*. 2000; 21:1487–1493. [PubMed: 11068095]
22. Heydendael W, Jacobson L. Widespread hypothalamic–pituitary–adrenocortical axis-relevant and mood-relevant effects of chronic fluoxetine treatment on glucocorticoid receptor gene expression in mice. *Eur J Neurosci*. 2010; 31:892–902. [PubMed: 20374287]
23. vanBeveren C, Straaten Fv, Curran T, Muller R, Verma IM. Analysis of FBJ-MuSV provirus and c-fos (mouse) gene reveals that viral and cellular fos gene products have different carboxy termini. *Cell*. 1983; 32:1241–1255. [PubMed: 6301687]
24. Domalik LJ, Chaplin DD, Kirkman MS, Wu RC, Liu W, Howard TA, et al. Different isozymes of mouse 11beta-hydroxylase produce mineralocorticoids and glucocorticoids. *Mol Endocrinol*. 1991; 5:1853–1861. [PubMed: 1686470]
25. Heydendael W, Jacobson L. Differential effects of imipramine and phenelzine on corticosteroid receptor gene expression in mouse brain: potential relevance to antidepressant response. *Brain Res*. 2008; 1238:93–107. [PubMed: 18761333]
26. Weintraub A, Singaravelu J, Bhatnagar S. Enduring and sex-specific effects of adolescent social isolation in rats on adult stress reactivity. *Brain Res*. 2010; 1343:83–92. [PubMed: 20438720]
27. Cryan JF, Mombereau C, Vassout A. The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. *Neurosci Biobehav Rev*. 2005; 29:571–625. [PubMed: 15890404]
28. Miyata K, Kamato T, Nishida A, Ito H, Yuki H, Yamano M, et al. Role of the serotonin3 receptor in stress-induced defecation. *J Pharmacol Exp Ther*. 1992; 261:297–303. [PubMed: 1560375]
29. Katz RJ. Animal model of depression: pharmacological sensitivity of hedonic deficit. *Pharmacol Biochem Behav*. 1982; 16:965–968. [PubMed: 7202217]
30. Levin N, Shinsako J, Dallman MF. Corticosterone acts on the brain to inhibit adrenalectomy-induced adrenocorticotropin secretion. *Endocrinology*. 1988; 122:694–701. [PubMed: 2828011]
31. Heydendael W, Jacobson L. Glucocorticoid status affects antidepressant regulation of locus coeruleus tyrosine hydroxylase and dorsal raphe tryptophan hydroxylase gene expression. *Brain Res*. 2009; 1288:69–78. [PubMed: 19577549]

32. Radley JJ, Arias CM, Sawchenko PE. Regional differentiation of the medial prefrontal cortex in regulating adaptive responses to acute emotional stress. *J Neurosci.* 2006; 26:12967–12976. [PubMed: 17167086]
33. Weinberg MS, Johnson DC, Bhatt AP, Spencer RL. Medial prefrontal cortex activity can disrupt the expression of stress response habituation. *Neuroscience.* 2010; 168:744–756. [PubMed: 20394807]
34. Bhatnagar S, Huber R, Nowak N, Trotter P. Lesions of the posterior paraventricular thalamus block habituation of hypothalamic–pituitary–adrenal responses to repeated restraint. *J Neuroendocrinol.* 2002; 14:403–410. [PubMed: 12000546]
35. Davis JF, Krause EG, Melhorn SJ, Sakai RR, Benoit SC. Dominant rats are natural risk takers and display increased motivation for food reward. *Neuroscience.* 2009; 162:23–30. [PubMed: 19393296]
36. Herman JP, Adams D, Prewitt C. Regulatory changes in neuroendocrine stress-integrative circuitry produced by a variable stress paradigm. *Neuroendocrinology.* 1995; 61:180–190. [PubMed: 7753337]
37. Boyle MP, Kolber BJ, Vogt SK, Wozniak DF, Muglia LJ. Forebrain glucocorticoid receptors modulate anxiety-associated locomotor activation and adrenal responsiveness. *J Neurosci.* 2006; 26:1971–1978. [PubMed: 16481429]
38. Swiergiel AH, Leskow IL, Dunn AJ. Effects of chronic and acute stressors and CRF on depression-like behavior in mice. *Behav Brain Res.* 2008; 186:32–40. [PubMed: 17716752]
39. VanDijken HH, Mos J, VanderHeyden AM, Tilders FJH. Characterization of stress-induced long-term behavioral changes in rats: evidence in favor of anxiety. *Physiol Behav.* 1992; 52:945–951. [PubMed: 1484851]
40. Kinsey SG, Bailey MT, Sheridan JF, Padgett DA, Avitsur R. Repeated social defeat causes increased anxiety-like behavior and alters splenocyte function in C57BL/6 and CD-1 mice. *Brain Behav Immun.* 2007; 21:458–466. [PubMed: 17178210]
41. Walsh RN, Cummins RA. The open-field test: a critical review. *Psychol Bull.* 1976; 83:482–504.
42. Krishnan V, Han MH, Graham DL, Berton O, Renthal W, Russo SJ, et al. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell.* 2007; 131:391–404. [PubMed: 17956738]
43. Lopez JF, Chalmers DT, Little KY, Watson SJ. A.E Bennett Research Award. Regulation of serotonin1A, glucocorticoid, and mineralocorticoid receptor in rat and human hippocampus: implications for the neurobiology of depression. *Biol Psychiatry.* 1998; 43:547–573. [PubMed: 9564441]
44. Kollack-Walker S, Don C, Watson SJ, Akil H. Differential expression of c-fos mRNA within neurocircuits of male hamsters exposed to acute or chronic defeat. *J Neuroendocrinol.* 1999; 11:547–559. [PubMed: 10444312]
45. Wood SK, Walker HE, Valentino RJ, Bhatnagar S. Individual differences in reactivity to social stress predict susceptibility and resilience to a depressive phenotype: role of corticotropin-releasing factor. *Endocrinology.* 2010; 151:1795–1805. [PubMed: 20160137]
46. Grissom N, Bhatnagar S. Habituation to repeated stress: get used to it. *Neurobiol Learn Mem.* 2009; 92:215–224. [PubMed: 18667167]
47. Kc P, Dick TE. Modulation of cardiorespiratory function mediated by the paraventricular nucleus. *Respir Physiol Neurobiol.* 2010; 174:55–64. [PubMed: 20708107]
48. Thase ME. Recognition and diagnosis of atypical depression. *J Clin Psychiatry.* 2007; 68(Suppl. 8):11–16. [PubMed: 17640153]
49. Cohen H, Zohar J, Gidron Y, Matar MA, Belkind D, Loewenthal U, et al. Blunted HPA axis response to stress influences susceptibility to posttraumatic stress response in rats. *Biol Psychiatry.* 2006; 59:1208–1218. [PubMed: 16458266]
50. Aerni A, Traber R, Hock C, Roozendaal B, Schelling G, Papassotiropoulos A, et al. Low-dose cortisol for symptoms of posttraumatic stress disorder. *Am J Psychiatry.* 2004; 161:1488–1490. [PubMed: 15285979]
51. Matza LS, Revicki DA, Davidson JR, Stewart JW. Depression with atypical features in the national comorbidity study. *Arch Gen Psychiatry.* 2003; 60:817–826. [PubMed: 12912765]

52. Yehuda R. Risk and resilience in posttraumatic stress disorder. *J Clin Psychiatry*. 2004; 65(Suppl. 1):29–36. [PubMed: 14728094]

Fig. 1.

Morning plasma ACTH (A) and corticosterone (B) levels collected by submandibular venipuncture within 1 h of lights-on in mice subjected for 13 days to daily handling (control; white bars) or 5-min episodes of social defeat (Intruder) by a resident male mouse. Some intruder mice exhibited plasma corticosterone levels that were more than 2 standard deviations above the mean of control mice (High Morning Corticosterone, or HMC Intruder; black bars), whereas a subset of intruder mice did not exhibit elevated corticosterone (low morning corticosterone, or LMC Intruder; hatched bars).*, $P < 0.05$ vs. control; $N = 5$ (control), 6 (HMC), and 3 (LMC).

Fig. 2.

Tests of emotion-related behavior in control, HMC and LMC mice. Immobility and defecation were scored during a 5-min tail suspension test (A and B, respectively) after 17 days of daily handling (control) or social defeat encounters (HMC and LMC). Sucrose preference (C) was scored on days 18 and 19 of daily handling or social defeat, and is expressed as the change in percent of sucrose consumed in total fluid intake over these two days. *, $P < 0.05$ vs. control; $N = 5$ (control), 6 (HMC), and 3 (LMC).

Fig. 3.

Characterization of the HPA axis after acute (control) or repeated (HMC, LMC) social defeat. Brains and blood were collected when mice were killed 30min after a 5-min social encounter with a resident male after 30 days of daily handling (control) or social defeat (HMC, LMC). The panels depict, from top to bottom: corticotropin-releasing hormone (CRH; A) mRNA in the paraventricular nucleus (PVN) of the hypothalamus; vasopressin heteronuclear RNA (hnRNA) in the PVN (B); plasma ACTH (C); plasma corticosterone (D); and 11 β -hydroxylase mRNA in the adrenal cortex (E).*, $P < 0.05$ vs. control; $N = 5$ (control), 6 (HMC), and 3 (LMC).

Fig. 4.

Glucocorticoid receptor (GR; A–H) and mineralocorticoid receptor (MR; I–M) mRNA in the CA1, CA2, and CA3 subfields of the hippocampus (A–C, I–K), the dentate gyrus (DG; D and L), the ventromedial prefrontal cortex (vmPFC; E and M), and glucocorticoid receptor mRNA in the paraventricular nucleus of the hypothalamus (PVN; F), the locus coeruleus (LC; G) and the dorsal raphe nucleus (DR; H) of control, HMC, and LMC mice, 30 min after acute (control) or 30 days of repeated social defeat (HMC, LMC). MR expression in the PVN, LC, and DR was negligible and is not shown. Note that because of differences in the sizes of templates used for densitometric analysis, absolute GR or MR expression levels are not comparable between brain regions. *, $P < 0.05$ vs. control; †, $P < 0.05$ vs. HMC. $N = 5$ (control), 6 (HMC), and 3 (LMC).

Fig. 5.

Levels of c-fos mRNA in the paraventricular hypothalamus (PVN; A), lateral septum (B), ventromedial prefrontal cortex (vmPFC; C), and posterior paraventricular nucleus of the thalamus (post. pvThal; D) of control, HMC, and LMC mice, 30min after acute (control) or 30 days of repeated social defeat (HMC, LMC). Semi-quantitative data analysis of the c-fos hybridization signal was performed as described in Experimental design for the regions indicated by the arrows or outline in the representative autoradiograms to the right of each graph. *, $P < 0.05$ vs. control; $N = 5$ (control), 6 (HMC), and 3 (LMC) for all measures except vmPFC, for which $N = 4$ (control), 5 (HMC), and 3 (LMC).

Fig. 6. Semi-quantitative analysis of prepro-orexin mRNA in the hypothalamus of control, HMC, and LMC mice, 30 min after acute (control) or 30 days of repeated social defeat (HMC, LMC). Inset: representative image of prepro-orexin in situ hybridization signal in the lateral hypothalamus. $N = 5$ (control), 5 (HMC), and 3 (LMC).