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Strain Differences in the Effects of Chronic Corticosterone Exposure in the Hippocampus

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Abstract

Stress hormones are thought to be involved in the etiology of depression, in part, because animal models show they cause morphological damage to the brain, an effect that can be reversed by chronic antidepressant treatment. The current study examined two mouse strains selected for naturalistic variation of tissue regeneration after injury for resistance to the effects of chronic corticosterone (CORT) exposure on cell proliferation and neurotrophin mobilization. The wound healer MRL/MpJ and control C57BL/6J mice were implanted subcutaneously with pellets that released CORT for 7 days. MRL/MpJ mice were resistant to reductions of hippocampal cell proliferation by chronic exposure to CORT when compared to vulnerable C57BL/6J mice. Chronic CORT exposure also reduced protein levels of brain derived neurotrophic factor (BDNF) in the hippocampus of C57BL/6J but not MRL/MpJ mice. CORT pellet exposure increased circulating levels of CORT in the plasma of both strains in a dose dependent manner although MRL/MpJ mice may have larger changes from baseline. The strains did not differ in circulating levels of corticosterone binding globulin (CBG). There were also no strain differences in CORT levels in the hippocampus, nor did CORT exposure alter glucocorticoid receptor or mineralocorticoid receptor expression in a strain dependent manner. Strain differences were found in the NMDA receptor, and BDNF I and IV promoter. Strain and CORT exposure interacted to alter tropomyosine-receptor-kinase B (TrkB) expression and this may be potential mechanism protecting MRL/MpJ mice. In addition, differences in the inflammatory response of matrix metalloproteinases (MMPs) may also contribute to these strain differences in resistance to the deleterious effects of CORT to the brain.

Keywords

Stress; Corticosterone; Neurogenesis; Neurotrophins; BDNF; NMDA receptor; Strain differences; Matrix metalloproteinases

INTRODUCTION

Depression is a debilitating disorder that affects 6.7% of the United States population within a given year (Kessler et al., 2005). Lifetime prevalence across cultures varies from 2-20% (Weissman et al., 1996). Currently 40% of patients experiencing depression do not respond to traditional treatments indicating a need for new treatments based on the underlying

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Conflicts of Interest

The authors have no conflicts of interest to report.

biology of vulnerability to depression (Culpepper, 2010). Tests of cortisol stimulation and feedback have been used to identify dysregulation of the hypothalamic-pituitary- adrenal axis (HPA axis) that occurs in half the population suffering from depression and other psychiatric disorders (Gold and Chrousos, 2002, Gillespie and Nemeroff, 2005). In mice, chronic exposure to elevated levels of the stress hormone corticosterone (CORT) has been used as a behavioral model of depression as it alters depression-associated behaviors (Gourley et al., 2008, Murray et al., 2008, David et al., 2009, Gourley et al., 2009).

One way that stress hormones may contribute to the etiology of depression is that they decrease cell proliferation (Cameron et al., 1998), dendritic arborization (Woolley et al., 1990) and the mobilization of neurotrophins in the hippocampus (Duman and Monteggia, 2006). Blocking glucocorticoid receptors can reverse detrimental effects of chronic stress on synaptic structure and function (Krugers et al., 2010) and prevent decreases in neurogenesis induced by exposure to CORT (Mayer et al., 2006). Chronic antidepressant treatment can also reverse decreased neurogenesis in both stress induced rodent models of depression (Malberg and Duman, 2003, Czeh et al., 2007) and in humans with depression (Boldrini et al., 2009) further indicating that decreases in neurogenesis can be used as a biomarker of a depressive like state. Stress hormones are known to decrease neurogenesis via the activation of NMDA receptors which are downstream of the glucocorticoid receptor (Cameron et al., 1998). In addition it is thought that changes in neurotrophin mobilization contribute to alterations in neurogenesis as stress decreases protein levels of a number of neurotrophins in the hippocampus including brain derived neurotrophic factor (BDNF) (Schmidt and Duman, 2007). BDNF depletion in the dentate gyrus has also been shown to attenuate the behavioral effects of antidepressants, although selective depletion of BDNF alone does not induce a depressive like behavioral phenotype (Adachi et al., 2008). These data suggest that neurotrophin mobilization most likely acts in concert with other stress pathways in the development of depression.

It has been proposed that both genetic and environmental factors contribute to the vulnerability to depression in humans (Caspi et al., 2003, Charney and Manji, 2004). As a model of genetic differences in response to stress hormones, we hypothesized that MRL/MpJ and C57BL/6J mice would differ in their resiliency and vulnerability to the deleterious effects of chronic elevations of CORT on cell proliferation and BDNF levels. MRL/MpJ mice exhibit enhanced wound healing and unusual regenerative capacities (Heber-Katz et al., 2004c). In addition, they are behaviorally responsive to antidepressants and show increased cell proliferation, cell survival and neurotrophin levels compared to C57BL/6J mice when exposed to chronic antidepressant treatment (Balu et al., 2009a). The current study compares the effects of chronic exposure to stress hormone CORT on cell proliferation, BDNF mobilization and stress hormone levels in MRL/MpJ and C57BL/6J mice. Then using a candidate gene approach based on published literature we examined a number of potential molecular mechanisms that may contribute to these strain differences in susceptibility to stress hormones.

METHODS AND MATERIALS

Animals

Adult male C57BL/6J and MRL/MpJ mice (Jackson Laboratories, Bar Harbor, ME, USA) were 7-10 weeks of age at the beginning of all studies. C57BL/6J mice were used as they are a common laboratory strain and are the background strain used in a majority of genetic manipulations. MRL/MpJ mice demonstrate enhanced regenerative responses to tissue injury and have increased neurogenesis following chronic treatment with both fluoxetine and desipramine compared to C57BL/6 mice (Heber-Katz et al., 2004b, Balu et al., 2009a). Mice were group housed (4 to a cage) in polycarbonate cages and maintained on a 12-h light/dark

cycle (lights on at 07:00 hours) in a temperature (22°C) and humidity-controlled colony. Animals were given *ad libitum* access to food and water. All procedures were conducted in accordance with the guidelines published in the NIH Guide for Care and Use of Laboratory Animals and all protocols were approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

Experiment 1 was a dose response study (n=84) performed to examine the effects of CORT exposure on cell proliferation in MRL/MpJ and C57BL/6J mice. Strains were run individually in cohorts of 20-30 animals (n=5-6 per CORT dose) due to limitations in tissue processing and equipment access. Two cohorts were run for each strain. Blood and tissue samples for dose response ELISAs, including BDNF, CORT and corticosterone binding globulin (CBG) (n= 40-44) were taken from a single cohort of animals in each strain (Figure 1a). Experiment 2 examined the effects of a single dose of CORT (2 pellets) on mRNA expression in the hippocampus and hypothalamus (n=30-32). ELISAs for CORT in the hippocampus and blood were run on samples taken from these animals (Figure 1b).

Pellet implantation

Mice were anesthetized via inhalation of isoflurane and 5 mg, 21 day release placebo and CORT pellets (1, 2 or 4 pellets; Innovative Research of America, Sarasota, FL) were implanted subcutaneously with a trochar. MRL/MpJ mice weighed on average 35 ± 0.7 g whereas C57BL/6J mice weighed on average 26 ± 3 g. For C57BL/6J mice the daily equivalent doses adjusted for body weight were as follows: 1 pellet= 9 mg/kg/ day, 2 pellets= 18 mg/kg/ day, 4 pellets= 36 mg/kg/day. For MRL/MpJ mice the daily equivalent doses were as follows: 1 pellet= 6 mg/kg/day, 2 pellets = 13 mg/kg/day, 4 pellets= 27 mg/kg/day. Mice were exposed to 7 days of constant CORT secretion prior to sacrifice.

BrdU Incorporation Using Flow Cytometry

On the seventh day of CORT exposure mice received a single injection of BrdU (200 mg/kg) and were sacrificed 24 hours later (n=84). Labeling of BrdU was measured in cells displaying the nuclear marker 7-aminoactinomycin D (7-AAD) by flow cytometry as previously described and validated (Balu et al., 2009a, Balu et al., 2009b). Mice were decapitated, their brains quickly removed, and the bilateral hippocampus was removed. The right hippocampus was analyzed for cell proliferation, as cell counts did not differ between hemispheres (Balu et al., 2009a). Tissue was placed in Hank's Balanced Salt Solution (HBSS, Gibco Grand Island, NY), and finely minced. Tissue was digested using an enzymatic cocktail (0.5 mL, 1 mg/mL papain, Roche Applied Sciences Indianapolis, IN; 0.1 M L-cysteine, Sigma St. Louis, MO) and incubated in a dry heat block at 37°C for 15 min. Hibernate-A (Brain Bits Springfield, IL) containing 10% heat-inactivated fetal bovine serum (FBS; Gibco Grand Island, NY) was added to stop the enzymatic digestion. Tissue was then mechanically triturated to form a single cell suspension and spun in a centrifuge at 2000 rpm for 5 min.

The supernatant was removed and the resultant cells were stained using the FITC BrdU Flow Kit (BD Biosciences San Jose, CA). Cells were initially fixed and permeabilized by resuspension in 100 μ L of Cytotfix/Cytoperm buffer. Cells were then washed in staining buffer (PBS, 3% FBS, 0.09% sodium azide), spun at 5000 rpm and aspirated. Cells were stored overnight in staining buffer. On day 2 of staining cells were spun at 5000 rpm, staining buffer was removed and cells permeabilized (10 min on ice), washed and refixed (5 min) and washed. The cells were then resuspended in 100 μ L of DNase (30 μ g; stock from kit was diluted in DPBS (Ca²⁺/Mg²⁺ free) containing 0.1 mM CaCl₂ and 10 mM MgCl₂) in a dry heat block at 37°C for one hour to break down DNA into a single strand and allow bonding of the BrdU antibody. Following washing and spinning, the cells were labeled with

50 μ L of FITC-conjugated anti-BrdU (1:50 dilution) in the dark at room temperature for 20 min. After the samples were washed, they were labeled with 20 μ L of the nuclear marker, 7-AAD, at room temperature in the dark. The cells were then resuspended in staining buffer (PBS, 3% FBS, 0.09% sodium azide). Prior to analysis, cells were filtered through a cell strainer cap (30 μ m) to remove debris. The data was collected on a BD FACS Canto system at the University of Pennsylvania Flow Cytometry Core Facility. Background signal was accounted for by staining tissue from animals that had not been injected with BrdU. All data was collected and analyzed using BD FACSDiva software (BD Biosciences, San Jose, CA).

ELISAs

One cohort of each strain was used in the dose response study (Fig. 3a,b,c: BDNF, CORT plasma, CBG/ Experiment 1 n = 40-44) and all animals from the second experiment were used to measure CORT in the hippocampus and blood of animals exposed to placebo vs. 2 CORT pellets. (Table 1: n= 30-32). The left hippocampus was flash frozen in isopentane and placed at -80° C until analysis. The tissue was homogenized in 0.75 mL of lysis buffer (100 mM PIPES pH 7.0, 500 mM NaCl, 2 mM EDTA, 0.1% sodium azide, 2% bovine serum albumin, 0.2% Triton X-100, 5 μ g/mL aprotinin, 0.1 μ g/mL pepstatin A, 0.5 μ g/mL antipain). The homogenate was centrifuged at 13,000 rpm for 30 min at 4° C. The supernatant was removed and the amount of BDNF protein or CORT in the supernatant from each sample was measured in duplicate by ELISA following the manufacturer's instructions. BDNF, CORT, CBG protein levels were quantified using commercially available sandwich ELISA kit ; BDNF (Promega, Madison, WI), CORT (ImmunoDiagnostic Systems, Fountain Hills, AZ), and CBG (Kamiya Biomedical Company, Seattle, WA). Intra assay variability for the BDNF kit ranged from 2.2%- 8.8%, mean assay sensitivity was 15.6 pg/mL, Inter-assay variability was not measured by the manufacturer. Intra assay variability for the CORT kit ranged from 3.8%-6.6%, inter assay variability ranged from 7.5%- 8.6%; mean assay sensitivity was 0.55 ng/mL. Inter-assay variability and intra assay variability were not measured by the manufacturer for the CBG ELISA, assay sensitivity was 2.75 ng/mL. BDNF and CORT levels taken from tissue were normalized to the wet tissue weight. Additionally CORT was measured in plasma, normalized to placebo to allow for unbiased analysis of samples run on separate plates and compared as fold changes. Samples analyzed on the same plate are listed as ng/mL (fig 1b, experiment 2). Trunk blood was collected at time of sacrifice in EDTA treated tubes (Sarstedt, Numbrecht, Germany). Blood was centrifuged at 3000 rpm for 20 min and plasma was removed and stored frozen (-80° C) until analysis. Samples were diluted and run in duplicate following the manufacturer's instructions.

Quantitative real-time polymerase chain reaction

MRL/MpJ (n= 16) and C57BL/6J mice (n=16) were surgically implanted with 2 CORT (5 mg) or placebo pellets (Experiment 2, Fig 1b). After 7 days of pellet implantation animals were sacrificed. The unilateral right hippocampus was dissected out and mRNA was isolated using the RNeasy spin kit for Isolation of DNA-free RNA (Qiagen, Austin, TX) following the manufacturer's instructions. RNA concentrations were measured and 150 ng/ μ L RNA was used as a template to synthesize c-DNA using the Superscript Vilo c-DNA Synthesis kit (Invitrogen, Carlsbad, CA). All reactions were performed with a master mix of SYBR green (Applied Biosystems, Austin, TX) and 300 nM primers (final concentration). Quantitative real-time polymerase chain reactions (qRT-PCR) were run using the Stratagene MX3000 and MXPro QPCR software. Cycling parameters were as follows: 95° C for 10 mins, 40 cycles at 95° C (30 s) and 60° C (1 min), ending with a melting curve analysis to control for amplification. All reactions were performed in triplicate and the mean cycle threshold was used for analysis. The mRNA levels of target genes were normalized to the housekeeping gene with the closest cycle threshold using the $2^{-\Delta\Delta C_t}$ method. The two house

keeping genes used were TATA binding protein (TBP) and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Primer sequences are available upon request.

Statistical Analysis

Statistical analysis for cell proliferation data and qRT PCR were performed using 2-way ANOVAs (strain $\times$ CORT exposure) with Newman-Keuls used for post hoc analysis. For ELISAs in which strains were run on separate plates at separate times (experiment 1, dose response studies: BDNF and CORT) data were normalized to within strain placebos and calculated as a fold change. In experiment 2, two-way ANOVA compared circulating levels of CORT in the blood and brains of the two strains given placebo or 2 CORT pellets with both strains run on the same plate within the same assay. Statistica software (StatSoft, Tulsa, OK) was used to perform all analysis. Statistical significance was set at $p < 0.05$.

RESULTS

Cell Proliferation

Exposure to 7 days of the stress hormone CORT reduced cell proliferation in the hippocampus of C57BL/6J mice at all doses but did not significantly reduce cell proliferation in MRL/MpJ mice at any dose as indicated by a significant interaction [$F_{3,74} = 3.92$, $p < 0.05$] (Fig. 2) Post hoc analysis indicated that MRL/MpJ mice treated with CORT at all doses had an average of 3-4-fold more cells than C57BL/6J mice treated with the same dose (p values < 0.001). There was also a significant main effect of CORT exposure [$F_{3,74} = 18.04$, $p < 0.001$ and of strain [$F_{1,74} = 50.81$, $p < 0.001$].

BDNF Levels

The contra-lateral lobe of the hippocampus from each cohort used for the cell proliferation study was examined for BDNF protein levels. ELISAs were run on separate strains at different times. To control for inter-assay variability BDNF levels were normalized to within strain placebo controls and reported as fold change. Two-way ANOVA indicated a significant interaction between CORT exposure and strain [$F_{3,33} = 3.03$, $p < 0.05$]. Post hoc analysis indicated that BDNF levels significantly differed between MRL/MpJ and C57BL/6J mice when implanted with 2 or 4 pellets (p values < 0.05) (Fig. 3a). C57BL/6J mice exposed to 4 CORT pellets had a significant reduction in BDNF levels compared to placebo pellets ($p < 0.05$). CORT exposure did not significantly alter BDNF levels in MRL/MpJ mice at any dose ($p > 0.05$).

CORT Levels

CORT levels in the plasma were examined to determine whether there were strain differences in circulating levels of CORT as a result of pellet implantation (Fig. 3b). Samples from experiment 1 (dose response study) were run on separate plates at separate times. To control for inter-assay variability samples were normalized to within strain controls and reported as fold change. Two-way ANOVA indicated a significant main effect of CORT exposure [$F_{3,36} = 109.05$, $p < 0.001$]. Post-hoc analysis indicated that CORT levels were significantly elevated in animals implanted with 2 or 4 pellets compared to mice implanted with placebo or 1 pellet. There was also a significant difference in circulating levels of CORT between animals implanted with 2 or 4 pellets ($p < 0.05$). There was a significant main effect of strain [$F_{3,20} = 6.25$, $p < 0.05$], with MRL/MpJ mice showing a larger increase in CORT than C57BL/6J mice, but there was no significant interaction between CORT exposure and strain.

In experiment 2, plasma from C57BL/6J and MRL/MpJ mice implanted with 2 pellets or placebo were taken at the same time, and processed and measured on the same plate. Two-

way ANOVA indicated that CORT was elevated in both strains [$F_{1,26} = 75.78, p < 0.001$] following implantation with 2 pellets but did not differ between strains [$F_{1,26} = 2.50, p > 0.05$] or interact with strain [$F_{1,26} = 0.87, p > 0.05$] (Table 1). Furthermore, hippocampal levels of CORT from the same animals were increased in both strains implanted with 2 pellets [$F_{1,25} = 16.21, p < 0.001$] but there was no significant main effect of strain or strain by CORT interaction (Table 1). CORT levels in the hippocampus were not measured following implantation of 1 or 4 pellets.

CBG levels

CBG levels were measured in plasma of MRL/MpJ and C57BL/6J mice exposed to placebo or 1, 2 and 4 pellets of CORT (Fig. 3c) as the majority of CORT in the body remains in a bound state and CBG can regulate the availability of CORT to tissue (Breuner and Orchinik, 2002). All samples were analyzed on the same plate at the same time. Two-way ANOVA indicated that there was no significant strain by CORT exposure interaction or main effects of CORT exposure or strain [$p > 0.05$] on CBG levels in the plasma of mice.

Gene Expression

Glucocorticoid (GR) mRNA expression was examined in the hippocampus (Fig. 4a/ Table 2) and hypothalamus (Fig. 4b/ Table 2) as a potential mechanism of strain differences in the effects of CORT on cell proliferation and neurotrophin mobilization. MRL/MpJ mice had significantly higher levels of GR expression in the hippocampus than C57BL/6J mice [$F_{1,26} = 14.71, p < 0.001$]. Exposure to 2 pellets of CORT significantly reduced GR expression in the hippocampus of both MRL/MpJ and C57BL/6J mice as demonstrated through a main effect of CORT exposure [$F_{1,26} = 83.41, p < 0.01$]. There was no strain by CORT exposure interaction [$F_{1,26} = 0.11, p > 0.05$]. Exposure to CORT for seven days significantly reduced GR expression in the hypothalamus of both MRL/MpJ and C57BL/6J mice [$F_{1,27} = 9.14, p < 0.01$] but there were no significant differences between strains [$F_{1,27} = 1.76, p > 0.05$] and no strain $\times$ CORT exposure interaction [$F_{1,27} = 0.55, p > 0.05$].

As CORT can also bind to mineralocorticoid receptors (MR) and down-regulate neurogenesis through this mechanism (Chang et al., 2008), the effects of 7 days of CORT exposure on MR gene expression in the hippocampus (Fig. 4c/ Table 2) and hypothalamus (Fig. 4d/ Table 2) were also examined. MRL/MpJ mice had higher expression levels of MR than C57BL/6J mice as indicated by a main effect of strain [$F_{1,26} = 5.72, p < 0.05$]. CORT pellet exposure decreased MR gene expression in the hippocampus of MRL/MpJ and C57BL/6J mice as indicated by a main effect of CORT [$F_{1,26} = 10.28, p < 0.01$], with no significant strain $\times$ treatment interaction [$F_{1,26} = 2.69, p > 0.05$]. MR expression in the hypothalamus did not differ between strains [$F_{1,27} = 0.09, p > 0.05$] and was unaltered by CORT pellet exposure [$F_{1,27} = 0.78, p > 0.05$] and there was no strain by CORT exposure interaction [$F_{1,27} = 0.005, p > 0.05$].

Reductions of hippocampal neurogenesis by stress are known to involve the NMDA receptor and its activation occurs downstream of the GR (Cameron et al., 1998). Gene expression of the NR1 and subunits for NR2A, NR2B and NR2C (Fig. 5a/ Table 2) were examined in the hippocampus to determine if CORT exposure altered mRNA levels in a strain-dependent manner. MRL/MpJ mice expressed higher NR1 levels of than C57BL/6J mice as indicated by a main effect of strain [$F_{1,28} = 28.70, p < 0.001$]. However, there were no effects of CORT exposure [$F_{1,28} = 0.005, p > 0.05$] and no strain $\times$ CORT exposure interaction [$F_{1,27} = 0.001, p > 0.05$]. There were no strain differences [$F_{1,26} = 1.16, p > 0.05$] or main effects of CORT [$F_{1,26} = 0.05, p > 0.05$] or interaction [$F_{1,26} = 0.15, p > 0.05$] on the NR2A subunit. MRL/MpJ mice had higher expression of the NR2B subunit than C57BL/6J mice as indicated by a main effect of strain [$F_{1,25} = 13.34, p < 0.01$]. Although a

main effect of CORT exposure indicated that CORT decreased NR2B expression in both strains, [$F_{1,25} = 20.16, p < 0.001$], there was no strain $\times$ treatment interaction [$F_{1,25} = 0.53, p > 0.05$]. MRL/MpJ mice also had higher expression of NR2C than C57BL/6J mice as indicated by a main effect of strain [$F_{1,28} = 13.36, p < 0.01$]. A main effect of CORT exposure indicated that CORT decreased NR2C expression in both strains [$F_{1,28} = 4.43, p < 0.05$] in the absence of a strain $\times$ treatment interaction [$F_{1,28} = 0.17, p > 0.05$].

Tropomyosine-receptor-kinase B (TrkB) mediates the effects of the neurotrophin BDNF, on hippocampal plasticity (Krystal et al., 2009), neurogenesis (Li et al., 2008) and cell survival (Sairanen et al., 2005). Neurotrophins are reduced following exposure to stress (Balu and Lucki, 2009; Duman and Monteggia, 2006). The TrkB receptor was examined as ELISA indicated there were potential strain differences in the effects of CORT exposure on BDNF levels. A significant interaction indicated that CORT exposure significantly reduced TrkB expression in C57BL/6J mice but not MRL/MpJ mice [$F_{1,25} = 9.48, p < 0.01$] (Fig. 5B/ Table 2). Post hoc analysis indicated that MRL/MpJ mice given placebo or CORT pellets had 1.2 and 1.7-fold more TrkB mRNA than C57BL/6J mice respectively. In addition there was a main effect of strain [$F_{1,25} = 52.02, p < 0.001$], MRL/MpJ mice had greater expression of the TrkB receptor than C57BL/6J. There was no significant main effect of CORT exposure [$F_{1,25} = 0.36, p > 0.05$].

To examine which BDNF promoters were involved in the strain differences on the effects of CORT on BDNF protein levels, BDNF gene expression at promoter I, IV and IX were examined. BDNF expression at promoter I (Fig. 5B/ Table 2) was elevated in both MRL/MpJ and C57BL/6J mice exposed to CORT as indicated by a main effect of CORT exposure [$F_{1,28} = 13.19, p < 0.05$]. A significant main effect of strain indicated that overall MRL/MpJ mice had higher expression of BDNF I than C57BL/6J mice [$F_{1,28} = 13.40, p < 0.05$], but there was no interaction between strain and CORT exposure [$F_{1,28} = 0.72, p > 0.05$]. BDNF expression at promoter IV (Fig. 5B) was significantly lower in both strains of mice exposed to CORT as indicated by a main effect of CORT exposure [$F_{1,28} = 7.44, p < 0.05$]. A significant main effect of strain indicated MRL/MpJ mice had higher levels of BDNF expression at promoter IV than C57BL/6J mice regardless of treatment [$F_{1,28} = 20.86, p < 0.05$]. There was no interaction between CORT exposure and strain on BDNF expression at promoter IV [$F_{1,28} = 0.73, p > 0.05$]. At BDNF promoter IX there was no interaction between CORT and strain [$F_{1,28} = 0.05, p > 0.05$] and no main effects of CORT [$F_{1,28} = 1.09, p > 0.05$] or strain [$F_{1,28} = 0.86, p > 0.05$] on BDNF expression at promoter IX.

The inflammatory markers MMP2 and MMP3 (Fig 5C) were examined as they have been shown to contribute to strain differences in wound healing (Gourevitch et al., 2003, Hampton et al., 2004). There was a significant interaction between CORT exposure and strain on mRNA levels for MMP2 [$F_{1,23} = 4.30, p < 0.05$]. Post hoc analysis indicated that MRL/MpJ mice expressed 2.5-fold higher levels of MMP2 expression than C57BL/6 mice but this marker was unaltered following exposure to CORT. In addition there was a main effect of strain [$F_{1,23} = 337.31, p < 0.001$] but no main effect of CORT exposure [$F_{1,23} = 0.08, p > 0.05$]. A significant main effect of strain indicated that MRL/MpJ mice also expressed overall higher levels of MMP3 than C57BL/6J mice [$F_{1,27} = 7.36, p < 0.05$]. There was no main effect of CORT exposure on MMP3 expression [$F_{1,27} = 0.04, p > 0.05$], there was a trend towards an interaction between strain and CORT exposure [$F_{1,27} = 3.17, p = 0.08$]. Post-hoc analysis determined that CORT treated MRL/MpJ mice had 1.4-fold higher expression of MMP3 than CORT treated C57BL/6J mice ($p < 0.05$) whereas placebo mice did not differ by strain ($p > 0.05$).

DISCUSSION

Stress has repeatedly been shown to reduce cell proliferation and neurogenesis in a number of animal models of depression (Tanapat et al., 1998, Malberg and Duman, 2003, Dranovsky and Hen, 2006, Stranahan et al., 2006). Cell proliferation is reduced in humans suffering from depression, an effect that can be reversed with antidepressant treatment (Boldrini et al., 2009). CORT exposure can also reduce neurogenesis and this effect can be reversed by antidepressant treatment as well (Murray et al., 2008). The present study demonstrates for the first time the existence of strain differences in the effects of CORT manipulation on cell proliferation and neurotrophin mobilization in mice. MRL/MpJ mice are resistant to the effects of chronic CORT exposure on cell proliferation and BDNF protein expression when compared to C57BL/6J mice. Strain differences in transcription for the NMDA receptor, TrkB receptor, BDNF protein and inflammatory response may contribute to these strain differences in proliferation and neurotrophin release, although functional relevance still needs to be addressed.

Two previous studies have examined strain differences in the relationship between CORT levels and cell proliferation in rodents. One study found that strain can effect cell proliferation and CORT levels in mice although a causal relationship was not examined (McCutcheon et al., 2008). An examination of neurokinin-1 receptor mice backcrossed on to a C57BL/6J or 129B6 backgrounds found differences in the CORT response of the two strains to acute restraint stress. In addition they found that the mutation increased neurogenesis in the 129B6 background but not the C57BL/6J background. A study in rats, reported strain differences in levels of cell proliferation between Sprague-Dawley and Lister-Hooded rats treated with fluoxetine along with corresponding differences in basal levels of CORT (Alahmed and Herbert, 2008). Sprague-Dawley rats had lower levels of CORT when data was collapsed across treatment and higher levels of cell proliferation. Removal of the adrenal glands increased cell proliferation equally in both strains indicating that CORT was necessary for this strain difference. Implantation of Sprague-Dawley rats with CORT pellets flattened the diurnal rhythm of CORT release and blocked a fluoxetine induced increase in cell proliferation, similar to effects that had previously been reported in Lister-Hooded rats although a direct comparison was not made. The current study suggests that differences in the effects of CORT pellet implantation on cell proliferation and BDNF levels between B6 and MRL mice are not due to strain differences in circulating levels of CORT or HPA activity as there were no interactions between CORT pellet exposure and strain, and no effects of strain or CORT pellet implantation on CBG levels.

Examination of CORT exposure in other strains of mice all show some reductions in cell proliferation similar to the effects reported here in C57BL/6J mice. This suggests that the wound healer MRL/MpJ mouse has a novel response to CORT exposure. Seven days of exposure to 4 CORT pellets reduced cell proliferation in CD 1 mice after 14 days of implantation (Murray et al., 2008). Stressors such as restraint and foot shock that increase CORT reduce cell proliferation in BALB/c mice when animals are stressed in the absence of companions (Cherng et al., 2010).

Pellet implantation increased circulating levels of CORT in both strains, indicating that the stress hormone was equally effective in both strains. A possible confound exists that even though the animals received the same number of pellets, the doses varied between strains due to weight differences. MRL/MpJ mice weigh on average 9 g more than C56BL/6J mice and differences between weight-based doses ranged from 3 mg/kg/day (1 pellet) to 9 mg/kg/day (4 pellets). It is unlikely that this could account for the differences in CORT pellet exposure on cell proliferation found between MRL/MpJ and C57BL/6J mice as cell proliferation was altered at a dose (1 pellet) that did not significantly increase circulating

CORT in either strain. Also regardless of the weight-based differences, the doses overlapped. For example, the four pellet dose in the MRL/MpJ mouse overlapped the 2 pellet dose in the C57BL/6J mouse. Furthermore, C57BL/6J mice did not receive more CORT than MRL/MpJ mice. In the dose-response study, MRL/MpJ mice actually had a significantly higher change from baseline CORT levels when data was collapsed across dose. However, in experiment 2, the strains did not differ in circulating levels of glucocorticoids in the hippocampus of mice given placebo or two CORT pellets. The possibility existed that CBG levels could contribute to strain differences in the effects of CORT exposure as more than 90% of CORT may exist in a bound state (Breuner and Orchinik, 2002). However, plasma levels of CBG were also not altered by CORT pellet implantation, strain or any interaction between these factors.

Examination of mRNA levels for the glucocorticoid receptor were similar and decreased to the same degree in hippocampus and hypothalamus of both strains suggesting that negative feedback on gene expression were not different between the strains. However, differences in corticotropin releasing factor (CRF) and adrenocorticotrophic hormone (ACTH) have not been examined and may still contribute to these strain differences. Mineralocorticoid receptor mRNA levels were also decreased in both strains by exposure to CORT but were not altered in the hypothalamus. Given that there were no differences in transcription, it seems unlikely that there were strain differences in transactivation, however the possibility is not ruled out that there could be indirect strain differences through transrepression of NF-kappa B and other transcription factors (Scheinman et al., 1995, Wu et al., 2004). Stress likely acts on multiple pathways to alter neurogenesis, as cytokines have been shown to alter neurotrophins and neurogenesis through the NF-Kappa B pathway in addition to the direct effects of glucocorticoids (Koo and Duman, 2008, Koo et al., 2010).

The effects of adrenal steroids on neurogenesis are thought to be dependent on the downstream effects of the NMDA receptor. Administration of the NMDA antagonist MK-801 can block the effects of CORT exposure on cell proliferation and application of NMDA can block the increase in cell proliferation induced by adrenalectomy (Cameron et al., 1998). Therefore NMDA receptor subunits were examined to determine whether they contributed to strain differences in vulnerability to stress. The NMDA receptor is an ionotropic glutamate receptor formed from a heteromeric assembly of a NR1 and NR2 subunit resulting in a voltage dependent receptor that and requires co-activation of both glycine and glutamate (Cull-Candy et al., 2001, Prybylowski and Wenthold, 2004). The NR1 binds glycine and the NR2 subunit binds glutamate; a few NMDA receptors also have a NR3 subunit that binds glycine and may modulate the activity of NR1 and 2 but occur rarely (Nacher et al., 2007, Low and Wee, 2010).

The present study examined the effects of 7 days of CORT exposure on NR 1 and NR 2a-c. NR 2d was not examined as it is only found on a subset of inter-neurons in the hippocampus and does not occur on granule or pyramidal cells (Standaert et al., 1996). NR1 and the 2b and 2c subunits were expressed at higher levels in MRL/MpJ mice than C57BL/6J mice. Exposure to two CORT pellets for 7 days did not alter NR1 or 2a levels in either strain. As animals mature, the majority of NMDA receptors switch from NR2b to NR2a assembly, however NR2b subunits are present on proliferating cells and newborn neurons in the adult hippocampus (Nacher et al., 2007). Gene expression of the 2b and 2c subunits were reduced in both strains exposed to glucocorticoids although there were higher levels in MRL/MpJ mice than C57BL/6J mice even with the stress induced reductions. It should be noted that NR2b expression may result in negative effects upon proliferation as application of a NR2b specific antagonist led to higher levels of cell proliferation and greater cell survival *in vivo* under un-stressed conditions (Hu et al., 2008). This would argue against the higher levels of NR2b found in the MRL/MpJ mouse as being beneficial. As the authors also found in this

study that MK-801 *in vitro* decreases cell proliferation whereas others have reported it to block the effects of CORT on cell proliferation *in vivo* (Cameron et al., 1998), it is possible that NMDA subunit effects on neurogenesis may differ between stressed and unstressed conditions. The NR2c subunit has been characterized as occurring on proliferating astrocytes in the adult hippocampus and does not co-localize with the mature neuronal marker NeuN (Karavanova et al., 2007).

The current study demonstrates strain differences in the effects of CORT on BDNF protein levels. BDNF levels were lower in C57BL/6J mice exposed to 2 or 4 CORT pellets than in MRL/MpJ mice. Within the hippocampus BDNF is made predominantly within granule cells (Binder et al., 2001) and the interesting possibility exists that differences in cell proliferation could affect BDNF transcription although generally it is more accepted that changes in BDNF levels lead to changes in neurogenesis (Sairanen et al., 2005, Li et al., 2008). Recent data has indicated that NMDA receptor activation alters transcription of BDNF at promoters I and IV via chromatin remodeling (Tian et al., 2010). CORT pellet exposure reduced expression of the BDNF IV promoter in C57BL/6J mice but not MRL/MpJ mice. BDNF IV has been shown to be involved with activity dependent translation of BDNF (Koppel et al., 2010, Gomez-Pinilla et al., 2011). Transgenic mice lacking BDNF promoter IV transcription show depression-like behavior including increased immobility in the tail-suspension test, increased anhedonia in the sucrose preferences test and learned helplessness behavior (Sakata et al., 2010). Furthermore, BDNF promoter IV is suppressed by hyper-methylation in the post-mortem brains of human suicides (Keller et al., 2010) suggesting a relationship between reductions in the expression of this promoter and susceptibility to stress. BDNF I activity was also altered by stress. Both strains had increased levels of transcription when exposed to CORT, but the MRL/MpJ mice had higher overall levels of BDNF I expression. An examination of a single restraint stress in rats found that expression of the BDNF promoter I and IV were both reduced 2 hours after the stressor but not 24 hours after stress (Fuchikami et al., 2009) and may reflect differences between acute stress and chronic CORT exposure. Interestingly, BDNF promoter I expression is increased by NMDA receptor activation (Tian et al., 2010) and is unregulated by contextual fear learning in rats (Lubin et al., 2008) suggesting that its up regulation may be involved in how an organism learns about threatening stimuli in the environment.

BDNF binds to and starts a molecular cascade at the TrkB receptor that can affect neurogenesis (Li et al., 2008), therefore TrkB receptor mRNA levels were examined. Exposure to two pellets of CORT for 7 days reduced TrkB expression in the hippocampus of C57BL/6 mice but did not decrease TrkB expression in MRL/MpJ mice. Chronic CORT exposure has previously been reported to reduce TrkB protein levels in the hippocampus and frontal cortex of mice (Kutyanawalla et al., 2011). In humans with mood disorders and schizophrenia, TrkB receptor mRNA has also been reported as reduced in post-mortem hippocampal tissue (Thompson Ray et al., 2011) indicating that alterations in the expression of this receptor are related to psychiatric illness.

In addition to direct regulation of neurotrophin mobilization through the BDNF pathway, these growth factors can be cleaved by enzymes such as matrix metalloproteinases (MMPs) involved in modification of the extra-cellular environment (Benekareddy et al., 2008). While MMPs have traditionally been investigated for their role in the inflammatory response, cell death and neurodegenerative disorders (Rosenberg, 2009), they can also have beneficial impacts and have been implicated in synaptic and structural plasticity (Benekareddy et al., 2008). The gelatinases MMP2 and 9 are involved with neurogenesis, angiogenesis, axon regeneration and remyelination in addition a role in apoptosis (Rosenberg, 2009). *In vitro*, the inhibition of MMPs can reduce cell proliferation and differentiation of human stem cells, and *in vivo* a relationship exists between increased activation of the MMPs and neurogenesis

following forebrain ischemia in gerbils (Wojcik et al., 2009). Earlier work demonstrated strain differences between MRL/MpJ and C57BL/6J mice in the expression of MMPs in cutaneous tissue and their inhibitors which played a causative role in wound healing (Heber-Katz et al., 2004a). Therefore we compared the effects of CORT treatment on the expression of MMP2 and MMP3 in the hippocampus between strains. While MMP 2 and 9 have similar functions and have been implicated in neurogenesis, MMP3 plays a role only in degrading the extracellular matrix. By examining these two members of this extensive family we attempted to infer whether MMP expression played a specific role in the effects of strain differences in response to CORT on cell proliferation or if in general there was just greater overall expression of this family. While expression of both proteinases were elevated in MRL/MpJ mice compared to controls, only MMP2 demonstrated a strain-dependent response after CORT treatment. CORT treated MRL/MpJ mice had 2-fold higher expression of MMP2 than their C57BL/6J counterparts. Given the role MMP2 has been proposed to play in neurogenesis and angiogenesis (Rosenberg, 2009, Wojcik et al., 2009), and may contribute to the strain differences that we report on cell proliferation.

The MRL/MpJ mouse has an unusual capacity for wound healing which is due to differences in its genetic profile from C57BL/6J mice (Heber-Katz et al., 2004b). The MRL/MpJ mouse also demonstrates greater increases in hippocampal cell proliferation, neurotrophin generation and anxiolytic behavioral effects after chronic antidepressant drug treatments than C57BL/6J mice (Balu et al. 2009a). The present study is the first to show that MRL/MpJ mice are also resistant to the detrimental effects of stress hormone exposure. Exposure to 7 days of CORT treatment increased plasma and tissue levels of CORT and resulted in similar transcriptional negative feedback in the hippocampus and hypothalamus of both strains. However, MRL/MpJ mice were resistant to the effects of chronic CORT pellet exposure on cell proliferation and BDNF protein levels in the hippocampus. The possibility remains that negative feedback through ACTH and CRF may still contribute to these strain differences in the effects of on hippocampal plasticity as they were not directly tested. Changes in transcriptional activity were small and a more thorough approach such as micro-array or RNA sequencing is probably needed to find novel mechanisms. Using a candidate gene approach the current study reports that CORT exposure had different effects on pathways involved in neuronal plasticity in MRL/MpJ and C57BL/6J mice including the NMDA receptor, the TrkB receptor, activity dependent BDNF transcription and MMPs. It is likely that these transcriptional pathways act in concert to protect MRL/MpJ mice from the deleterious effects of stress.

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References

- Adachi M, Barrot M, Autry AE, Theobald D, Monteggia LM. Selective loss of brain-derived neurotrophic factor in the dentate gyrus attenuates antidepressant efficacy. *Biol Psychiatry*. 2008; 63:642–649. [PubMed: 17981266]
- Alahmed S, Herbert J. Strain differences in proliferation of progenitor cells in the dentate gyrus of the adult rat and the response to fluoxetine are dependent on corticosterone. *Neuroscience*. 2008; 157:677–682. [PubMed: 18930787]
- Balu DT, Hodes GE, Anderson BT, Lucki I. Enhanced sensitivity of the MRL/MpJ mouse to the neuroplastic and behavioral effects of chronic antidepressant treatments. *Neuropsychopharmacology*. 2009a; 34:1764–1773. [PubMed: 19177066]
- Balu DT, Hodes GE, Hill TE, Ho N, Rahman Z, Bender CN, Ring RH, Dwyer JM, Rosenzweig-Lipson S, Hughes ZA, Schechter LE, Lucki I. Flow cytometric analysis of BrdU incorporation as a

- high-throughput method for measuring adult neurogenesis in the mouse. *J Pharmacol Toxicol Methods*. 2009b; 59:100–107. [PubMed: 19121403]
- Benekareddy M, Mehrotra P, Kulkarni VA, Ramakrishnan P, Dias BG, Vaidya VA. Antidepressant treatments regulate matrix metalloproteinases-2 and -9 (MMP-2/MMP-9) and tissue inhibitors of the metalloproteinases (TIMPS 1-4) in the adult rat hippocampus. *Synapse*. 2008; 62:590–600. [PubMed: 18509851]
- Binder DK, Croll SD, Gall CM, Scharfman HE. BDNF and epilepsy: too much of a good thing? *Trends Neurosci*. 2001; 24:47–53. [PubMed: 11163887]
- Boldrini M, Underwood MD, Hen R, Rosoklija GB, Dwork AJ, John Mann J, Arango V. Antidepressants increase neural progenitor cells in the human hippocampus. *Neuropsychopharmacology*. 2009; 34:2376–2389. [PubMed: 19606083]
- Brazel CY, Linke TL, Osborne JK, Miura T, Cai J, Pevny L, Rao MS. Sox2 expression defines a heterogeneous population of neurosphere-forming cells in the adult murine brain. *Aging Cell*. 2005; 4:197–207. [PubMed: 16026334]
- Breuner CW, Orchinik M. Plasma binding proteins as mediators of corticosteroid action in vertebrates. *J Endocrinol*. 2002; 175:99–112. [PubMed: 12379494]
- Cameron HA, McKay RD. Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. *J Comp Neurol*. 2001; 435:406–417. [PubMed: 11406822]
- Cameron HA, Tanapat P, Gould E. Adrenal steroids and N-methyl-D-aspartate receptor activation regulate neurogenesis in the dentate gyrus of adult rats through a common pathway. *Neuroscience*. 1998; 82:349–354. [PubMed: 9466447]
- Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, Poulton R. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science*. 2003; 301:386–389. [PubMed: 12869766]
- Chang YT, Chen YC, Wu CW, Yu L, Chen HI, Jen CJ, Kuo YM. Glucocorticoid signaling and exercise-induced downregulation of the mineralocorticoid receptor in the induction of adult mouse dentate neurogenesis by treadmill running. *Psychoneuroendocrinology*. 2008; 33:1173–1182. [PubMed: 18760539]
- Charney DS, Manji HK. Life stress, genes, and depression: multiple pathways lead to increased risk and new opportunities for intervention. *Sci STKE*. 2004; 2004:re5. [PubMed: 15039492]
- Cherng CG, Lin PS, Chuang JY, Chang WT, Lee YS, Kao GS, Lai YT, Yu L. Presence of conspecifics and their odor-impregnated objects reverse stress-decreased neurogenesis in mouse dentate gyrus. *J Neurochem*. 2010; 112:1138–1146. [PubMed: 19943847]
- Christie BR, Cameron HA. Neurogenesis in the adult hippocampus. *Hippocampus*. 2006; 16:199–207. [PubMed: 16411231]
- Cull-Candy S, Brickley S, Farrant M. NMDA receptor subunits: diversity, development and disease. *Curr Opin Neurobiol*. 2001; 11:327–335. [PubMed: 11399431]
- Culpepper L. Why do you need to move beyond first-line therapy for major depression? *J Clin Psychiatry* 71 Suppl. 2010; 1:4–9.
- Czeh B, Muller-Keuker JJ, Rygula R, Abumaria N, Hiemke C, Domenici E, Fuchs E. Chronic social stress inhibits cell proliferation in the adult medial prefrontal cortex: hemispheric asymmetry and reversal by fluoxetine treatment. *Neuropsychopharmacology*. 2007; 32:1490–1503. [PubMed: 17164819]
- Czeh B, Simon M, Schmelting B, Hiemke C, Fuchs E. Astroglial plasticity in the hippocampus is affected by chronic psychosocial stress and concomitant fluoxetine treatment. *Neuropsychopharmacology*. 2006; 31:1616–1626. [PubMed: 16395301]
- David DJ, Samuels BA, Rainer Q, Wang JW, Marsteller D, Mendez I, Drew M, Craig DA, Guiard BP, Guilloux JP, Artymyshyn RP, Gardier AM, Gerald C, Antonijevic IA, Leonardo ED, Hen R. Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. *Neuron*. 2009; 62:479–493. [PubMed: 19477151]
- Dranovsky A, Hen R. Hippocampal neurogenesis: regulation by stress and antidepressants. *Biol Psychiatry*. 2006; 59:1136–1143. [PubMed: 16797263]
- Duman RS, Monteggia LM. A neurotrophic model for stress-related mood disorders. *Biol Psychiatry*. 2006; 59:1116–1127. [PubMed: 16631126]

- Encinas JM, Enikolopov G. Identifying and quantitating neural stem and progenitor cells in the adult brain. *Methods Cell Biol.* 2008; 85:243–272. [PubMed: 18155466]
- Encinas JM, Vaahtokari A, Enikolopov G. Fluoxetine targets early progenitor cells in the adult brain. *Proc Natl Acad Sci U S A.* 2006; 103:8233–8238. [PubMed: 16702546]
- Fuchikami M, Morinobu S, Kurata A, Yamamoto S, Yamawaki S. Single immobilization stress differentially alters the expression profile of transcripts of the brain-derived neurotrophic factor (BDNF) gene and histone acetylation at its promoters in the rat hippocampus. *Int J Neuropsychopharmacol.* 2009; 12:73–82. [PubMed: 18544182]
- Gage FH. Molecular and cellular mechanisms contributing to the regulation, proliferation and differentiation of neural stem cells in the adult dentate gyrus. *Keio J Med.* 2010; 59:79–83. [PubMed: 20881448]
- Gillespie CF, Nemeroff CB. Hypercortisolemia and depression. *Psychosom Med.* 2005; 67(Suppl 1):S26–28. [PubMed: 15953796]
- Gold PW, Chrousos GP. Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs low CRH/NE states. *Mol Psychiatry.* 2002; 7:254–275. [PubMed: 11920153]
- Gomez-Pinilla F, Zhuang Y, Feng J, Ying Z, Fan G. Exercise impacts brain-derived neurotrophic factor plasticity by engaging mechanisms of epigenetic regulation. *Eur J Neurosci.* 2011; 33:383–390. [PubMed: 21198979]
- Gourevitch D, Clark L, Chen P, Seitz A, Samulewicz SJ, Heber-Katz E. Matrix metalloproteinase activity correlates with blastema formation in the regenerating MRL mouse ear hole model. *Dev Dyn.* 2003; 226:377–387. [PubMed: 12557216]
- Gourley SL, Kedves AT, Olausson P, Taylor JR. A history of corticosterone exposure regulates fear extinction and cortical NR2B, GluR2/3, and BDNF. *Neuropsychopharmacology.* 2009; 34:707–716. [PubMed: 18719621]
- Gourley SL, Kiraly DD, Howell JL, Olausson P, Taylor JR. Acute hippocampal brain-derived neurotrophic factor restores motivational and forced swim performance after corticosterone. *Biol Psychiatry.* 2008; 64:884–890. [PubMed: 18675955]
- Hampton DW, Seitz A, Chen P, Heber-Katz E, Fawcett JW. Altered CNS response to injury in the MRL/MpJ mouse. *Neuroscience.* 2004; 127:821–832. [PubMed: 15312895]
- Hattiangady B, Shetty AK. Aging does not alter the number or phenotype of putative stem/progenitor cells in the neurogenic region of the hippocampus. *Neurobiol Aging.* 2008; 29:129–147. [PubMed: 17092610]
- Heber-Katz E, Chen P, Clark L, Zhang XM, Troutman S, Blankenhorn EP. Regeneration in MRL mice: further genetic loci controlling the ear hole closure trait using MRL and M.m. Castaneus mice. *Wound Repair Regen.* 2004a; 12:384–392. [PubMed: 15225218]
- Heber-Katz E, Leferovich J, Bedelbaeva K, Gourevitch D, Clark L. The scarless heart and the MRL mouse. *Philos Trans R Soc Lond B Biol Sci.* 2004b; 359:785–793. [PubMed: 15293806]
- Heber-Katz E, Leferovich JM, Bedelbaeva K, Gourevitch D. Spallanzani's mouse: a model of restoration and regeneration. *Curr Top Microbiol Immunol.* 2004c; 280:165–189. [PubMed: 14594211]
- Hu M, Sun YJ, Zhou QG, Chen L, Hu Y, Luo CX, Wu JY, Xu JS, Li LX, Zhu DY. Negative regulation of neurogenesis and spatial memory by NR2B-containing NMDA receptors. *J Neurochem.* 2008; 106:1900–1913. [PubMed: 18624924]
- Karavanova I, Vasudevan K, Cheng J, Buonanno A. Novel regional and developmental NMDA receptor expression patterns uncovered in NR2C subunit-beta-galactosidase knock-in mice. *Mol Cell Neurosci.* 2007; 34:468–480. [PubMed: 17276696]
- Keller S, Sarchiapone M, Zarrilli F, Videtic A, Ferraro A, Carli V, Sacchetti S, Lembo F, Angiolillo A, Jovanovic N, Pisanti F, Tomaiuolo R, Monticelli A, Balazic J, Roy A, Marusic A, Cocozza S, Fusco A, Bruni CB, Castaldo G, Chiariotti L. Increased BDNF promoter methylation in the Wernicke area of suicide subjects. *Arch Gen Psychiatry.* 2010; 67:258–267. [PubMed: 20194826]
- Kessler RC, Chiu WT, Demler O, Merikangas KR, Walters EE. Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry.* 2005; 62:617–627. [PubMed: 15939839]

- Komitova M, Eriksson PS. Sox-2 is expressed by neural progenitors and astroglia in the adult rat brain. *Neurosci Lett*. 2004; 369:24–27. [PubMed: 15380301]
- Koo JW, Duman RS. IL-1 β is an essential mediator of the antineurogenic and anhedonic effects of stress. *Proc Natl Acad Sci U S A*. 2008; 105:751–756. [PubMed: 18178625]
- Koo JW, Russo SJ, Ferguson D, Nestler EJ, Duman RS. Nuclear factor- κ B is a critical mediator of stress-impaired neurogenesis and depressive behavior. *Proc Natl Acad Sci U S A*. 2010; 107:2669–2674. [PubMed: 20133768]
- Koppel I, Aid-Pavlidis T, Jaanson K, Sepp M, Palm K, Timmusk T. BAC transgenic mice reveal distal cis-regulatory elements governing BDNF gene expression. *Genesis*. 2010; 48:214–219. [PubMed: 20186743]
- Krusters HJ, Lucassen PJ, Karst H, Joels M. Chronic stress effects on hippocampal structure and synaptic function: relevance for depression and normalization by anti-glucocorticoid treatment. *Front Synaptic Neurosci*. 2010; 2:24. [PubMed: 21423510]
- Krystal JH, Tolin DF, Sanacora G, Castner SA, Williams GV, Aikins DE, Hoffman RE, D'Souza DC. Neuroplasticity as a target for the pharmacotherapy of anxiety disorders, mood disorders, and schizophrenia. *Drug Discov Today*. 2009; 14:690–697. [PubMed: 19460458]
- Kutiyanawalla A, Terry AV Jr, Pillai A. Cysteamine attenuates the decreases in TrkB protein levels and the anxiety/depression-like behaviors in mice induced by corticosterone treatment. *PLoS One*. 2011; 6:e26153. [PubMed: 22039440]
- Li Y, Luikart BW, Birnbaum S, Chen J, Kwon CH, Kernie SG, Bassel-Duby R, Parada LF. TrkB regulates hippocampal neurogenesis and governs sensitivity to antidepressant treatment. *Neuron*. 2008; 59:399–412. [PubMed: 18701066]
- Low CM, Wee KS. New insights into the not-so-new NR3 subunits of N-methyl-D-aspartate receptor: localization, structure, and function. *Mol Pharmacol*. 2010; 78:1–11. [PubMed: 20363861]
- Lubin FD, Roth TL, Sweatt JD. Epigenetic regulation of BDNF gene transcription in the consolidation of fear memory. *J Neurosci*. 2008; 28:10576–10586. [PubMed: 18923034]
- Malberg JE, Duman RS. Cell proliferation in adult hippocampus is decreased by inescapable stress: reversal by fluoxetine treatment. *Neuropsychopharmacology*. 2003; 28:1562–1571. [PubMed: 12838272]
- Malberg JE, Eisch AJ, Nestler EJ, Duman RS. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J Neurosci*. 2000; 20:9104–9110. [PubMed: 11124987]
- Mayer JL, Klumpers L, Maslam S, de Kloet ER, Joels M, Lucassen PJ. Brief treatment with the glucocorticoid receptor antagonist mifepristone normalises the corticosterone-induced reduction of adult hippocampal neurogenesis. *J Neuroendocrinol*. 2006; 18:629–631. [PubMed: 16867184]
- McCutcheon JE, Fisher AS, Guzdar E, Wood SA, Lightman SL, Hunt SP. Genetic background influences the behavioural and molecular consequences of neurokinin-1 receptor knockout. *Eur J Neurosci*. 2008; 27:683–690. [PubMed: 18279320]
- Murray F, Smith DW, Hutson PH. Chronic low dose corticosterone exposure decreased hippocampal cell proliferation, volume and induced anxiety and depression like behaviours in mice. *Eur J Pharmacol*. 2008; 583:115–127. [PubMed: 18289522]
- Nacher J, Varea E, Miguel Blasco-Ibanez J, Gomez-Climent MA, Castillo-Gomez E, Crespo C, Martinez-Guijarro FJ, McEwen BS. N-methyl-D-aspartate receptor expression during adult neurogenesis in the rat dentate gyrus. *Neuroscience*. 2007; 144:855–864. [PubMed: 17157994]
- Prybylowski K, Wenthold RJ. N-Methyl-D-aspartate receptors: subunit assembly and trafficking to the synapse. *J Biol Chem*. 2004; 279:9673–9676. [PubMed: 14742424]
- Rosenberg GA. Matrix metalloproteinases and their multiple roles in neurodegenerative diseases. *Lancet Neurol*. 2009; 8:205–216. [PubMed: 19161911]
- Sairanen M, Lucas G, Ernfors P, Castren M, Castren E. Brain-derived neurotrophic factor and antidepressant drugs have different but coordinated effects on neuronal turnover, proliferation, and survival in the adult dentate gyrus. *J Neurosci*. 2005; 25:1089–1094. [PubMed: 15689544]
- Sakata K, Jin L, Jha S. Lack of promoter IV-driven BDNF transcription results in depression-like behavior. *Genes Brain Behav*. 2010; 9:712–721. [PubMed: 20528954]

- Scheinman RI, Gualberto A, Jewell CM, Cidlowski JA, Baldwin AS Jr. Characterization of mechanisms involved in transrepression of NF-kappa B by activated glucocorticoid receptors. *Mol Cell Biol*. 1995; 15:943–953. [PubMed: 7823959]
- Schmidt HD, Duman RS. The role of neurotrophic factors in adult hippocampal neurogenesis, antidepressant treatments and animal models of depressive-like behavior. *Behav Pharmacol*. 2007; 18:391–418. [PubMed: 17762509]
- Standaert DG, Landwehrmeyer GB, Kerner JA, Penney JB Jr, Young AB. Expression of NMDAR2D glutamate receptor subunit mRNA in neurochemically identified interneurons in the rat neostriatum, neocortex and hippocampus. *Brain Res Mol Brain Res*. 1996; 42:89–102. [PubMed: 8915584]
- Stranahan AM, Khalil D, Gould E. Social isolation delays the positive effects of running on adult neurogenesis. *Nat Neurosci*. 2006; 9:526–533. [PubMed: 16531997]
- Tanapat P, Galea LA, Gould E. Stress inhibits the proliferation of granule cell precursors in the developing dentate gyrus. *Int J Dev Neurosci*. 1998; 16:235–239. [PubMed: 9785120]
- Thompson Ray M, Weickert CS, Wyatt E, Webster MJ. Decreased BDNF, trkB-TK+ and GAD67 mRNA expression in the hippocampus of individuals with schizophrenia and mood disorders. *J Psychiatry Neurosci*. 2011; 36:195–203. [PubMed: 21223646]
- Tian F, Marini AM, Lipsky RH. NMDA receptor activation induces differential epigenetic modification of Bdnf promoters in hippocampal neurons. *Amino Acids*. 2010; 38:1067–1074. [PubMed: 19565326]
- Weissman MM, Bland RC, Canino GJ, Faravelli C, Greenwald S, Hwu HG, Joyce PR, Karam EG, Lee CK, Lellouch J, Lepine JP, Newman SC, Rubio-Stipec M, Wells JE, Wickramaratne PJ, Wittchen H, Yeh EK. Cross-national epidemiology of major depression and bipolar disorder. *JAMA*. 1996; 276:293–299. [PubMed: 8656541]
- Wojcik L, Sawicka A, Rivera S, Zalewska T. Neurogenesis in gerbil hippocampus following brain ischemia: focus on the involvement of metalloproteinases. *Acta Neurobiol Exp (Wars)*. 2009; 69:52–61. [PubMed: 19325641]
- Woolley CS, Gould E, McEwen BS. Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. *Brain Res*. 1990; 531:225–231. [PubMed: 1705153]
- Wu J, Li Y, Dietz J, Lala DS. Repression of p65 transcriptional activation by the glucocorticoid receptor in the absence of receptor-coactivator interactions. *Mol Endocrinol*. 2004; 18:53–62. [PubMed: 14551261]
- Ye Y, Wang G, Wang H, Wang X. Brain-derived neurotrophic factor (BDNF) infusion restored astrocytic plasticity in the hippocampus of a rat model of depression. *Neurosci Lett*. 2011; 503:15–19. [PubMed: 21843601]

Figure 1. Schematic of experimental design

A. In experiment 1, C57BL/6J and MRL/MpJ mice were exposed to Placebo or CORT releasing pellets (1, 2 or 4 pellets) for 7 days. Mice were injected with 200 mg/kg of BrdU on day 7 and then sacrificed on day 8. Tissue was used to measure cell proliferation and BDNF levels in hippocampus. CORT and CBG levels were measured in plasma.

B. In experiment 2 C57BL/6J and MRL/MpJ mice were exposed to placebo or CORT releasing pellets (2 pellets) for 7 days. Animals were sacrificed on Day 8 and mRNA and CORT was measured in the hippocampus. Circulating levels of CORT were measured in plasma.

Figure 2.

Seven days of exposure to CORT pellets significantly reduced cell proliferation in C57BL/6J mice at all doses (Placebo: $n = 11$, 1 pellet: $n = 8$, 2 pellets: $n = 10$, 4 pellets: $n = 10$) but did not significantly reduce cell proliferation in MRL/MpJ mice (Placebo: $n = 13$, 1 pellet: $n = 12$, 2 pellets: $n = 10$, 4 pellets: $n = 10$) as indicated by a significant interaction of CORT exposure and strain [$F_{3,74} = 3.92$, $p < 0.05$]. * denotes strain $\times$ CORT difference indicated by post-hoc test. ^ denotes a main effect of strain. # denotes a main effect of CORT

Figure 3. Dose response curve of protein levels of BDNF in hippocampus and CORT and CBG in plasma for C57BL/6J and MRL/MpJ mice

A. CORT pellet exposure reduced BDNF levels in the hippocampus of C57BL/6J (Placebo: $n = 6$, 1 pellet: $n = 6$, 2 pellets: $n = 5$, 4 pellets: $n = 6$) mice compared to MRL/MpJ (Placebo: $n = 5$, 1 pellet: $n = 5$, 2 pellets: $n = 4$, 4 pellets: $n = 5$) as indicated by a strain $\times$ CORT exposure interaction [$F_{3,33} = 3.03$, $p < 0.05$], Post-hoc analysis indicated that levels were lower in C57BL/6J mice given 2 or 4 CORT pellets than MRL/MpJ mice given the same number of pellets. Exposure to 4 pellets significantly reduced BDNF levels in C57BL/6J mice compared to their Placebo treated controls.

B. CORT pellet exposure increased CORT levels in both strains [$F_{3,36} = 109.05$, $p < 0.001$], Post-hoc analysis indicated that mice given 2 or 4 pellets had higher circulating levels of CORT than those given placebo or 1 pellet. Mice given 4 pellets had higher levels of CORT than those given 2 pellets. MRL/MpJ mice (Placebo: $n = 5$, 1 pellet: $n = 5$, 2 pellets: $n = 5$, 4 pellets: $n = 5$) have higher overall normalized CORT values than C57BL/6J mice (Placebo: $n = 6$, 1 pellet: $n = 6$, 2 pellets: $n = 6$, 4 pellets: $n = 6$) when data was collapsed across dose [$F_{3,20} = 6.25$, $p < 0.05$] but there were no strain $\times$ CORT interactions [$F_{3,20} = 2.28$, $p > 0.05$].

C. C57BL/6J (Placebo: $n = 5$, 1 pellet: $n = 5$, 2 pellets: $n = 5$, 4 pellets: $n = 5$) and MRL/MpJ mice (Placebo: $n = 5$, 1 pellet: $n = 5$, 2 pellets: $n = 5$, 4 pellets: $n = 5$) did not differ in plasma levels of CBG. There was no strain $\times$ CORT exposure interaction [$F_{1,32} = 1.14$, $p > 0.05$] and no main effects of CORT exposure [$F_{1,32} = 0.96$, $p > 0.05$] or strain [$F_{1,32} = 0.88$, $p > 0.05$]. * denotes strain $\times$ CORT differences indicated by post-hoc test. ^ denotes a main effect of strain. # denotes a main effect of CORT.

Figure 4. The effects of 7 days of CORT exposure with 2 CORT pellets on glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) expression in the hippocampus and hypothalamus

A. Exposure to CORT pellets significantly reduced GR expression the hippocampus of C57BL/6J (Placebo: $n = 7$, CORT: $n = 8$) and MRL/MpJ mice (Placebo: $n = 7$, CORT: $n = 8$) as indicated by a main effect of CORT pellet implantation [$F_{1,26} = 83.41$, $p < 0.01$]. A significant main effect of strain indicated MRL/MpJ mice had higher overall expression of GR [$F_{1,26} = 14.71$, $p < 0.001$].

B. CORT exposure significantly reduced GR expression in the hypothalamus of both C57BL/6J (Placebo: $n = 8$, CORT: $n = 7$) and MRL/MpJ mice (Placebo: $n = 8$, CORT: $n = 8$) as indicated by a main effect of CORT pellet implantation [$F_{1,27} = 9.14$, $p < 0.01$], GR expression did not differ between strains in the hypothalamus [$F_{1,27} = 1.76$, $p > 0.05$] and no strain $\times$ CORT exposure interaction [$F_{1,27} = 0.55$, $p > 0.05$].

C. CORT exposure significantly reduced MR expression the hippocampus of both C57BL/6J (Placebo: $n = 7$, CORT: $n = 8$) and MRL/MpJ mice (Placebo: $n = 8$, CORT: $n = 8$) [$F_{1,26} = 10.28$, $p < 0.01$]. MRL/MpJ mice had higher expression of MR in the hippocampus than C57BL/6J mice [$F_{1,26} = 5.72$, $p < 0.05$] with no significant interaction between strain $\times$ CORT exposure [$F_{1,26} = 2.69$, $p > 0.05$]. **D.** There was no significant interaction between CORT exposure and strain for MR mRNA levels in the hypothalamus [$F_{1,27} = 0.005$, $p > 0.05$] of C57BL/6J (Placebo: $n = 8$, CORT: $n = 8$) and MRL/MpJ mice (Placebo: $n = 7$, CORT: $n = 8$). There were no effects of strain [$F_{1,27} = 0.09$, $p > 0.05$] or CORT pellet exposure [$F_{1,27} = 0.78$, $p > 0.05$]. * denotes strain $\times$ CORT differences indicated by post-hoc test. ^ denotes a main effect of strain. # denotes a main effect of CORT.

Figure 5. Potential molecular mechanisms of strain differences in resiliency to CORT

A. The effects of chronic exposure to 2 pellets of CORT on NMDA receptor subunit (NR) expression in the hippocampus of MRL/MpJ and C57BL/6J mice. MRL/MpJ mice (Placebo: $n = 8$, CORT: $n = 8$) had higher expression of the NR1 subunit than C57BL/6J mice (Placebo: $n = 8$, CORT: $n = 8$) as indicated by a main effect of strain [$F_{1,28} = 28.70$, $p < 0.001$]. There were no effects of CORT [$F_{1,28} = 0.005$, $p > 0.05$] and no strain by CORT exposure interactions [$F_{1,27} = 0.001$, $p > 0.05$] on NR1 expression. There were no effects of CORT exposure [$F_{1,26} = 1.16$, $p > 0.05$] or strain [$F_{1,26} = 0.05$, $p > 0.05$] or interactions [$F_{1,26} = 0.15$, $p > 0.05$] on NR2a expression in C57BL/6J (Placebo: $n = 8$, CORT: $n = 8$) or MRL/MpJ mice (Placebo: $n = 7$, CORT: $n = 8$). CORT exposure significantly reduced

NR2b expression in both strains [$F_{1,25} = 13.34$, $p < 0.01$], but MRL/MpJ mice (Placebo: $n = 8$, CORT: $n = 7$) still had higher overall NR2b expression [$F_{1,25} = 20.16$, $p < 0.001$] than C57BL/6J mice (Placebo: $n = 8$, CORT: $n = 6$). There was no interaction between CORT exposure and strain on NR2b expression [$F_{1,25} = 0.53$, $p > 0.05$]. CORT exposure significantly reduced NR2c expression in both strains [$F_{1,28} = 13.36$, $p < 0.01$] but MRL/MpJ mice (Placebo: $n = 8$, CORT: $n = 8$) had significantly higher expression of NR2c [$F_{1,28} = 4.43$, $p < 0.05$] compared to C57BL/6J mice (Placebo: $n = 8$, CORT: $n = 8$). There was no strain $\times$ CORT exposure interaction on NR2c expression [$F_{1,28} = 0.17$, $p > 0.05$].

B. The effects of chronic CORT exposure on TrkB and BDNF promoter expression in the hippocampus. CORT exposure decreased TrkB expression in the hippocampus of C57BL/6J (Placebo: $n = 8$, CORT: $n = 8$) mice but did not reduce expression in MRL/MpJ mice (Placebo: $n = 6$, CORT: $n = 7$) as indicated by a significant interaction between CORT exposure and strain [$F_{1,25} = 9.48$, $p < 0.01$]. CORT exposure increased levels of BDNF promoter I [$F_{1,28} = 13.40$, $p < 0.05$]. The activity dependent promoter expression was higher in MRL/MpJ mice (Placebo: $n = 8$, CORT: $n = 8$) compared to C57BL/6J mice (Placebo: $n = 8$, CORT: $n = 8$) as indicated by a main effect of strain [$F_{1,28} = 13.19$, $p < 0.05$]. There was no interaction between CORT exposure and strain [$F_{1,28} = 0.73$, $p > 0.05$]. CORT exposure increased levels of BDNF promoter IV [$F_{1,28} = 20.86$, $p < 0.05$]. The activity dependent promoter expression of BDNF IV was higher in MRL/MpJ mice (Placebo: $n = 8$, CORT: $n = 8$) compared to C57BL/6J mice (Placebo: $n = 8$, CORT: $n = 8$) as indicated by a main effect of strain [$F_{1,28} = 7.44$, $p < 0.05$]. There were no significant interactions between CORT exposure and strain [$F_{1,28} = 0.73$, $p > 0.05$]. There were no significant effects of CORT exposure [$F_{1,28} = 0.86$, $p > 0.05$] or strain [$F_{1,28} = 1.09$, $p > 0.05$] on BDNF promoter IX in C57BL/6J (Placebo: $n = 8$, CORT: $n = 8$) or MRL/MpJ mice (Placebo: $n = 8$, CORT: $n = 8$). There was no interaction between CORT exposure and strain [$F_{1,28} = 0.05$, $p > 0.05$].

C. The effects of chronic CORT exposure on expression of MMPs. MMP2 was elevated in the hippocampus of MRL/MpJ mice (Placebo: $n = 7$, CORT: $n = 7$) given both placebo and CORT pellets whereas CORT decreased MMP2 in the hippocampus of C57BL/6J mice (Placebo: $n = 6$, CORT: $n = 7$) as indicated by a significant interaction [$F_{1,23} = 4.30$, $p < 0.05$]. MMP3 levels were elevated in the hippocampus of MRL/MpJ mice (Placebo: $n = 8$, CORT: $n = 8$) compared to C57BL/6J mice (Placebo: $n = 7$, CORT: $n = 8$) as indicated by a main effect of strain [$F_{1,27} = 7.36$, $p < 0.05$]. There was also a trend towards an interaction between CORT exposure and strain [$F_{1,27} = 3.17$, $p = 0.08$] but there was no main effect of CORT [$F_{1,27} = 0.04$, $p > 0.05$]. * denotes strain $\times$ CORT differences indicated by post-hoc test. ^ denotes a main effect of strain. # denotes a main effect of CORT.

Table 1

Effects of 7 day exposure to Placebo or 2 CORT pellets on levels of CORT in the plasma and hippocampus of C57BL/6J and MRL/MpJ mice

	C57BL/6J Placebo	C57BL/6J CORT Pellets	MRL/MpJ Placebo	MRL/MpJ CORT Pellets
CORT Plasma Ng/mL	49 ± 8	345 ± 57 [#]	30 ± 5	268 ± 23 [#]
CORT Hippocampus Ng/g tissue	1475 ± 341	2373 ± 343 [#]	1089 ± 241	2551 ± 220 [#]

Seven days of CORT pellet exposure (2 pellets) exposure significantly increased circulating levels of CORT in both C57BL/6J and MRL/MpJ mice ($p < 0.05$). There were no strain differences. Pellet exposure also significantly increased levels of CORT measured in the hippocampus ($p < 0.05$). There were no strain differences. Asterisks denote significant effect of corticosterone exposure.

* denotes strain × CORT difference indicated by post-hoc test.

^ denotes a main effect of strain.

denotes a main effect of CORT

Table 2

The effects of exposure to Placebo or 2 CORT pellets on mRNA expression in the hippocampus of C57BL/6J (C57) and MRL/MpJ (MRL) mice.

	CORT/Placebo	MRL/C57	Interaction
GR (hippocampus)	0.71 FC [#]	1.15 FC [^]	NC
GR (hypothalamus)	0.71 FC [#]	1.14 FC [^]	NC
MR (hippocampus)	0.67 FC [#]	1.40 FC [^]	NC
MR (hypothalamus)	0.87 FC [#]	NC	NC
NR1	NC	1.30 FC [^]	NC
NR2a	NC	NC	NC
NR2b	0.83 FC [#]	1.14 FC [^]	NC
NR2c	0.97 FC [#]	1.20 FC [^]	NC
TrkB	NC	$p < 0.001$	$F_{1,25} = 9.48, p < 0.01$ C57 CORT < C57 Placebo: 0.8 FC [*] MRL CORT > C57 CORT: 1.68 FC [*] MRL Placebo > C57 Placebo: 1.21 FC [*] MRL CORT > C57 Placebo: 1.35 FC [*]
BDNF exon I	1.29 FC [#]	1.29 FC [^]	NC
BDNF exon IV	0.90 FC [#]	1.18 FC [^]	NC
BDNF exon IX	NC	NC	$F_{1,28} = 0.05, p > 0.05$
MMP2	NC	$p < 0.001$	$F_{1,23} = 4.30, p < 0.05$. MRL Placebo > C57 Placebo: 2.6 FC [*] MRL Placebo > C57 CORT: 2.16 FC [*] MRL CORT > C57 CORT: 2.0 FC [*] MRL CORT > C57 Placebo: 2.4 FC [*]
MMP3	NC	1.2 FC [^]	$F_{1,27} = 3.17, p = 0.08$

* denotes strain $\times$ CORT difference by post-hoc test.

[^] denotes a main effect of strain.

[#] denotes a main effect of CORT. NC denotes no significant change. FC denotes fold change.