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Glucocorticoid sensitizers *Bag1* and *Ppid* are regulated by adolescent stress in a sex-dependent manner

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Abstract

Early life stress precipitates dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and this effect is most pronounced in females. The mechanisms that mediate female sensitivity to stress-induced HPA axis dysregulation are unknown. The purpose of this study was to determine whether sex moderates the effects of chronic adolescent stress on glucocorticoid receptor (GR) translocation and moderators of the GR system. Female adolescent rats with a history of chronic stress exposure demonstrated a delayed resolution of the plasma corticosterone response to an acute stressor and this delay was accompanied by attenuated GR translocation compared to control adolescent females. The chronic stress-induced phenotype in females was similar to the baseline phenotype in male adolescent rats. Conversely, the expression patterns of GR moderators/co-chaperones became more sexually dimorphic following chronic stress, suggesting divergent function of the GR system between male and female adolescent rats. Gene expression of *Ppid*, a positive regulator of the GR, was predicted by plasma estradiol and 34% lower in control adolescent females than males, indicating that sex steroids may play a role in the sexually dimorphic response. After chronic adolescent stress, females displayed elevated hippocampal expression of *Bag1* and *Ppid* genes that was not observed in males. Overall, the GR output to an acute stressor, illustrated by transcription of *Nr3c1* (encoding the GR), *Bag1, Fkbp5, Ppid,* and *Src1,* was significantly upregulated and differed in a sex-specific and chronic stress-dependent

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Contributors

CHB, GNN, and EBB participated in the research design. CHB, MQR, SM, and CAB conducted experiments. CHB performed data analysis. All authors contributed and have approved the final manuscript.

Conflicts of Interest

CHB, MQR, SM, and CAB have nothing to disclose. EB Binder receives funding from NIMH, the Doris Duke Charitable Foundation, the Behrens-Weise foundation, the NeuroNova G GmbH and PharmaNeuroBoost. Dr. Binder is also a consultant for PharmaNeuroBoost. GN Neigh receives grant funding from NIMH, AHA, NARSAD, GSK, and Emory University.

manner. This study provides new evidence for sex differences during development and adaptation of the glucocorticoid receptor chaperone system.

Keywords

Cyp40; Ncoa1; Fkbp51; social defeat; adolescent stress

Introduction

The response to stress is a highly adaptable and evolutionarily conserved biological process vital to the long-term survival of an organism (Owens and Nemeroff, 1991). The hypothalamic-pituitary-adrenal (HPA) axis mediates this biological process to control the behavioral, endocrine, and gene expression responsivity to stress (Owens and Nemeroff, 1991; Bourke et al., 2012). Negative feedback on the HPA axis is mediated by glucocorticoids binding to the glucocorticoid receptor (Bourke et al., 2012). The function of the glucocorticoid receptor (GR) is dependent on a large number of proteins, including chaperones, co-chaperones and co-activators. These proteins influence receptor sensitivity, translocation to the nucleus, DNA binding and transcriptional effects of the GR on target genes (Grad and Picard, 2007; Pratt and Toft, 1997). We hypothesized that the mediators of the GR system are intricately regulated by stress and may be involved in the development of stress disorders.

Nuclear receptors such as GRs are shuttled into the nucleus by a chaperone system, bind to the DNA at GR response elements and alter gene expression in concert with coactivation or corepression proteins. Positive modulators of the GR system include the co-chaperone peptidylprolyl isomerase D (*Ppid*) and the nuclear receptor coactivator 1 (*Src1*). *Ppid* is thought to assist in GR translocation by modulating the interaction with dynein to facilitate nuclear shuttling of the complex (Ratajczak et al., 2003). *Src1* co-activates the GR inside the nucleus to facilitate changes in gene expression. Comparatively, negative modulator co-chaperones BCL-2 associated athanogene (*Bag1*) and FK506 binding protein 51 (*Fkbp5*) mainly act to tune glucocorticoid sensitivity. *Bag1* is believed to modulate the folding of the GR complex by competing for binding of regulatory chaperones, but also by inhibiting GR translocation and altering transactivation by interfering with DNA-binding (Schmidt et al., 2003; Grad and Picard, 2007). *Fkbp5* is among the best characterized GR co-chaperones. *Fkbp5* decreases the affinity of the receptor for cortisol and prevents receptor translocation resulting in GR insensitivity to circulating glucocorticoids (Binder, 2009). Functional genetic polymorphisms in *Fkbp5* have been shown to interact with early life stress in humans to predict post-traumatic stress disorder, major depression and suicide attempts in adults (Binder et al., 2008; Brent et al., 2010; Xie et al., 2010; Appel et al., 2011; Zimmermann et al., 2011). Findings with *Fkbp5* suggest that co-chaperones of GR can moderate the long-term effects of early life stress on the HPA-axis, as well as consequent psychiatric symptoms in adults.

In addition to genetic and environmental factors, developmental stages and sex differences influence function of the HPA axis. Subsequent to perinatal development, adolescence comprises a sensitive developmental window characterized by extensive growth and maturation in the brain, during which chronic stress can modify stress-related physiology (McCormick and Mathews, 2007). In addition, there is significant interaction between HPA and hypothalamic-pituitary-gonadal (HPG) pathways (Weiser et al., 2010; Evaul et al., 2010). Gonadal hormones moderate GR sensitivity through competitive inhibition at the GR by progesterone (Rousseau et al., 1972, Duncan and Duncan, 1979, Keller-Wood et al., 1988), direct competition at common steroid response elements, and sex-steroid induced

alterations in GR co-chaperones and co-activators. This interaction may be particularly prominent during adolescence. Specifically, *Ppid* is upregulated by estradiol while *Fkbp5* is transcriptionally upregulated by glucocorticoids, progestins, and androgens (Ratajczak et al., 2003; Hubler et al., 2003; Hubler and Scammell, 2004; Jääskeläinen et al., 2011). Therefore, the marked surges in sex steroids during puberty could alter/impact the expression of co-chaperones that regulate the GR complex. These changes in expression could lead to downstream changes in GR activation and translocation and thereby altered expression of genes regulated by the GR. Additionally, the presence of increased sex steroids during adolescence may interact with chronic stress exposure to mediate sex specific effects of adolescent stress.

Chronic adolescent stress alters behavior and HPA axis function in female, but not male, rats (Bourke and Neigh, 2011). Given the effects of gonadal hormones on the GR system, we assessed the effects of sex and chronic adolescent stress exposure on GR translocation and several distinct targets related to GR translocation effectors (*Gr, Ppid, Src1)* and inhibitors (*Bag1, Fkbp5)*. In addition, because chronic stress effects may not be apparent when the HPA axis is at rest, we assessed the response of the GR system to an acute stress challenge following a history of chronic adolescent stress.

Methods

Animals

Timed pregnant Wistar rats (Charles River, Wilmington, MA) arrived on gestational day 12 (n = 24). Shipping stress during puberty has been shown to alter behavioral responses (Laroche et al., 2009), but shipping of pregnant dams has not been shown to alter developmental outcomes unless a pharmacological challenge was also introduced (Ogawa et al., 2007); therefore shipping was conducted during *in utero* development. As an additional measure of control, littermates were assigned to control and stress groups. Rats were housed on a 14:10 reverse light:dark cycle in a facility controlled for humidity (60%) and temperature (20°C–23°C). Rodent diet 5001 chow (Purina Mills, Richmond, IN) and water were available *ad libitum* throughout the study. Three days after birth, rat pups were sexed and litters were culled to four male and four female pups. No more than two pups per litter were assigned to a group in order to prevent litter effects. Pups were weighed and weaned on postnatal day (PND) 21 and housed two per cage in same sex groups. All animal experiments were approved by Emory University's Institutional Animal Care and Use Committee and carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Resources, 1996). All efforts were made to minimize animal suffering and to reduce the number of animals used.

Chronic Mixed-Modality Stress

The chronic mixed-modality stress (Stress) was performed as previously described (Bourke and Neigh, 2011). Briefly, Stress consisted of individual housing beginning at PND 36 and continuing throughout the study combined with randomly alternating daily exposure to social defeat or restraint from PND 37–49. Individual housing is a potent stressor in juvenile rats (Ibi et al., 2008) and individual housing is necessary for the stress-induced pathophysiology of social defeat (Von Frijtag et al., 2000). Rats in the chronic stress groups underwent 6 random exposures to each social defeat and restraint (60 min; Braintree Sci. Inc.) in a pseudorandom order to prevent habituation. In the social defeat paradigm, rats are exposed to a same sex dominant adult rat that demonstrated aggressive behavior toward the experimental rat (female adults are ovariectomized to control estrous cycle variations in behavior). Although physical injury to the experimental rat rarely occurs (rat excluded from study if injury occurs), exposure to the dominant rat elicits a sustained stress response and

repeated exposure alters the HPA axis (Bhatnagar et al., 2006). The collective impact of these individual stressors comprise the chronic mixed-modality stress paradigm (Bourke and Neigh, 2011). All stressors occurred during the light cycle to coincide with the nadir of the circadian cycle of corticosterone to maximally increase corticosterone. On PND53, four days after the conclusion of the chronic mixed-modality stress model, blood samples were collected. Baseline blood samples were collected immediately upon removal from the colony room. Post-stress blood samples were collected after rats were placed in a clear acrylic beaker (60 cm high × 22 cm in diameter) filled with 30°C water for five minutes during the light cycle. Five, 30, or 120 minutes after initiation of the acute forced swim stress, animals were rapidly decapitated. For the 30 and 120 minutes groups, rats were returned to their home cages between the end of the forced swim and tissue collection. Unless part of the baseline group, naïve littermates that were not exposed to chronic adolescent stress (Control) were exposed to the acute forced swim stress and sacrificed in parallel to Stress groups at the designated timepoints. Separate cohorts of rats were used for each time point in the endocrine assessment/real time PCR and the western blot experiments.

Endocrine and Tissue Analyses

Following decapitation, trunk blood was collected immediately in BD Vacutainer EDTA collection tubes (BD, Franklin Lakes, NJ). Blood was spun down at 1,800 rcf and the plasma fraction was collected. Plasma corticosterone was assayed with the ImmuChem ^{125}I Corticosterone RIA Kit (sensitivity: 1 ng/mL, MP Biomedicals, Orangeburg, NY). Plasma progesterone was assayed with the Progesterone ActiveRIA Kit (sensitivity: 0.12 ng/mL, Beckman-Coulter, Brea, CA). Plasma testosterone was assayed with the DSL-4100 RIA Kit (sensitivity: 0.05 ng/mL, DSL, Webster, TX). Plasma estradiol was assayed using 50 μL of plasma with the DSL-4400 RIA Kit (sensitivity: 4.7 pg/mL, DSL, Webster, TX). Samples were run in duplicate for all endocrine assays. Reproductive tissues were collected and wet weights were assessed to establish sexual maturity (Pignatelli et al., 2006). Body mass was recorded throughout the study. Tissue weights (adrenals, uterine weights, and testes) were normalized to body weight.

Cytosolic and Nuclear Fraction Extraction

Following decapitation and brain removal, brains were frozen on dry ice and stored at −80°C. Later, the hippocampus was dissected on dry ice and the left side was taken for western blotting. Frozen hippocampi (~100 mg) were homogenized in 1 mL of homogenization buffer containing 50 mM Tris (pH 7.2), 1 mM EDTA, 6 mM $MgCl_2$, 10% sucrose, and 1:1,000 protease inhibitor cocktail (Sigma Aldrich, St. Louis, MO) with a hand-held 2 mL Kontes Tenbroek tissue grinder (Fisher Scientific, Pittsburgh, PA) on ice. Homogenate was spun at 2,000 × *g* for 5 min at 4°C. The supernatant was separated for cytosolic extraction and the pellet was kept on ice for nuclear extraction. The cytosolic supernatant was spun at 105,000 × *g* for 30 min at 4°C and this supernatant was saved as the cytosolic extract. The nuclear extract pellet was washed twice by resuspending the pellet in 0.5 mL of homogenization buffer and spun at 2,000 × *g* for 5 min at 4°C. The pellet was resuspended in nuclear extraction buffer containing homogenization buffer with 0.5 M NaCl and incubated on ice for 1 hour. Homogenates were spun down for 10 minutes at 8,000 × *g* at 4°C and the supernatant was saved as the nuclear extract. Protein concentrations were determined with the BCA assay (Pierce Biotechnology, Rockford, IL).

Glucocorticoid Receptor Western Blot

Protein extracts (10μg) were loaded onto a 10% Bis-Tris gel (Invitrogen, Carlsbad, CA) and separated by gel electrophoresis. The gel was blotted onto a PVDF membrane (Invitrogen, Carlsbad, CA) and blocked in 7.5% skim milk-TBST nutating at 4°C overnight. Blots were

incubated for 2 hrs with primary antibody for GR M-20 (1:2,500), GAPDH FL-335 (1:50,000) or NF-YA H-209 (1:2,500) (Santa Cruz Biotechnology, Santa Cruz, CA). GAPDH was used as a loading control for cytosolic extracts and NF-YA was used for nuclear extracts. Blots were incubated with goat anti-rabbit IgG-HRP (Santa Cruz Biotechnology, Santa Cruz, CA) and visualized with the SuperSignal ECL Kit (Pierce Biotechnology, Rockford, IL). Blots were exposed to ECL Hyperfilm (GE Healthcare Biosciences, Pittsburgh, PA) and analyzed with AIS 6.0 Imaging Software (Imaging Research, St. Catharines, Canada). Translocation is reported as (nuclear GR) / (cytosolic GR + nuclear GR).

Real Time PCR

Rats were killed via rapid decapitation. Brains were immediately removed and rapidly frozen on dry ice. The hippocampus was dissected on dry ice. RNA was extracted with the TRIzol method (Invitrogen, Carlsbad, CA) and RNA integrity was assessed by a BioRad Spectrophotometer (Biorad, Hercules, CA) and an Agilent Bioanalyzer 2100 (Agilent, Santa Clara, CA). RNA was reverse transcribed with the High Capacity RNA to cDNA kit (Applied Biosystems, Foster City, CA). cDNA was quantified with the PicoGreen Assay (Invitrogen, Carlsbad, CA). A rat endogenous control plate (Applied Biosystems, Foster City, CA) was run in order to determine the ideal endogenous control, which was found to be *Tfrc* based on the lowest deviation between treatment groups. *Tfrc* (the transferrin receptor) regulates iron homeostasis and is not expect to be altered by experimental treatments. The selection of an ideal endogenous control is important to the interpretation of real time PCR experiments (Schmittgen and Zakrajsek, 2000) and while many groups commonly use housekeeping genes such as *Gapdh* or *Actb*, the endogenous control plate found these to have a high deviation between treatment groups. Primers for *Gr, Bag1, Fkbp5, Ppid,* and *Src1* were purchased from Assays on Demand (Applied Biosystems, Foster City, CA). Real time-PCR was run on 7900HT system (Applied Biosystems, Foster City, CA). Fold change in gene expression = $2^{-\Delta\Delta Ct}$.

Statistical Analyses

GraphPad Prism 4.0 was used for all statistical analyses, but correlations were analyzed with R. Student's t-tests were used for comparisons of two groups. Multivariate ANOVAs were used in the case of comparisons with more than two groups (chronic stress × sex) or (chronic stress × acute stress). If an ANOVA main effect was found, a Bonferroni post hoc test was used to assess individual group differences. The alpha value was set to 0.05.

Results

Chronic Adolescent Stress Increased Baseline Progesterone in Adolescent Female Rats

Body mass was measured throughout the study to determine if stress affected weight gain. Males exposed to adolescent stress had decreased weight gain compared to control males ($F_{(1, 205)} = 44.88$; $p < 0.05$; Table 1) but females exposed to stress did not exhibit impaired weight gain ($F_{(1, 175)} = 1.21$; $p = 0.274$; Table 1). Throughout adolescence, all males ($F_{(4, 205)} = 1115$; $p < 0.05$) and females ($F_{(4, 175)} = 714.1$; $p < 0.05$) gained weight over the course of the study. Terminal baseline measurements (BL) of several steroid hormones were taken to determine if chronic adolescent stress affects output of the adrenals and gonads. Uterine and adrenal weights were unaffected by adolescent stress, but testicular mass was increased due to adolescent stress (Table 1). Baseline plasma corticosterone was unaltered due to chronic stress or sex ($p > 0.05$; Fig. 1A and B). Chronic stress significantly increased baseline plasma progesterone in females compared to control females ($t_{14} = 2.5$; $p < 0.05$; Fig. 1D). Baseline progesterone was unaffected by chronic stress in males ($t_{14} = 0.0$; $p >$

0,05; Fig. 1C). Chronic stress did not affect baseline plasma testosterone in males (t_{14} = 1.4; p > 0.05; Fig. 1E) or baseline plasma estradiol in females (t_{16} = 1.1; p > 0.05; Fig. 1F).

Chronic Adolescent Stress Prolonged the Response to a Novel Acute Stressor in Female Rats

Chronic stress did not alter the peak corticosterone response but an acute stressor in female rats increased plasma corticosterone, leading to an observed peak in plasma corticosterone at 30 minutes ($F_{3, 59}$ = 13.2; p < 0.05; Fig. 1B). At 30 minutes after the acute stressor, females exposed to chronic adolescent stress displayed elevated plasma corticosterone as compared to control females at the same time point (t_{15} = 2.7; p < 0.05). Although baseline progesterone was increased in female rats exposed to chronic stress, acute stress did not further alter plasma progesterone in female rats ($F_{3,47}$ = 2.1; p > 0.05; Fig. 1D) regardless of stress history ($F_{1, 47}$ = 2.4; p > 0.05; Fig. 1D). While females without a history of chronic adolescent stress suppressed plasma estradiol after a swim stressor (t_{14} = 2.3; p < 0.05), this effect was absent in females exposed to chronic adolescent stress (baseline compared to 30 minutes after forced swim; t_{16} = 1.1; p > 0.05). Although the decreases differed in magnitude, all female rats demonstrated decreased plasma estradiol concentrations after the acute stressor ($F_{3, 58}$ = 6.7; p < 0.05; Fig. 1F).

Chronic Adolescent Stress Alters the Testosterone Response to Acute Stress in Male Rats

Both male groups demonstrated a hormonal response to the acute stressor, but chronic adolescent stress blunted the acute stress-induced increase in plasma testosterone ($F_{1, 62}$ = 6.22; p < 0.05; Fig. 1E) most prominently at 30 minutes (t_{18} = 2.6, p < 0.05). Chronic adolescent stress did not alter plasma corticosterone ($F_{1, 64}$ = 0.4; p > 0.05; Fig. 1A) or plasma progesterone in male rats ($F_{1, 61}$ = 0.5; p > 0.05; Fig. 1C). Male plasma corticosterone and plasma progesterone concentrations increased following acute stress (Corticosterone: $F_{3, 64}$ = 41.87; p < 0.05; Fig. 1A, Progesterone: $F_{3, 61}$ = 12.2; p < 0.05; Fig. 1C).

Chronic Adolescent Stress Attenuates GR Translocation in the Hippocampus of Female Rats

The proportion of GR in the nucleus was altered in a sex and chronic stress-dependent manner. There was no effect of either acute or chronic stress exposure on GR translocation in male rats (p > 0.05; Fig. 2A). Control female rats exposed to acute swim stress exhibited an increase in GR translocation to the nucleus as compared to control counterparts that were not exposed to the acute stress challenge (t_{10} = 2.4; p < 0.05; Fig. 2B). A history of chronic adolescent stress prevented the acute stress-induced increase in GR translocation in female rats (p > 0.05; Fig. 2B). These effects are not due to changes in total GR protein content between experimental groups in the cytosolic or nuclear fractions (p > 0.05) or amount of loading protein because GAPDH and NFYA were unchanged (p > 0.05). GAPDH was detectable only in the cytosolic fraction while NFYA was detectable only in the nuclear fraction (Fig. 2), validating the extraction procedure.

Female Adolescent Rats Express Less Ppid in the Hippocampus than Male Adolescent Rats

To determine baseline sex differences in gene expression in the hippocampus, male and female controls were compared by normalizing gene expression to male controls. There were no sex differences in *Gr*, *Bag1*, *Fkbp5*, or *Src1* (Fig. 3A). *Ppid* was 34% lower in control adolescent females compared to control adolescent males (t_{13} = 2.3; p < 0.05; Fig. 3A).

Chronic Adolescent Stress Increased Baseline Expression of *Ppid* and *Bag1* in the Hippocampus of Adolescent Female Rats

Baseline gene expression differences due to chronic adolescent stress were compared by using each sex's control as the normalization value. Chronic adolescent stress elevated basal expression of *Bag1* ($F_{1, 28} = 5.9$; $p < 0.05$), *Src1* ($F_{1, 29} = 4.6$; $p < 0.05$), and caused a trend toward elevation of *Ppid* ($F_{1, 28} = 5.5$; $p = 0.06$). Post hoc analysis demonstrated that chronic adolescent stress elevated basal expression of *Bag1* ($t_{14} = 2.6$; $p < 0.05$; Fig. 3C) and *Ppid* ($t_{14} = 2.8$; $p < 0.05$; Fig. 3E) in female but not male rats.

Chronic Stress Modifies the Gene Expression Response to Acute Stress in Male Rats

After acute stress, all transcripts increased expression over two hours ($p < 0.05$; Fig. 4A–E). Chronic adolescent stress in males did not alter the acute stress-induced expression of *Bag1*, *Fkbp5*, or *Ppid* ($p > 0.05$; Fig. 4B–D). *Gr* expression had a trend towards an increase due to chronic stress ($F_{1, 45} = 3.3$; $p = 0.07$; Fig. 4A). At 120 minutes, males exposed to chronic stress had increased *Gr* gene expression compared to controls ($t_{15} = 2.6$; $p < 0.05$). While chronic adolescent stress did not independently affect *Fkbp5* gene expression, acute stress caused a steady increase in gene expression of controls that was not displayed in animals previously exposed to chronic adolescent stress ($F_{2, 44} = 3.6$; $p < 0.05$; Fig. 4C). This effect was most apparent at 120 minutes after the stressor, with chronic adolescent stress males returning to baseline while control animals had elevated gene expression of *Fkbp5* ($t_{13} = 2.8$; $p < 0.05$). Chronic adolescent stress increased *Src1* expression ($F_{1, 45} = 4.6$; $p < 0.05$; Fig. 4E). At 120 minutes after acute stress, males exposed to chronic adolescent stress had elevated *Src1* expression while expression in controls had decreased ($t_{14} = 3.0$; $p < 0.05$).

Chronic Stress Modifies the Gene Expression Response to Acute Stress in Female Rats

After acute stress, all transcripts increased expression over two hours ($p < 0.05$; Fig. 4F–J). *Gr* expression increased due to chronic adolescent stress ($F_{(1, 38)} = 5.6$; $p < 0.05$; Fig. 4F). At 30 minutes after an acute stress, females with a history of chronic adolescent stress had 50% higher *Gr* expression than controls ($t_{11} = 2.7$; $p < 0.05$). Females had a trend towards an increase in *Bag1* expression due to chronic adolescent stress ($F_{1, 38} = 3.7$; $p = 0.06$; Fig. 4G). At 30 minutes, *Bag1* expression was significantly increased by 275% compared to control values at the same time point ($t_{12} = 3.0$; $p < 0.05$). Chronic adolescent stress had a trend towards an increase in *Fkbp5* expression ($F_{1, 39} = 4.0$; $p = 0.05$; Fig. 4H). At 30 minutes post-stress, *Fkbp5* was increased by 150% due to chronic adolescent stress compared to control values at the same time point ($t_{13} = 2.9$; $p < 0.05$). *Ppid* expression was increased due to chronic adolescent stress, peaking at 540% of baseline at 30 minutes in adolescent stress females compared to baseline control females ($F_{1, 38} = 5.7$; $p < 0.05$; Fig. 4I). *Src1* expression was unaltered due to chronic adolescent stress ($F_{1, 41} = 0.0$; $p > 0.05$; Fig. 4J).

Plasma Estradiol Predicts *Ppid* Expression in Female Rats Exposed to Chronic Adolescent Stress

Linear regression analysis was used to determine if gene expression was correlated with plasma hormones. In females exposed to chronic adolescent stress, plasma estradiol levels predicted *Ppid* expression ($r^2 = 0.213$, $t_{31} = 2.9$, $p < 0.05$, Fig. 5B). Females without a history of chronic stress did not show this correlation ($r^2 = 0.015$, $t_{24} = 0.6$, $p > 0.05$, Fig. 5A). Co-varying for time after acute stress reduced this correlation to a trend ($r^2 = 0.277$, $t_{30} = 1.8$, $p = 0.086$). Time after acute stress did not account for the correlation of estradiol and *Ppid* expression in this model ($p > 0.05$). No other transcripts correlated with plasma hormones for males or females ($p > 0.05$).

Discussion

Sex-specific regulation of the GR is evident in adolescent rats and this sexual dimorphism is augmented by exposure to chronic stress. Although basal corticosterone concentrations and GR distribution were similar between male and female adolescent rats, basal expression of GR co-chaperones and moderators differed in a sex-dependent fashion. Functional sex differences in the GR system became evident when adolescent rats were exposed to an acute stress challenge. Nuclear translocation of GR increased following acute stress challenge in female, but not male, adolescent rats. In addition, there were temporally discordant changes between the sexes in terms of the corticosterone response and gene expression within the GR system following acute stress exposure. The sex difference in acute stress-induced GR translocation was mitigated by a history of chronic stress exposure in the adolescent females and the profile of the corticosterone response became temporally concordant with male adolescent rats. Contrary to this apparent masculinization of the stress response by chronic adolescent stress as observed by others (Kaiser et al., 2003), changes in gene expression of the GR system became increasingly sexually dimorphic with the combination of a chronic stress history and acute stress challenge. Collectively, these data indicate that sex-specific effects of adolescent stress may be mediated at the level of molecular regulation of GR function.

Chronic Adolescent Stress Exacerbates Molecular Sex Differences in the GR System

Glucocorticoid sensitivity in this study refers to the output of GR function measured by GR translocation and gene expression of GR responsive genes. Sensitivity of the GR system is exquisitely regulated through steps in translocation and nuclear coactivation. We chose to examine the GR system in the hippocampus because recent data highlight the importance of forebrain-specific GR in mediating affective-like behaviors (Wei et al., 2004, Wei et al., 2012) and the established role of the hippocampus in negative feedback on the HPA axis (Sapolsky et al., 1985). Sensitizers of the glucocorticoid receptor to the molecular response to stress, such as *Bag1* and *Ppid*, may mediate the HPA axis alterations seen in females. *Bag1* has been implicated in manic-like behavior and resilience to stress (Maeng et al., 2008). Specifically, knock-down of this gene has been shown to result in fewer anxiety-like and depressive-like behavior measures. Given that chronic stress elevates female rats' basal expression of *Bag1*, the possibility exists that sex and stress interact to mediate GR insensitivity via *Bag1*. Upregulation of this system may oppose the effects of chronic corticosterone exposure due to chronic adolescent stress.

An analysis of moderators of GR action found that females expressed *Ppid*, a positive moderator of GR translocation, at a lower level than males in the hippocampus. While females have lower basal expression of *Ppid* in the hippocampus than males, chronic adolescent stress induced elevated expression of *Ppid* in females compared to female controls. *Ppid* has an elusive role in GR action and may participate by interacting with GR chaperone proteins (Renoir et al., 1995) or by possibly potentiating GR action by acting on nuclear export of glucocorticoids (Davies et al., 2005; Periyasamy et al., 2010). Agonists of *Ppid* rapidly induce GR-mediated transcription (Renoir et al., 1995) but association of *Ppid* with the pathogenesis of mood disorders has yet to be elucidated. In normal pregnancy, expression of *Ppid* is upregulated with rising sex-steroids during pregnancy. This upregulation is blunted in pregnant women with clinically significant depressive symptoms, possibly contributing to the observed altered GR sensitivity (Katz et al., 2011). However, the un-opposed effects of *Bag1* on the progesterone receptor (Knapp et al., 2012) may explain the observed differences in baseline progesterone following exposure to chronic adolescent stress (Figure 1D).

Chronic Adolescent Stress Modifies the Response of the GR System to an Acute Challenge

At the peak of acute HPA axis activation, females exposed to chronic adolescent stress demonstrated a robust elevation in *Gr* (2-fold), *Bag1* (6-fold), and *Fkbp5* (3-fold) compared to baseline. It has been shown that GR activation can rapidly induce the transcription of its own moderators, most prominently *Fkbp5* within an ultra-short negative feedback loop (Binder, 2009). The enhanced transcription of these GR target genes tracked with the higher corticosterone levels at the 30 minutes timepoint but may also indicate an enhanced sensitivity to GR activation in chronically-stressed females, very much in line with the observed increase in GR translocation following the forced swim stress (see Figure 2). This may be mediated by an increase sensitivity of the receptor itself via differences in the co-chaperone composition of the complex in concert with epigenetic changes in the target genes. In fact, prolonged exposure to corticosterone in mice as well as early trauma have been shown to lead to DNA de-methylation of GR response elements in *Fkbp5* (Lee et al., 2010, 2011, Binder personal communication), resulting in an enhanced transcription of this gene following subsequent GR activation. In fact, this enhanced sensitivity to GR activation is in line with increased *Ppid* expression evident in the females exposed to chronic adolescent stress (Renoir et al., 1995) and its effects may not be sufficiently counteracted by the parallel increase of *Bag1*. Indeed, we also observed a positive correlation of *Ppid* with estradiol levels, but only in chronically stress females, supporting an interaction of sex steroids and chronic stress in shaping GR function.

A GR supersensitivity, as possibly observed in the chronically stressed females, has been associated with post-traumatic stress disorder in human studies (Rohleder et al., 2004; Yehuda et al., 2004). While chronically-stressed female rats in our study have elevated corticosterone levels at 30 minutes following the acute stressor, the recovery at 120 minutes is more profound than in controls, which could be an endocrine reflection of the enhanced GR sensitivity observed at the molecular level.

Males with a history of chronic adolescent stress demonstrated elevated *Gr* and *Src1* expression compared to controls during the recovery from the acute stressor, even though corticosterone levels were back to baseline in both groups. *Src1* is a nuclear coactivator of GR, and *Src1* activates GR after nuclear import to mediate gene expression (Kurihara et al., 2002; Meijer et al., 2005). Knockout of *Src1* decreases expression of corticotropin-releasing factor in the amygdala and knockouts are insensitive to the effects of dexamethasone, supporting a role of *Src1* in GR negative feedback (Lachize et al., 2009). These data may suggest that elevated *Src1* in the recovery phase could lead to an enhancement of GR function at later time points. In addition to these molecular differences, males exposed to chronic adolescent stress displayed impaired weight gain and increased testicular weight.

Overall, these results illustrate an important sex difference in GR function in the hippocampus. Males exposed to chronic adolescent stress displayed prolonged changes in GR regulated and regulating genes in response to an acute stressor, while females exposed to chronic adolescent stress displayed an immediate enhancement of GR function and target gene regulation.

Baseline differences in HPA axis activation

All animals, regardless of chronic stress exposure or sex, demonstrated a high baseline concentration of plasma corticosterone. This effect was likely due to the collection time, as all animals were sacrificed during a peak of the circadian cycle for corticosterone, ten hours into the light cycle. Additionally, time course experiments spanned two hours during this stage of the circadian cycle for corticosterone. Therefore, our results can only be interpreted

in reference to this stage of the circadian cycle. Additionally, the rapid recovery of the stress response in adolescent females was further examined with an injection of corticosterone (Fig. S1). High baseline level and fast recovery after stress were observed in a separate experiment examining serial sampling after injection of corticosterone in adolescent female rats (Fig. S1). A rapid recovery of plasma corticosterone was also observed in these experiments (Fig. S1 and Fig. 1), indicating an altered metabolic regulation of corticosterone during adolescence in females as shown by others (Doremus-Fitzwater et al., 2009). Discrepancies among our results and other studies (Doremus-Fitzwater et al., 2009; Goel and Bale, 2010) may be attributable to the circadian time of sample collection, the difference in the type of stressor, and/or the age of the animals.

The chaperone complex proteins and *Src1* do not discretely work on GR but can also regulate sensitivity of other hormone receptors. The complexity of this system and the exact relationship between other hormone receptors and the observed sex-specific changes is beyond the scope of the present study. However, we hypothesize that the interaction of hormone receptors and the chaperone complex likely contributes to the observed effects.

In conclusion, we found sexually dimorphic effects of chronic adolescent stress on the GR system. We chose to focus specifically on the glucocorticoid receptor chaperone complex composed of positive moderators of GR function (*Ppid* and *Src1*) and negative moderators of GR function (*Bag1* and *Fkbp5*). We hypothesized that sensitivity of the GR system can be manipulated by stress by acting on positive and negative moderators to ultimately regulate GR function. We also found that sex plays a prominent role in the sensitivity of GR, although the exact direct or indirect mechanisms through gonadal hormones are unclear at present. These data suggest that alterations in the moderators of GR contribute to an enhanced glucocorticoid sensitivity in females and stress resilience in males and may be responsible for the sex-dependent effects of chronic adolescent stress. This study adds to the extant literature which documents a stress-sensitive phenotype in females and points to adolescence as a sensitive developmental window for the effects of stress.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Appel K, Schwahn C, Mahler J, Schulz A, Spitzer C, Fenske K, Stender J, Barnow S, John U, Teumer A, Biffar R, Nauck M, Völzke H, Freyberger HJ, Grabe HJ. Moderation of adult depression by a polymorphism in the FKBP5 gene and childhood physical abuse in the general population. Neuropsychopharmacology. 2011; 36(10):1982–1991. [PubMed: 21654733]

Bhatnagar S, Vining C, Iyer V, Kinni V. Changes in hypothalamic-pituitary-adrenal function, body temperature, body weight and food intake with repeated social stress exposure in rats. J. Neuroendocrinol. 2006; 18:13–24. [PubMed: 16451216]

Binder EB. The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. Psychoneuroendocrinology. 2009; 34(Suppl 1):S186–S195. [PubMed: 19560279]

Binder EB, Bradley RG, Liu W, Epstein MP, Deveau TC, Mercer KB, Tang Y, Gillespie CF, Heim CM, Nemeroff CB, Schwartz AC, Cubells JF, Ressler KJ. Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. JAMA. 2008; 299:1291–1305. [PubMed: 18349090]

Bourke CH, Neigh GN. Behavioral effects of chronic adolescent stress are sustained and sexually dimorphic. Horm. Behav. 2011; 60:112–120. [PubMed: 21466807]

Bourke CH, Harrell CS, Neigh GN. Stress-induced sex differences: Adaptations mediated by the glucocorticoid receptor. Horm. Behav. 2012

Brent D, Melhem N, Ferrell R, Emslie G, Wagner KD, Ryan N, Vitiello B, Birmaher B, Mayes T, Zelazny J, Onorato M, Devlin B, Clarke G, DeBar L, Keller M. Association of FKBP5 polymorphisms with suicidal events in the Treatment of Resistant Depression in Adolescents (TORDIA) study. Am. J. Psychiatry. 2010; 167:190–197. [PubMed: 20008943]

Davies TH, Ning Y-M, Sánchez ER. Differential control of glucocorticoid receptor hormone-binding function by tetratricopeptide repeat (TPR) proteins and the immunosuppressive ligand FK506. Biochemistry. 2005; 44:2030–2038. [PubMed: 15697228]

Doremus-Fitzwater TL, Varlinskaya EI, Spear LP. Social and non-social anxiety in adolescent and adult rats after repeated restraint. Physiol. Behav. 2009; 97:484–494. [PubMed: 19345235]

Duncan MR, Duncan GR. An in vivo study of the action of antiglucocorticoids on thymus weight ratio, antibody titre and the adrenal-pituitary-hypothalamus axis. J. Steroid Biochem. 1979; 10(3): 245–259. [PubMed: 222960]

Evaul K, Li R, Papari-Zareei M, Auchus RJ, Sharifi N. 3beta-hydroxysteroid dehydrogenase is a possible pharmacological target in the treatment of castration-resistant prostate cancer. Endocrinology. 2010; 151:3514–3520. [PubMed: 20534728]

Goel N, Bale TL. Sex differences in the serotonergic influence on the hypothalamic-pituitary-adrenal stress axis. Endocrinology. 2010; 151:1784–1794. [PubMed: 20185764]

Grad I, Picard D. The glucocorticoid responses are shaped by molecular chaperones. Mol. Cell. Endocrinol. 2007; 275:2–12. [PubMed: 17628337]

Hubler TR, Denny WB, Valentine DL, Cheung-Flynn J, Smith DF, Scammell JG. The FK506-binding immunophilin FKBP51 is transcriptionally regulated by progestin and attenuates progestin responsiveness. Endocrinology. 2003; 144:2380–2387. [PubMed: 12746298]

Hubler TR, Scammell JG. Intronic hormone response elements mediate regulation of FKBP5 by progestins and glucocorticoids. Cell Stress Chaperones. 2004; 9:243–252. [PubMed: 15544162]

Ibi D, Takuma K, Koike H, Mizoguchi H, Tsuritani K, Kuwahara Y, Kamei H, Nagai T, Yoneda Y, Nabeshima T, Yamada K. Social isolation rearing-induced impairment of the hippocampal neurogenesis is associated with deficits in spatial memory and emotion-related behaviors in juvenile mice. J. Neurochem. 2008; 105(3):921–932. [PubMed: 18182044]

Institute for Laboratory Animal Resources. Guide for the Care and Use of Laboratory Animals 7th ed. Washington DC: Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council; 1996.

Jääskeläinen T, Makkonen H, Palvimo JJ. Steroid up-regulation of FKBP51 and its role in hormone signaling. Curr. Opin. Pharmacol. 2011; 11(4):326–331. [PubMed: 21531172]

Kaiser S, Kruijver FP, Swaab DF, Sachser N. Early social stress in female guinea pigs induces a masculinization of adult behavior and corresponding changes in brain and neuroendocrine function. Behav. Brain Res. 2003; 144(1–2):199–210. [PubMed: 12946610]

Katz ER, Stowe ZN, Newport DJ, Kelley ME, Pace TW, Cubells JF, Binder EB. Regulation of mRNA expression encoding chaperone and co-chaperone proteins of the glucocorticoid receptor in peripheral blood: association with depressive symptoms during pregnancy. Psychol Med. 2011; 14:1–14.

Keller-Wood M, Silbiger J, Wood CE. Progesterone attenuates the inhibition of adrenocorticotropin responses by cortisol in nonpregnant ewes. Endocrinology. 1988; 123(1):647–651. [PubMed: 2838268]

Knapp RT, Steiner A, Schmidt U, Hafner K, Holsboer F, Rein T. BAG-1 diversely affects steroid receptor activity. Biochem. J. 2012; 441(1):297–303. [PubMed: 21919886]

Kurihara I, Shibata H, Suzuki T, Ando T, Kobayashi S, Hayashi M, Saito I, Saruta T. Expression and regulation of nuclear receptor coactivators in glucocorticoid action. Mol. Cell Endocrinol. 2002; 189:181–189. [PubMed: 12039076]

Lachize S, Apostolakis EM, Laan S van der, Tijssen AMI, Xu J, Kloet ER de, Meijer OC. Steroid receptor coactivator-1 is necessary for regulation of corticotropin-releasing hormone by chronic stress and glucocorticoids. Proc. Natl. Acad. Sci. U.S.A. 2009; 106:8038–8042. [PubMed: 19416907]

Laroche J, Gasbarro L, Herman JP, Blaustein JD. Reduced behavioral response to gonadal hormones in mice shipped during the peripubertal/adolescent period. Endocrinology. 2009; 150(5):2351–2358. [PubMed: 19131570]

Lee RS, Tamashiro KL, Yang X, Purcell RH, Harvey A, Willour VL, Huo Y, Rongione M, Wand GS, Potash JB. Chronic corticosterone exposure increases expression and decreases deoxyribonucleic acid methylation of Fkbp5 in mice. Endocrinology. 2010; 151(9):4332–4343. [PubMed: 20668026]

Lee RS, Tamashiro KL, Yang X, Purcell RH, Huo Y, Rongione M, Potash JB, Wand GS. A measure of glucocorticoid load provided by DNA methylation of Fkbp5 in mice. Psychopharmacology (Berl). 2011; 218(1):303–312. [PubMed: 21509501]

Maeng S, Hunsberger JG, Pearson B, Yuan P, Wang Y, Wei Y, McCammon J, Schloesser RJ, Zhou R, Du J, Chen G, McEwen B, Reed JC, Manji HK. BAG1 plays a critical role in regulating recovery from both manic-like and depression-like behavioral impairments. Proc. Natl. Acad. Sci. U.S.A. 2008; 105(25):8766–8771. [PubMed: 18562287]

McCormick CM, Mathews IZ. HPA function in adolescence: role of sex hormones in its regulation and the enduring consequences of exposure to stressors. Pharmacol. Biochem. Behav. 2007; 86:220–233. [PubMed: 16901532]

Meijer OC, Kalkhoven E, Laan S van der, Steenbergen PJ, Houtman SH, Dijkmans TF, Pearce D, Kloet ER de. Steroid receptor coactivator-1 splice variants differentially affect corticosteroid receptor signaling. Endocrinology. 2005; 146:1438–1448. [PubMed: 15564339]

Ogawa T, Kuwagata M, Hori Y, Shioda S. Valproate-induced developmental neurotoxicity is affected by maternal conditions including shipping stress and environmental change during early pregnancy. Toxicol. Lett. 2007; 174(1–3):18–24. [PubMed: 17900830]

Owens MJ, Nemeroff CB. Physiology and pharmacology of corticotropin-releasing factor. Pharmacol. Rev. 1991; 43:425–473. [PubMed: 1775506]

Periyasamy S, Hinds T, Shemshedini L, Shou W, Sanchez ER. FKBP51 and Cyp40 are positive regulators of androgen-dependent prostate cancer cell growth and the targets of FK506 and cyclosporin A. Oncogene. 2010; 29:1691–1701. [PubMed: 20023700]

Pignatelli D, Xiao F, Gouveia AM, Ferreira JG, Vinson GP. Adrenarche in the rat. J. Endocrinol. 2006; 191:301–308. [PubMed: 17065412]

Pratt WB, Toft DO. Steroid receptor interactions with heat shock protein and immunophilin chaperones. Endocr. Rev. 1997; 18(3):306–360. [PubMed: 9183567]

Ratajczak T, Ward BK, Minchin RF. Immunophilin chaperones in steroid receptor signalling. Curr. Top. Med. Chem. 2003; 3:1348–1357. [PubMed: 12871166]

Renoir JM, Mercier-Bodard C, Hoffmann K, Bihan S Le, Ning YM, Sanchez ER, Handschumacher RE, Baulieu EE. Cyclosporin A potentiates the dexamethasone-induced mouse mammary tumor virus-chloramphenicol acetyltransferase activity in LMCAT cells: a possible role for different heat shock protein-binding immunophilins in glucocorticosteroid receptor-mediated gene expression. Proc. Natl. Acad. Sci. U.S.A. 1995; 92:4977–4981. [PubMed: 7539138]

Rohleder N, Joksimovic L, Wolf JM, Kirschbaum C. Hypocortisolism and increased glucocorticoid sensitivity of pro-Inflammatory cytokine production in Bosnian war refugees with posttraumatic stress disorder. Biol. Psychiatry. 2004; 55:745–751. [PubMed: 15039004]

Rousseau GG, Baxter JD, Tomkins GM. Glucocorticoid receptors: relations between steroid binding and biological effects. J. Mol. Biol. 1972; 67(1):99–115. [PubMed: 4402888]

Sapolsky RM, Meaney MJ, McEwen BS. The development of the glucocorticoid receptor system in the rat limbic brain. III. Negative-feedback regulation. Brain Res. 1985; 350(1–2):169–173. [PubMed: 3986611]

Schmidt U, Wochnik GM, Rosenhagen MC, Young JC, Hartl FU, Holsboer F, Rein T. Essential role of the unusual DNA-binding motif of BAG-1 for inhibition of the glucocorticoid receptor. J. Biol. Chem. 2003; 278:4926–4931. [PubMed: 12482863]

Schmittgen TD, Zakrajsek BA. Effect of experimental treatment on housekeeping gene expression: validation by real-time, quantitative RT-PCR. J. Biochem. Biophys. Methods. 2000; 46(1–2):69–81. [PubMed: 11086195]

Von Frijtag JC, Reijmers LG, Van der Harst JE, Leus IE, Van den Bos R, Spruijt BM. Defeat followed by individual housing results in long-term impaired reward- and cognition-related behaviours in rats. Behav. Brain Res. 2000; 117(1–2):137–146. [PubMed: 11099767]

Wei Q, Fentress HM, Hoversten MT, Zhang L, Hebda-Bauer EK, Watson SJ, Seasholtz AF, Akil H. Early-life forebrain glucocorticoid receptor overexpression increases anxiety behavior and cocaine sensitization. Biol. Psychiatry. 2012; 71(3):224–231. [PubMed: 21872848]

Wei Q, Lu XY, Liu L, Schafer G, Shieh KR, Burke S, Robinson TE, Watson SJ, Seasholtz AF, Akil H. Glucocorticoid receptor overexpression in forebrain: a mouse model of increased emotional lability. Proc. Natl. Acad. Sci. U.S.A. 2004; 101(32):11851–11856. [PubMed: 15280545]

Weiser MJ, Foradori CD, Handa RJ. Estrogen receptor beta activation prevents glucocorticoid receptor-dependent effects of the central nucleus of the amygdala on behavior and neuroendocrine function. Brain Res. 2010; 1336:78–88. [PubMed: 20381466]

Xie P, Kranzler HR, Poling J, Stein MB, Anton RF, Farrer LA, Gelernter J. Interaction of FKBP5 with childhood adversity on risk for post-traumatic stress disorder. Neuropsychopharmacology. 2010; 35(8):1684–1692. [PubMed: 20393453]

Yehuda R, Golier JA, Yang R-K, Tischler L. Enhanced sensitivity to glucocorticoids in peripheral mononuclear leukocytes in posttraumatic stress disorder. Biol. Psychiatry. 2004; 55:1110–1116. [PubMed: 15158431]

Zimmermann P, Brückl T, Nocon A, Pfister H, Binder EB, Uhr M, Lieb R, Moffitt TE, Caspi A, Holsboer F, Ising M. Interaction of FKBP5 gene variants and adverse life events in predicting depression onset: results from a 10-year prospective community study. Am. J. Psychiatry. 2011; 168(10):1107–1116. [PubMed: 21865530]

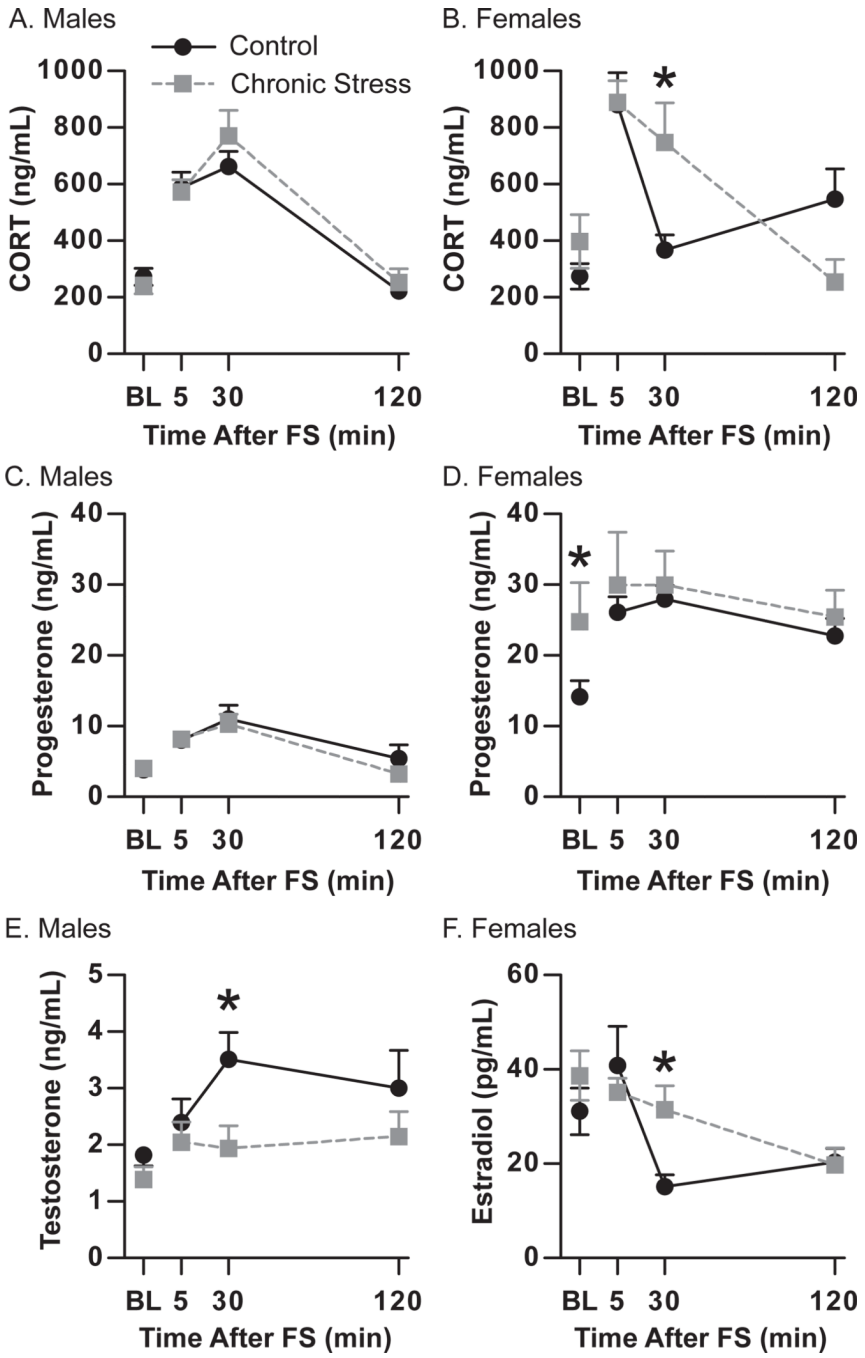

Figure 1.

Effect of acute stress challenge on endocrine parameters over time. Rats were exposed to a five minute forced swim stress and trunk blood was collected post decapitation. Plasma corticosterone (CORT) in males was unaltered due to chronic adolescent stress (A), but females exposed to chronic stress displayed a prolonged increase in CORT (B) compared to unexposed controls. Compared to same sex controls, chronic stress increased plasma progesterone concentration in females (D) but not males (C). (E) Chronic stress in males caused a blunted increase in plasma testosterone compared to males without a history of chronic stress. (F) Chronic stress in females caused an impaired recovery of plasma estradiol

compared to females without a history of chronic stress. N = 7–10. * $p < 0.05$ Bonferroni *post hoc* test. Data are presented as mean ± SEM.

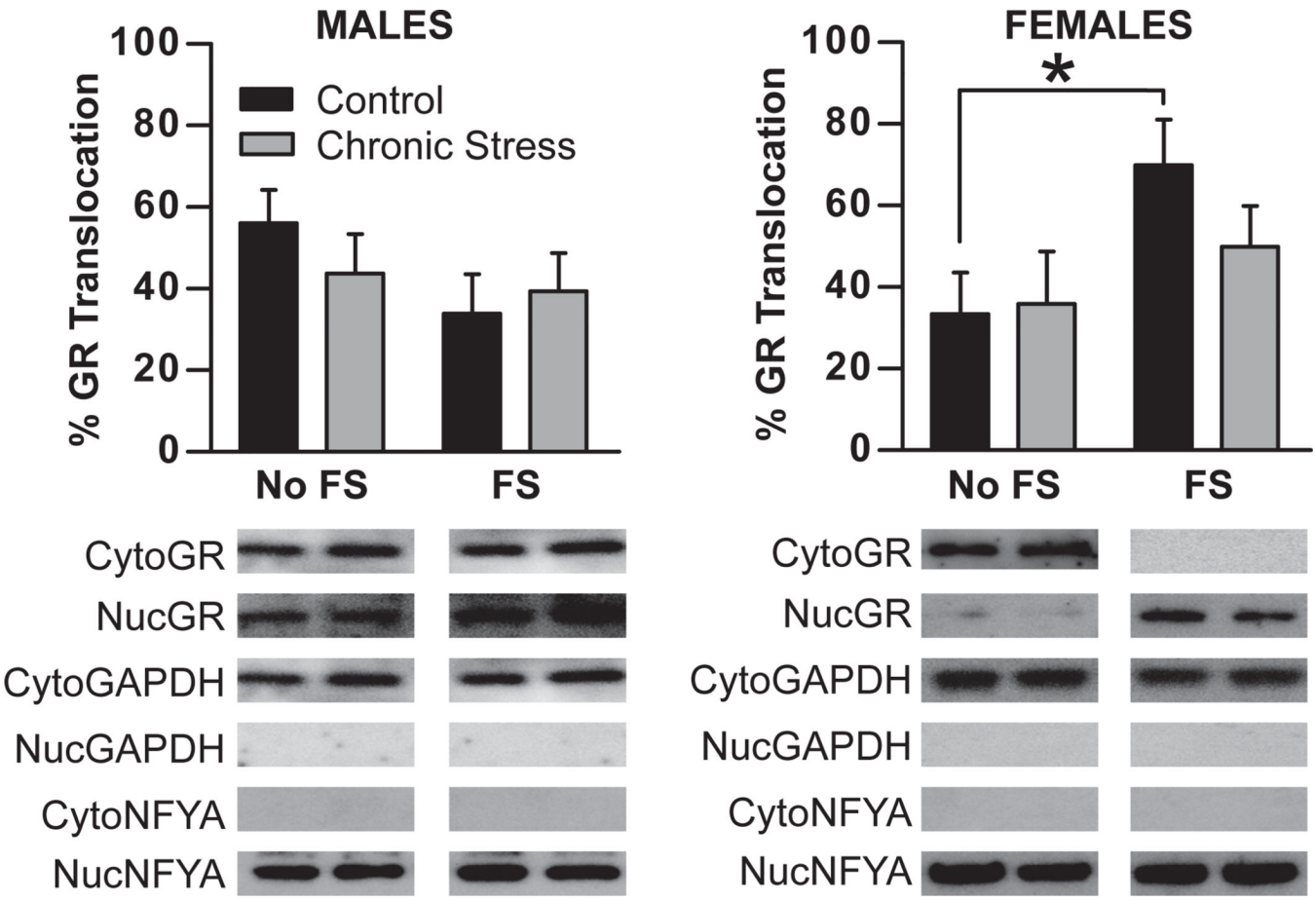

Figure 2.

Glucocorticoid receptor translocation. Subcellular fractionation and western blotting were carried out to determine the percent of GR in the nucleus versus total GR (cytosolic + nuclear). A five minute forced swim stress (FS) was used to induce GR translocation. Neither chronic stress, nor acute, nor the combination altered GR translocation in the hippocampi of male rats (A). Females exposed to acute stress displayed increased GR translocation, but this acute stress effect was attenuated by a history of chronic adolescent stress (B). N = 6–7. * $p < 0.05$ t test. Data are presented as mean ± SEM.

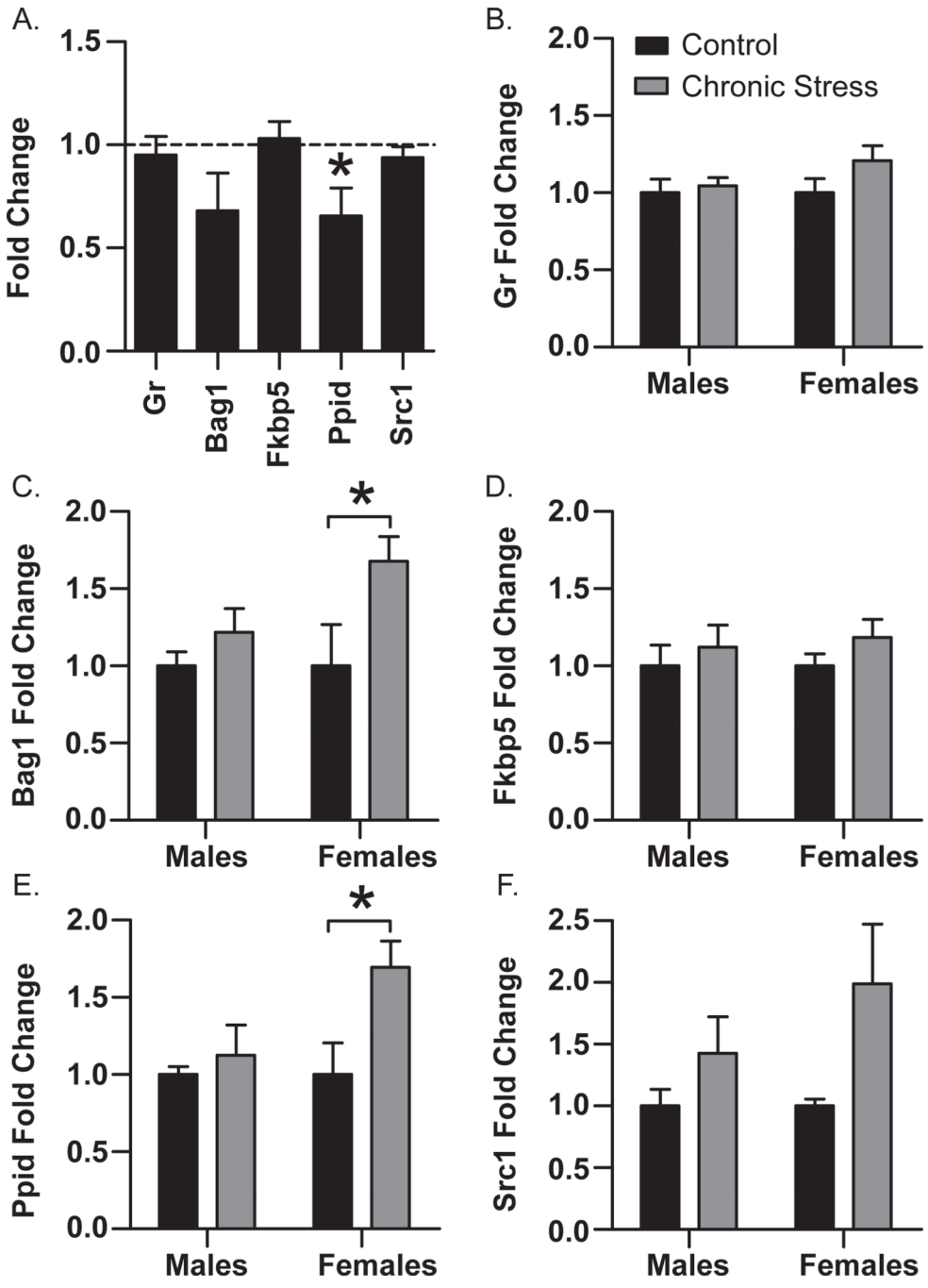

Figure 3.

Baseline sex differences in gene expression of *Gr, Bag1, Fkbp5, Ppid,* and *Src1* following chronic adolescent stress. Samples were collected immediately upon removal from the colony room four days after the final chronic stress exposure. RT-PCR of transcripts in the hippocampus was conducted. Female control expression values were normalized to male control expression values to determine inherent sex differences in gene expression (A). Female adolescent rats express less *Ppid* in the hippocampus than male adolescent rats (dotted line) (A). Gene expression values were normalized to same sex controls for subsequent comparisons. (B) Baseline *Gr* and (D) *Fkbp5* were unaltered due to adolescent stress in either sex. (C) Baseline *Bag1* and (E) *Ppid* were increased due to adolescent stress

in female, but not male, adolescent rats. Chronic adolescent stress increased *Src1* expression compared to controls, but no difference was found between individual groups. * $p < 0.05$ t test (A). * $p < 0.05$ Bonferroni *post hoc* test (C, E). N = 7–8. Data are presented as mean ± SEM.

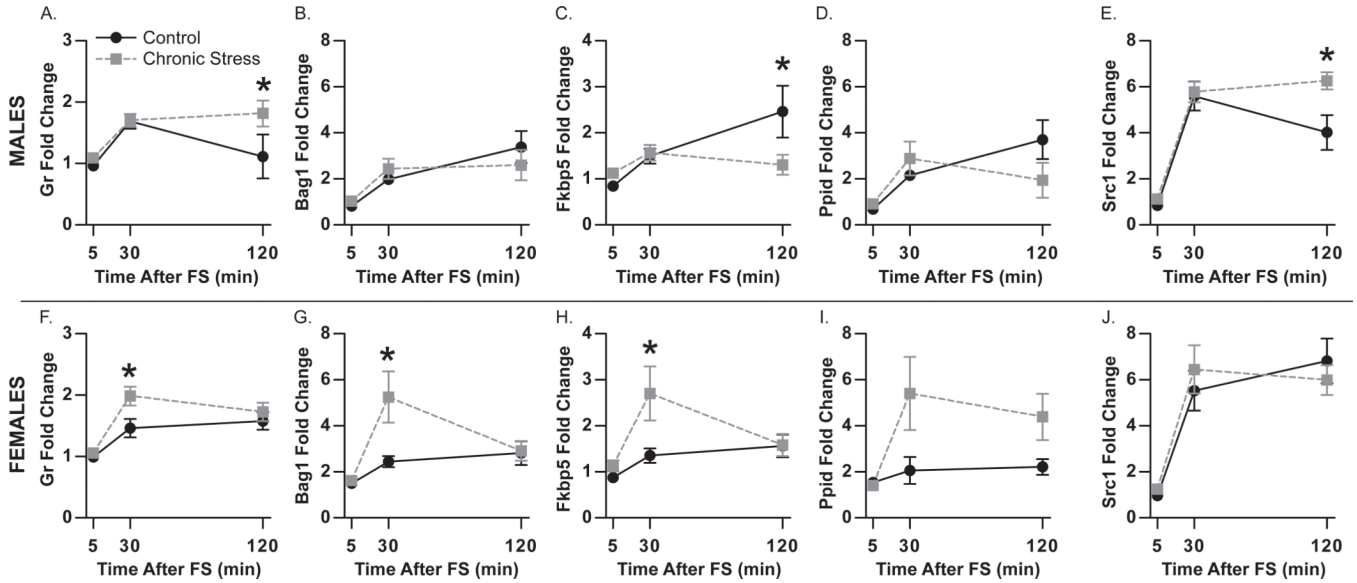

Figure 4.

Male and female gene expression time course of *Gr, Bag1, Fkbp5, Ppid,* and *Src1*. Rats were exposed to a five minute forced swim stress (FS) and decapitated 5, 30, or 120 minutes after initiation of the stressor. Male (A–E) or female (F–J) gene expression values were normalized to same sex controls at baseline without exposure to an acute stressor. N = 5–11. * $p < 0.05$ Bonferroni *post hoc* test. Data are presented as mean ± SEM.

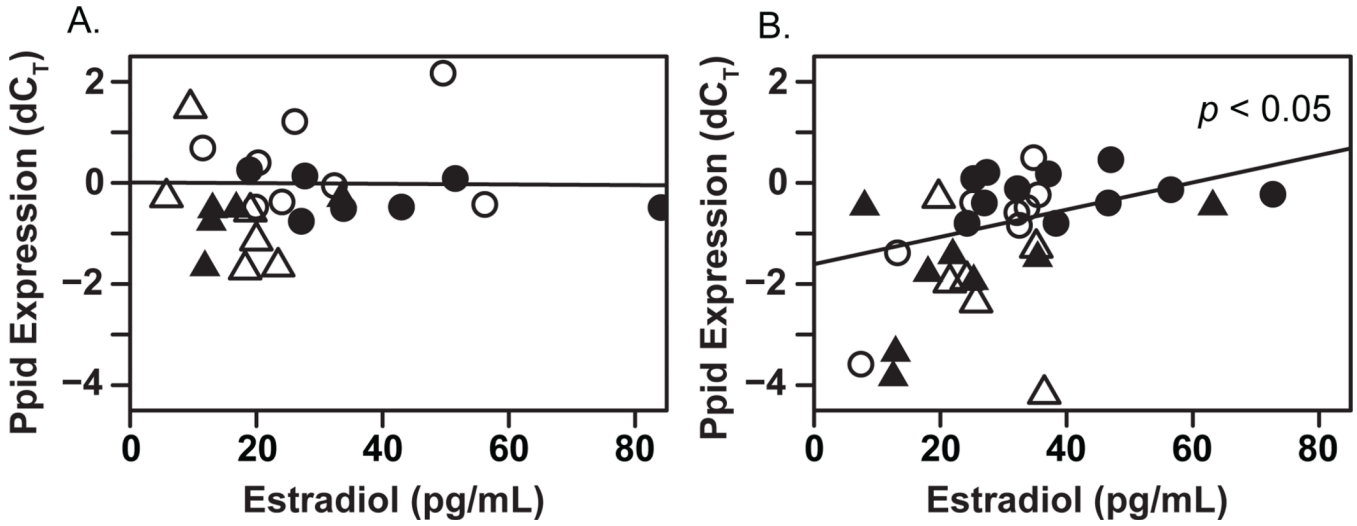

Figure 5.

Regression analysis of *Ppid* expression and plasma estradiol. Linear regression analysis was performed to determine if the delta C_T (dC_T) value of *Ppid* correlated with plasma estradiol. Females without a history of chronic adolescent stress had no correlation of *Ppid* and plasma estradiol (A). In females with a history of chronic adolescent stress, plasma estradiol predicted expression of *Ppid* ($p < 0.05$) (B). Symbols: baseline (open circle), 5 minutes post acute stressor (closed circle), 30 minutes post acute stressor (open triangle), 120 minutes post acute stressor (closed triangle).

Table 1

Tissue weights and body mass assessment. Chronic adolescent stress in males caused impaired weight gain after initiation of the stress model. Chronic adolescent stress in females caused no differences in weight gain.

	N	Male Control	Stress	Female Control	Stress
PND 23 (g)	20–23	80.3 ± 1.6	79.0 ± 1.7	72.7 ± 1.8	73.3 ± 0.9
PND 35 (g)	20–23	182.4 ± 2.8	178.7 ± 3.1	142.4 ± 2.5	146.5 ± 2.0
PND 40 (g)	20–23	224.8 ± 3.4	208.3 ± 3.7 *	161.9 ± 3.1	160.7 ± 2.2
PND 47 (g)	20–23	284.8 ± 4.3	253.7 ± 4.2 *	186.1 ± 3.4	185.7 ± 2.9
PND 49 (g)	20–23	305.5 ± 4.9	282.3 ± 4.3 *	194.1 ± 3.2	200.0 ± 3.0
Adrenal weight (mg)	17–20	86.70 ± 4.50	90.37 ± 2.32	90.81 ± 3.30	88.18 ± 3.20
Testes weight (g)	20–23	1.89 ± 0.03	2.07 ± 0.06 †	–	–
Uterine weight (mg)	14–17	–	–	358.6 ± 12.72	378.8 ± 17.89

* $p < 0.05$ Bonferroni *post hoc* test,

† $p < 0.05$ Student's t test. Data are presented as mean ± SEM.