Published in final edited form as:

Physiol Behav. 2015 October 15; 150: 8–15. doi:10.1016/j.physbeh.2015.02.011.

HIV-1 Proteins Accelerate HPA Axis Habituation in Female Rats

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Abstract

Congenital infection by the Human Immunodeficiency Virus (HIV) has been shown to lead to multiple co-morbidities, and people living with HIV have a higher incidence of affective and anxiety disorders. A marked increase in mood disorders is evident during the sensitive phase of adolescence and this is further pronounced in females. Depression has been linked to dysfunction of the intracellular response system to corticosteroids at the level of the hippocampus (HC) and prefrontal cortex (PFC) with a notable role of the glucocorticoid receptor (GR) and its co-chaperones (FKBP5 and FKBP4). The current study examined the extent to which HIV protein expression in adolescent female rats altered the stress response at both the level of corticosterone output and molecular regulation of the glucocorticoid receptor in the brain. WT and HIV-1 genotype female rats were randomly allocated in control, acute stress and repeat stress groups. Corticosterone plasma levels and expression of GR, FKBP4, and FKBP5 in the HC and PFC were measured. The presence of HIV-1 proteins facilitate habituation of the corticosterone response to repeated stressors, such that HIV-1 TG rats habituated to repeated restraint and WT rats did not. This was reflected by interactions between stress exposure and HIV-1 protein expression at the level of GR co-chaperones. Although expression of the GR was similarly reduced after acute and repeat stress in both genotypes, expression of FKBP5 and FKBP4 was altered in a brain-region specific manner depending on the duration of the stress exposure and the presence or absence of HIV-1 proteins. Collectively, the data presented demonstrate that HIV-1 proteins accelerate habituation to repeated stressors and modify the influence of acute and repeat stressors on GR co-chaperones in a brain region-specific manner.

Keywords

HIV; FKBP5; FKBP4; stress; female; hippocampus; prefrontal cortex

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1) Introduction

Treatment advances have greatly increased the life expectancy of people living with HIV (Smith, Ryom et al. 2014). However, individuals living with HIV have a higher incidence of stress-associated disorders. The development of these disorders has been proposed to be due to the psychosocial burden that is associated with the diagnosis of HIV positive status (Lichtenstein, Laska et al. 2002), stress stemming from occupational-related problems, lack of social support, cultural biases associated with infection, and/or resource-poor environments (Brown 2001; Wohl, Galvan et al. 2010; Kamau, Kuria et al. 2012). It is estimated that 30 - 50% of people living with HIV have a stress-related mood disorder such as depression, compared to only 6.5% of the general population (Hinkin, Castellon et al. 2002; Lopes, Olfson et al. 2012). Psychological problems can arise at a very young age in patients chronically infected with HIV (Gadow, Angelidou et al. 2012), with anxiety and depression being more common in females (Mellins, Elkington et al. 2012). Furthermore, adolescents living with HIV are at an even higher risk than adult HIV patients of developing multiple psychiatric co-morbidities, amongst which is depression (Gadow, Angelidou et al. 2012; Smith, Chernoff et al. 2012).

Brain studies focusing on structural changes seen with HIV patients have revealed that HIV infection produces changes in the subcortical white matter and the frontal lobe networks (Schweinsburg, Scott et al. 2012). Studies have linked untreated HIV infection with increasing amounts of neuroinflammation in the early parts of the disease process (Suh, Sinclair et al. 2014), which is believed to be a major contributor of HIV-related cognitive effects (Hong and Banks 2014). In addition, the presence of HIV-related proteins in model animals, without viral infectivity, can cause depressive-like and anxiety-like behaviors in adolescent female rats (Nemeth, Glasper et al. 2014), produce changes in the medial PFC of male rats (Wayman, Dodiya et al. 2012), and increase oxidative stress driven apoptosis in the rat hippocampus (Kruman, Nath et al. 1998). These results suggest that both HIV and HIV proteins produce a susceptibility to the development of psychiatric co-morbidities.

Evidence suggests that the increased impact of stress on behavior during adolescence is facilitated by the increased plasticity of the hypothalamic-pituitary-adrenal (HPA) axis during this developmental phase (McCormick, Robarts et al. 2005; Eiland and Romeo 2013). The HPA axis mediates this biological process to control the behavioral, endocrine, and gene expression responsivity to stress. The initial drive originates from Corticotropin Releasing Factor (CRF) secreting neurons in the hypothalamus, which in turn stimulate Adrenocorticotrophic Hormone (ACTH) production from the anterior pituitary (Rivier, Brownstein et al. 1982). The latter then acts on the adrenal cortex to stimulate production of corticosteroids (Faust, Goldstein et al. 1977; Chung, Guo et al. 1997), the main effectors of the system, and also exert negative feedback control mainly at the level of the hypothalamus, the anterior pituitary, and the hippocampus (Keller-Wood and Dallman 1984; Myers, McDonald et al. 1992). It should be noted that control of ACTH secretion is complex and under the control of many more substances other than CRF; for a detailed review see previously published reviews on the subject (Panagiotakopoulos and Neigh 2014).

Intracellular glucocorticoid activity is mediated by actions of the glucocorticoid receptor (GR) which works in concert with multiple proteins, including chaperones, co-chaperones and co-activators (Panagiotakopoulos and Neigh 2014) (Figure 1). Most notably, interchange on the GR of Immunophilin FK506 Binding Protein 5 (FKBP5; this protein is also referred in the literature as FKBP51) with FKBP4 (this protein is also referred in the literature as FKBP52) after ligand binding occurs (Davies, Ning et al. 2002), allows for interaction with dynein and translocation of the GR-Chaperone heterocomplex into the nucleus (Wochnik, Ruegg et al. 2005), where nuclear transcription can be modulated (McNally, Muller et al. 2000). The GR cannot function as a transcription factor without the appropriate function of FKBP4 which requires dissociation of FKBP5. FKBP5 polymorphisms have been linked with suicidality (Brent, Melhem et al. 2010), depression (Sarginson, Lazzaroni et al. 2010; Shinozaki, Jowsey et al. 2011; Menke, Klengel et al. 2013) and anxiety disorders (Kang, Chung et al. 2012; Minelli, Maffioletti et al. 2013), making it an emerging consideration in the efforts focused on successful pharmacological treatment of these disorders (Binder 2009; Niitsu, Fabbri et al. 2013). Specifically, in depressed adults living with HIV there is an increase in FKBP4 in the PFC, whereas in HIV adults without depression, FKBP5 is increased instead (Tatro, Everall et al. 2009). Therefore, the output of the HPA axis (amount of circulating glucocorticoids) and the plasticity of its target tissue (conferred by GR-related co-chaperones) are tightly associated with the development of psychiatric co-morbidities, and may be further de-regulated in persons living with HIV.

Investigation of the HPA axis in humans living with HIV is challenging due to the multiple confounding factors of everyday life that can affect its function and serve as stressors (Chrousos 1998). Animal research has provided for a valuable platform of experimental design under controlled conditions, where this sensitive system can be efficiently studied. The HIV-1 transgenic (TG) rat model, as described in Reid *et al* (2001), is a non-replicating model of HIV infection that mimics the pathogenesis of HIV-infected patients on long-term highly active antiretroviral therapy (HAART) with no detectable levels of virus replication (Reid, Sadowska et al. 2001; Peng, Vigorito et al. 2010), without potential side effects resulting from HAART itself. In short, no infectious virus particles are produced, but tissues express HIV-1 related proteins. The HIV-1 TG rat model allows evaluation of the effect that the HIV virus might have on the organism through the process of HIV-1 protein expression. The aim of the current study was to determine the extent to which the expression of HIV proteins in adolescent female rats alters the HPA axis under baseline non-stress conditions, after a single acute stressor, and following repeated stressor exposures.

2) Methods

Animals

HIV-1 TG male rats were bred in-house to Fisher females to generate the progeny for this study. Only female offspring were studied. The offspring were reared by their mother until weaning at postnatal day (PND) 25. At PND 25 the litters were sexed and genotyped. They were then pair housed as same-sex pairs within the same genotypes. Littermates were assigned to control and stress groups with no more than two pups per litter assigned to each

group in order to prevent litter effects (Holson and Pearce 1992). Rats were housed on a 14:10 reverse light:dark cycle in a facility controlled for humidity (60%) and temperature (20–23 °C). Rodent diet 5001 chow (Purina Mills, Richmond, IN) and water were available *ad libitum* throughout the study. Experiments began in late adolescence on PND 55. 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 (National Research Council 2011). All efforts were made to minimize animal suffering and to reduce the number of rats used.

Experimental Protocol

Rats of both genotypes were exposed to one of the following conditions: control non-stress, acute stress, or repeat stress. All rats were euthanized via rapid decapitation. Rats assigned to the control groups were euthanized immediately upon removal from their cage. Rats in the acute stress groups were euthanized immediately following their first exposure to stress. Rats in the repeat stress group were euthanized immediately following their eighth exposure to stress. All groups had an $n = 11$, apart from repeatedly stressed wild-type (WT) rats ($n = 10$).

Restraint Stress

Acute restraint stress has been long established to be a significant stressor for the laboratory rat (Perhach and Barry 1970), and repeated restraint stress has been used in various modalities to simulate repeated physiological stress (Grissom and Bhatnagar 2009). The latter, separate from its capacity to test for the production of a stress response, allows for the investigation of habituation to the same repeated stressful stimulus (Kant, Eggleston et al. 1985). Rats assigned to the acute stress group were exposed to a single bout of restraint stress for 20 minutes, immediately prior to decapitation. Rats assigned to the repeat stress group underwent 8 exposures of 30 minutes of restraint on consecutive days and were killed immediately following the eighth exposure. Animals were restrained in acrylic rat restraints (BrainTree Scientific, Braintree, MA) which prevented head to tail turns but did not compress the rat. All stress experiments occurred during the light cycle to coincide with the nadir of the circadian cycle of corticosterone to maximally increase corticosterone during stressor exposure.

Corticosterone Assay

Following decapitation, trunk blood was collected immediately in BD Vacutainer EDTA collection tubes (BD, Franklin Lakes, NJ). Blood was spun down at 1800rcf and the plasma fraction was collected. Plasma corticosterone levels were assayed via ELISA: corticosterone (sensitivity: 27 pg/ml, Enzo Life Sciences, Farmingdale, NY, USA). Samples were run in duplicate.

Quantitative Real Time PCR

Following decapitation, brains were immediately removed and rapidly frozen on dry ice. The hippocampus and PFC were dissected on dry ice. Hippocampal and PFC RNA were extracted with the TRIzol method (Invitrogen, Carlsbad, CA) using an RNeasy Mini kit

(Qiagen, Valencia, CA, USA). RNA concentration and integrity were assessed by a NanoDrop 2000 spectrophotometer (ThermoScientific, Wilmington, DE, USA). 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) and then standardized so that all samples started quantitative RT-PCR with 5pg of cDNA. The selection of an ideal endogenous control is important to the interpretation of real time PCR experiments (Schmittgen and Zakrajsek 2000). All DNA primers were designed using the Primer3Plus online software (Andreas Untergasser 2007) (see Table 1 in Supplementary materials). The primers were ordered and manufactured from Integrated DNA Technologies (San Diego, CA, USA). Primers were validated using decreasing cDNA concentrations by a factor of 5, to the standard of -3.3 for the slope of the association between threshold cycle and log cDNA concentration (Bookout and Mangelsdorf 2003). qRT-PCR was performed using Absolute Blue qPCR SYBER Green ROM Mix (Thermo Scientific, Wilmington, DE, USA). The universal two-step RT-PCR cycling conditions used on the 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) were: 50 °C (2 min), 95 °C (10 min), 40 cycles of 95 °C (15 sec) and 60 °C (1 min), then 95 °C (15 sec), 60 °C (15sec). The expression of 5 candidate endogenous control genes was assessed (see table 2 in supplementary materials). The ddCt of an individual endogenous control gene (HPRT1 for the HC) or the geometric mean of 5 endogenous control genes (B2M, bACTIN, RPL13A, HPRT1, and LDHA for the PFC) was used, whichever provided for a lower deviation between groups. Relative gene expression of individual samples run in triplicate (with coefficient of variation cut-off set to 4%) was calculated by the comparative Cycle Threshold quantification method relative to the change of endogenous control genes (2^{-CT}), as described previously (Livak and Schmittgen 2001).

Statistical Analysis

Statistical analysis of differences in corticosterone concentrations was made using two-way ANOVA with post hoc analysis using Sidak's multiple comparisons test. Statistical analysis of differences in gene expression was made using two-way ANOVA with post hoc analysis using Holm-Sidak's multiple comparisons test. All statistical analyses were performed using GraphPad Prism v.6 (La Jolla, CA). All data were determined to be statistically significant when $p < 0.05$ and are presented as a mean $\pm$ standard error of the mean (SEM).

3) Results

3.1 HIV-1 TG rats, but not WT rats, habituated to repeated restraint

Corticosterone values were measured at baseline and after acute or repeat restraint stress (Figure 2). There was a significant effect of stress ($F_{2, 59} = 56.23$, $p < 0.0001$), as well as an interaction between stress and genotype ($F_{2, 59} = 3.851$, $p = 0.0268$). There was no observed effect of genotype alone ($F_{1,59} = 2.679$, $p > 0.1$). Using a post hoc analysis we yielded a significant decrease in corticosterone concentrations following repeat stress between the two genotypes ($p < 0.05$), and, within HIV-1 TG rats, acute stress concentrations were significantly higher than repeat stress concentrations ($p < 0.05$). Corticosterone concentrations between acute and repeat stress conditions did not vary for wild-type rats ($p > 0.05$).

3.2 Acute and repeat stress similarly impact GR expression

Expression of GR was measured in the HC and PFC of WT and HIV-1 TG animals at baseline, after acute, or after repeated stress (Figures 3A, 4A).

In the PFC (Figure 3A), there was a significant effect of stress ($F_{2, 58} = 21.47$, $p < 0.0001$), no observed effect of genotype alone ($F_{1, 58} = 0.6642$, $p = 0.42$), and no interaction between stress and genotype ($F_{2, 58} = 0.5756$, $p = 0.57$). Post hoc analyses yielded significant differences between groups. Compared to WT control, there was a significant decrease in GR gene expression for WT acutely stressed ($t_{(58)} = 4.115$, $p = 0.0006$), HIV-1 acutely stressed ($t_{(58)} = 3.368$, $p = 0.0047$), WT repeatedly stressed ($t_{(58)} = 3.107$, $p = 0.0058$), and HIV-1 repeatedly stressed ($t_{(58)} = 3.414$, $p = 0.0047$) groups. Compared to HIV-1 control, HIV-1 acutely stressed ($t_{(58)} = 4.340$, $p = 0.0002$) and HIV-1 repeatedly stressed ($t_{(58)} = 4.365$, $p = 0.0002$) rats had equally lower GR gene expression levels. There was no difference observed between acute stress and repeat stress groups in either the WT or the HIV-1 genotype (all $p > 0.05$). No genotype effect was observed between WT and HIV-1 rats between the control, acute stress, and repeat stress animals (all $p > 0.05$).

In the HC (Figure 4A), there was a significant effect of stress ($F_{2, 57} = 15.68$, $p < 0.0001$), no observed effect of genotype alone ($F_{1, 57} = 0.05344$, $p = 0.82$), and no interaction between stress and genotype ($F_{2, 57} = 0.1430$, $p = 0.87$). Post hoc analyses yielded significant differences between groups. Against WT control, there was a significant decrease in GR gene expression for WT acutely stressed ($t_{(57)} = 3.057$, $p = 0.0068$), HIV-1 acutely stressed ($t_{(57)} = 3.806$, $p = 0.0017$), WT repeatedly stressed ($t_{(57)} = 3.449$, $p = 0.0043$), and HIV-1 repeatedly stressed ($t_{(57)} = 3.302$, $p = 0.0050$) groups. Against HIV-1 control, HIV-1 acutely stressed ($t_{(57)} = 3.915$, $p = 0.0007$) and HIV-1 repeatedly stressed ($t_{(57)} = 3.408$, $p = 0.0024$) rats had equally lower GR gene expression levels. There was no difference observed between acute stress and repeat stress groups in either the WT or the HIV-1 genotype (all $p > 0.05$). No genotype effect was observed between WT and HIV-1 rats between the control, acute stress, and repeat stress animals (all $p > 0.05$).

3.3 FKBP5 expression is modulated in a region-specific manner in WT rats

Expression of FKBP5 was measured in the HC and PFC of WT and TG animals at baseline, after acute, or after repeated stress (Figures 3B, and 4B).

In the PFC (Figure 3B), there was a significant effect of stress ($F_{2, 58} = 14.05$, $p < 0.0001$), no observed effect of genotype alone ($F_{1, 58} = 0.4660$, $p = 0.50$), and no interaction between stress and genotype ($F_{2, 58} = 0.09113$, $p = 0.91$). Post hoc analyses yielded significant differences between groups. Against WT control, there was a significant decrease in FKBP5 gene expression for WT acutely stressed ($t_{(58)} = 2.646$, $p = 0.0311$), WT repeatedly stressed ($t_{(58)} = 3.344$, $p = 0.0072$), and HIV-1 repeatedly stressed ($t_{(58)} = 3.188$, $p = 0.0092$) groups. Against HIV-1 control, HIV-1 acutely stressed ($t_{(58)} = 2.828$, $p = 0.0128$) and HIV-1 repeatedly stressed ($t_{(58)} = 3.771$, $p = 0.0012$) rats had equally lower FKBP5 gene expression levels. There was no difference observed between acute stress and repeat stress groups in either the WT or the HIV-1 genotype (all $p > 0.05$). No genotype effect was observed

between WT and HIV-1 rats between the control, acute stress, and repeat stress animals (all $p > 0.05$).

In the HC (Figure 4B), there was a significant effect of stress ($F_{2,56} = 9.666$, $p=0.0002$), no observed effect of genotype alone ($F_{1,56} = 0.1442$, $p=0.71$), and no interaction between stress and genotype ($F_{2,56} = 1.268$, $p = 0.2893$). Post hoc analyses yielded significant differences between groups. Against WT control, there was a significant decrease in FKBP5 gene expression for WT repeatedly stressed ($t_{(56)}=3.299$, $p=0.0084$) alone. WT acutely stressed and all HIV-1 rats did not have different levels of expression compared to WT control rats (all $p>0.05$). Against HIV-1 control, HIV-1 acutely stressed ($t_{(56)}=2.726$, $p=0.017$) and HIV-1 repeatedly stressed ($t_{(56)}=3.037$, $p=0.0108$) rats had equally lower FKBP5 gene expression levels. There was no difference observed between acute stress and repeat stress groups in either the HIV-1 genotype ($p>0.05$), but there was a decrease in expression in WT repeat stress animals compared to WT acute stress rats ($t_{(56)}=2.299$, $p=0.0499$). No genotype effect was observed between WT and HIV-1 rats between the control, acute stress, and repeat stress animals (all $p > 0.05$).

3.4 FKBP4 expression patterns are altered in the PFC of HIV-1 TG rats in response to stress, conferring a genotype specific expression pattern

Expression of FKBP4 was measured in the HC and PFC of WT and HIV-1 TG animals at baseline, after acute, or after repeated stress (Figures 3C, 4C).

In the PFC (Figure 3C), there was a significant effect of stress ($F_{2,58} = 9.772$, $p = 0.0002$), and no observed effect of genotype alone ($F_{1,58} = 0.3511$, $p=0.5558$). There was, however, a significant interaction between stress and genotype ($F_{2,58} = 6.597$, $p = 0.0026$). Post hoc analyses yielded significant differences between groups. Against WT control, there was a significant decrease in FKBP4 gene expression for WT acutely stressed ($t_{(58)}=3.055$, $p=0.0135$), and HIV-1 repeatedly stressed ($t_{(58)}=4.261$, $p=0.0004$) groups. WT repeatedly stressed and HIV-1 acutely stressed rats did not have different levels of FKBP4 expression compared to WT control rats (all $p>0.05$). Against HIV-1 control, only HIV-1 repeatedly stressed ($t_{(58)}=4.647$, $p<0.0001$) rats had lower FKBP4 gene expression levels, and acutely stressed HIV-1 rats had similar levels of FKBP4 expression ($p>0.05$). Even though no difference was observed between acute stress and repeat stress groups in the WT genotype ($p>0.05$), within the HIV-1 genotype repeatedly stressed animals had lower FKBP4 levels compared to acutely stressed animals ($t_{(58)}=3.200$, $p=0.0087$).

In the HC (Figure 4C), there was a significant effect of stress ($F_{2,58} = 20.73$, $p < 0.0001$), no observed effect of genotype alone ($F_{1,58} = 0.03751$, $p=0.8471$), and no interaction between stress and genotype ($F_{2,58} = 0.6338$, $p = 0.5342$). Post hoc analyses yielded significant differences between groups. Against WT control, there was a significant decrease in FKBP4 gene expression for WT acutely stressed ($t_{(58)}=3.240$, $p=0.0040$), HIV-1 acutely stressed ($t_{(58)}=3.374$, $p=0.0040$), WT repeatedly stressed ($t_{(58)}=3.464$, $p=0.0040$), and HIV-1 repeatedly stressed ($t_{(58)}=4.309$, $p=0.0003$) groups. Against HIV-1 control, HIV-1 acutely stressed ($t_{(58)}=4.038$, $p=0.0003$) and HIV-1 repeatedly stressed ($t_{(58)}=4.957$, $p<0.0001$) rats had equally lower FKBP4 gene expression levels. There was no difference observed between acute stress and repeat stress groups in either the WT or the HIV-1 genotype (all

$p > 0.05$). No genotype effect was observed between WT and HIV-1 rats among the control, acute stress, and repeat stress animals (all $p > 0.05$).

4) Discussion

The current body of work assessed the degree to which development with HIV-1 proteins altered the HPA axis response of female rats to acute and repeat stress at both the systemic and molecular levels. In summary, we found that restraint stress produced an adequate acute stress response, with a lesser stimulation of the HPA axis following repeated stress exposures in HIV-1 TG rats compared to the WT genotype (Figure 2). The presence of HIV-1 proteins did not alter expression of the GR directly, such that the GR expression was uniformly affected by acute and repeat stress regardless of genotype (Figures 3A, 4A). Therefore, the altered corticosterone response to repeated restraint does not appear to be mediated by altered regulation of GR expression. The molecular influence of HIV-1 proteins is evident at the level of the glucocorticoid co-chaperones FKBP5 (Figures 3B, 4B) and FKBP4 (Figures 3C, 4C), and these may be potential mechanisms for the facilitation of the rapid habituation of the corticosterone response in HIV-1 TG rats. The demonstration of HIV-1 protein influence on HPA axis reactivity and changes in co-chaperones in this study may provide context for discrepancies in earlier observations of HPA axis function in the presence of HIV infection, given the reported influence of acute versus repeat stress.

To-date, only a small study in congenital HIV infected children has been published, showing no change in the HPA axis function (Lala, Palomba et al. 1996). Early studies on the function of the HPA axis in older human subjects with HIV infection without Acquired Immunodeficiency Syndrome (AIDS), demonstrated intact HPA axis function in only 50% of study participants (Azar and Melby 1993), with the remainder of the subjects having some degree of reduced pituitary or adrenal reserves. Other studies have shown that HPA axis function is normal in patients with AIDS (Dobs, Dempsey et al. 1988). Moreover, another finding is that HPA axis function mostly correlates with viral load and disease severity (Raffi, Brisseau et al. 1991). Along those lines, pharmacological stimulation of the HPA axis in HIV positive patients with early disease produces a normal cortisol output in many more individuals than if the testing is performed in patients with chronic HIV infection (Membreno, Irony et al. 1987; Verges, Chavanet et al. 1989). Furthermore, blunting of the ACTH response to exogenous CRF is especially noted in advanced uncontrolled infection (Hoshino, Yamashita et al. 2002). However this effect is rarely observed in the early stages or well controlled HIV-1 infection (Biglino, Limone et al. 1995), which is modelled by the HIV-1 rat. Our study showed no change in corticosterone output after daily repetition of the restraint stressor in wildtype adolescent female rats. This is in accordance to existing literature, where there is a clear description of the development and maturation of the habituation response to a repeated stressor after the adolescent period (Eiland and Romeo 2013; Klein and Romeo 2013). Adult female rats have been found to habituate to repeat restraint stress (Babb, Masini et al. 2014), however studies using adolescent female rats have shown no evidence of habituation to repeat restraint stress (Barha, Brummelte et al. 2011). The results reported here focus on a single point in development, late adolescence, and therefore the effects of chronicity were not directly assessed. The data presented here do enable us to conclude that in the absence of active viral

replication, HIV-1 protein expression is sufficient to alter the dynamics of the HPA axis response to repeated stressors. These findings may have implications for individuals living with HIV who are on adequate antiretroviral therapy with suppressed viral loads but still suffering from stress-related conditions and suggest that co-chaperones of the GR are a candidate mechanism for HIV-associated changes in HPA axis function.

As detailed in the introduction, the main effectors of the HPA axis, glucocorticoids, function at their receptor with the aid of a series of chaperone and co-chaperones (Figure 1). The association of HPA axis modulation and HIV infection has been partially characterized at the level of these intracellular components. Work by Tatro et al (2009) in adults with HIV, showed selective regulation of FKBP4 or FKBP5, depending on whether an HIV patient was depressed or non-depressed respectively (Tatro, Everall et al. 2009). Follow up work by the same group showed that the measurable changes in blood FKBP5 levels correlated clinically with improvement in the Beck Depression Inventory (BDI) for major depressive disorder (Tatro, Nguyen et al. 2010). However, it is still unclear if the functional link between the HIV-related dysregulation of glucocorticoid co-chaperones and depressive behaviors is cause or effect. The data presented here suggest that HIV proteins are, in and of themselves, sufficient to alter the stress-induced expression patterns of FKBP4 and FKBP5 in a brain-region specific manner.

Considering the entire picture of expression changes of GR, FKBP5, and FKBP4, and the available literature on the interactions of these proteins, some hypotheses can be drawn regarding the path to functional differences in glucocorticoid output in HIV-1 TG rats. Our studies showed a universal decrease in GR expression throughout genotypes and stress modalities in the PFC and the HC. Existing literature demonstrates that a decrease in GR expression following stress exposure serves as a means of physiological adaptation of the stress response (Tritos, Kitraki et al. 1999; McKlveen, Myers et al. 2013). The timing of the immediate GR mRNA amount reduction following an acute stress in our study, was also similar in previously reported work in the literature (Romeo, Kaplowitz et al. 2013). However, the reduction in GR expression in the current data set is not coupled to habituation of the corticosterone response to restraint because both genotypes demonstrate the reduction in GR expression following even a single acute stressor. This uncoupling of GR expression from corticosterone concentrations may indicate that the effects are mediated at an alternate level of the HPA axis, at the level of GR translocation or DNA binding, or perhaps at the level of GR co-chaperones. It is important to note that the lack of difference in GR expression modulation in this study only applies to the HC and the PFC. Other parts of the HPA axis, such as the hypothalamus and/or anterior pituitary, could be involved in producing the difference in the habituation response observed between genotypes, at the level of GR expression.

The relationship between FKBP5, GR function, and stress phenotypes has been detailed in both human investigation and work with animal models. Some of the support of the relationship between the negative regulation of GR by FKBP5 and the effects on the response to stress stems from work with FKBP5 knockout mice which have been shown to adopt more active coping behaviors in response to various stressors (Touma, Gassen et al. 2011). In the current data set, we observed a reduction in expression of FKBP5 regardless of

genotype or stress duration in the PFC. An equal reduction of FKBP5 was also noted in the HC following repeat stress exposure for both genotypes. However, only HIV-1 TG rats exhibited a reduction in FKBP5 expression in the HC following a single acute stressor. Furthermore, wildtype animals had significantly lower FKBP5 levels after repeat stress compared to acute stress. The HIV-1 TG rats appear to be more sensitive to the effects of a single acute stress and may be exhibiting an accelerated level of adaptation to stress given that repeat stress eventually caused reduced FKBP5 expression in hippocampal tissue of WT rats. The reduction in expression of FKBP5 in the HC may be a normal physiological adaptive intracellular response to promote glucocorticoid-related signaling; eventually this would help propagate the body's stress response in the face of prolonged exposure to stress. Based on the available evidence regarding FKBP5 function, decreased expression of FKBP5 in HIV-1 TG rats would promote GR translocation into the nucleus of HIV-1 TG rats after acute stress, and therefore would potentiate the transcription power of corticosteroids under these conditions. Perhaps the accelerated reduction in FKBP5 expression in the HIV-1 TG rats contributes to the increased habituation that the HIV-1 phenotype shows following repeated stressor exposure. It is noteworthy that in our study, the effect of acute stress on WT animals differs between the PFC and the HC, with preservation of FKBP5 levels in the HC, suggesting the need for the inhibitory presence of FKBP5 in this critical brain region, and eluding to the notion that subcellular manipulation of the HPA axis in the CNS can be achieved in a region-specific manner. Indeed, in the hippocampus of mice, FKBP5 expression remains stable following restraint stress but expression is increased after other various forms of acute stress (Scharf, Liebl et al. 2011). These results highlight the importance of FKBP5 in intracellular glucocorticoid signaling and the interaction of GR co-chaperones with HIV, in accordance to previous studies demonstrating differences in FKBP5 expression in HIV-infected humans (Tatro, Everall et al. 2009; Tatro, Nguyen et al. 2010).

Although less has been reported in the literature regarding FKBP4, changes in FKBP4 can also alter stress reactivity and FKBP4 is responsive to HIV. Previous studies using FKBP4 (+/−) mice, have demonstrated that a state of heterozygosity, and thus reduced levels of FKBP4, indeed directly lead to altered sensitivity to environmental stress, in a subset of behavioral and neuroendocrine parameters (Hartmann, Wagner et al. 2012). In depressed adults living with HIV there is an increase in FKBP4 in the PFC, whereas in HIV adults without depression, FKBP5 is increased instead (Tatro, Everall et al. 2009). In the current study we demonstrate that although hippocampal expression of FKBP4 is similarly reduced following acute and repeat stress exposure regardless of the presence of HIV-1 proteins (Figure 4C), FKBP4 is differentially responsive to stress in the presence of HIV-1 proteins in the PFC (Figure 3C). WT rats demonstrated a reduction in FKBP4 expression in the PFC following an acute stressor that resolved with repeated exposures to restraint. This transient decrease in FKBP4 expression in the PFC of WT rats suggests that under conditions of repeat stress that the target tissue would be more sensitive to glucocorticoid signaling than in an acute stress condition. Furthermore, the decrease in FKBP4 expression after acute stress in both the PFC and HC in WT rats alludes to the presence of a possible unidentified ultra-short feedback loop between incoming glucocorticoids and cytoplasmic FKBP4. Thus far,

only FKBP5 is considered to be involved in this type of ultra-short feedback loop (Binder 2009).

In contrast to effects of acute stress on FKBP4 in the PFC of WT animals, expression of FKBP4 in the PFC of HIV-1 TG rats exhibited a distinctly different pattern: expression remained unaltered after acute stress, indicating a potential shift in the molecular ultra-short feedback loop (Figure 3C). This shift compared to the WT genotype, would allow for a faster glucocorticoid signaling transduction during acute stress. Moreover, the presence of HIV-1 proteins in the PFC changed the pattern of FKBP4 expression under repeat stress conditions, such that, following repeated stress, expression of FKBP4 in the PFC is reduced when compared to the control HIV-1 and acutely stressed HIV-1 animals. This effect would repress the ability of the GR system to confer a cellular effect under repeat stress, and may be related to the increased capacity of HIV-1 rats to habituate to repeat stress. In conclusion, HIV-1 proteins modify the timing of stress-induced changes in PFC FKBP4 gene expression, but a causative association will require further inquiry.

5) Conclusion

In this study we demonstrated the extent to which HIV-1 proteins modify HPA axis output in female adolescent rats, as well as evaluated the expression of the GR and its negative (FKBP5) and positive (FKBP4) co-chaperones in the PFC and HC under non-stress, acute stress, and repeated stress conditions. Our results illustrate the influence of HIV-1 proteins on the output the HPA axis, as well as modulation of gene expression in diverse manners depending on the brain region and duration of stress. Two changes in FKBP5 and FKBP4 gene expression stand out in our study as potential clues to the increased pathology of stress-related co-morbidities in patients with HIV infection. In the HC, acute stress reduced FKBP5 levels (inhibitory co-chaperone) only in HIV-1 rats, leading to a potential loss of inhibitory buffering capacity on the intracellular signaling of the GR system. In the PFC, FKBP4 (stimulatory co-chaperone) is not decreased in TG rats after acute stress, as seen in the WT rats, and the tissue therefore inappropriately retains its efficiency in propagating incoming corticosteroid-related stress signals. Collectively, these findings suggest that the discrepancies in the existing literature regarding HIV and the HPA axis may be due to nuances in the timing and chronicity of stressor exposure and incomplete representation of the molecular machinery of the HPA axis. A more complete appreciation for the mediators and modifiers that contribute to interactions between HIV and the stress response will provide the framework necessary to more adequately address and treat stress-related co-morbidities in people living with HIV.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This research was supported by the Creative and Novel Ideas in HIV Research Program (CNIHR) through a supplement to the University of Alabama at Birmingham (UAB) Center For AIDS Research funding (P30 AI027767-24). This funding was made possible by collaborative efforts of the Office of AIDS Research, the

National Institutes of Allergies and Infectious Diseases, and the International AIDS Society. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

6) References

- Andreas Untergasser HN, Rao Xiangyu, Bisseling Ton, Geurts René, Jack AM. Leunissen: Primer3Plus, an enhanced web interface to Primer3 Nucleic Acids Research. 2007
- Azar ST, Melby JC. Hypothalamic-pituitary-adrenal function in non-AIDS patients with advanced HIV infection. *Am J Med Sci*. 1993; 305(5):321–325. [PubMed: 8484393]
- Babb JA, Masini CV, et al. Habituation of hypothalamic-pituitary-adrenocortical axis hormones to repeated homotypic stress and subsequent heterotypic stressor exposure in male and female rats. *Stress*. 2014; 17(3):224–234. [PubMed: 24635729]
- Barha CK, Brummelte S, et al. Chronic restraint stress in adolescence differentially influences hypothalamic-pituitary-adrenal axis function and adult hippocampal neurogenesis in male and female rats. *Hippocampus*. 2011; 21(11):1216–1227. [PubMed: 20665592]
- Biglino A, Limone P, et al. Altered adrenocorticotropin and cortisol response to corticotropin-releasing hormone in HIV-1 infection. *Eur J Endocrinol*. 1995; 133(2):173–179. [PubMed: 7655641]
- 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):S186–195. [PubMed: 19560279]
- Bookout AL, Mangelsdorf DJ. Quantitative real-time PCR protocol for analysis of nuclear receptor signaling pathways. *Nucl Recept Signal*. 2003; 1:e012. [PubMed: 16604184]
- Brent D, Melhem N, et al. Association of FKBP5 polymorphisms with suicidal events in the Treatment of Resistant Depression in Adolescents (TORDIA) study. *Am J Psychiatry*. 2010; 167(2):190–197. [PubMed: 20008943]
- Brown G. The impact of HIV/AIDS on the African American woman and child: epidemiology, cultural and psychosocial issues and nursing management. *ABNF J*. 2001; 12(3):60–62. [PubMed: 11760500]
- Chrousos GP. Stressors, stress, and neuroendocrine integration of the adaptive response. The 1997 Hans Selye Memorial Lecture. *Ann N Y Acad Sci*. 1998; 851:311–335.
- Chung BC, Guo IC, et al. Transcriptional regulation of the CYP11A1 and ferredoxin genes. *Steroids*. 1997; 62(1):37–42. [PubMed: 9029712]
- Davies TH, Ning YM, et al. A new first step in activation of steroid receptors: hormone-induced switching of FKBP51 and FKBP52 immunophilins. *J Biol Chem*. 2002; 277(7):4597–4600. [PubMed: 11751894]
- Dobs AS, Dempsey MA, et al. Endocrine disorders in men infected with human immunodeficiency virus. *Am J Med*. 1988; 84:611–616. 3 Pt 2. [PubMed: 3348269]
- Eiland L, Romeo RD. Stress and the developing adolescent brain. *Neuroscience*. 2013; 249:162–171. [PubMed: 23123920]
- Faust JR, Goldstein JL, et al. Receptor-mediated uptake of low density lipoprotein and utilization of its cholesterol for steroid synthesis in cultured mouse adrenal cells. *J Biol Chem*. 1977; 252(14):4861–4871. [PubMed: 194897]
- Gadow KD, Angelidou K, et al. Longitudinal study of emerging mental health concerns in youth perinatally infected with HIV and peer comparisons. *J Dev Behav Pediatr*. 2012; 33(6):456–468. [PubMed: 22772819]
- Grissom N, Bhatnagar S. Habituation to repeated stress: get used to it. *Neurobiol Learn Mem*. 2009; 92(2):215–224. [PubMed: 18667167]
- Hartmann J, Wagner KV, et al. Fkbp52 heterozygosity alters behavioral, endocrine and neurogenetic parameters under basal and chronic stress conditions in mice. *Psychoneuroendocrinology*. 2012; 37(12):2009–2021. [PubMed: 22641006]
- Hinkin CH, Castellon SA, et al. Medication adherence among HIV+ adults: effects of cognitive dysfunction and regimen complexity. *Neurology*. 2002; 59(12):1944–1950. [PubMed: 12499488]
- Holson RR, Pearce B. Principles and pitfalls in the analysis of prenatal treatment effects in multiparous species. *Neurotoxicol Teratol*. 1992; 14(3):221–228. [PubMed: 1635542]

- Hong S, Banks WA. Role of the immune system in HIV-associated neuroinflammation and neurocognitive implications. *Brain Behav Immun*. 2014
- Hoshino Y, Yamashita N, et al. Prospective examination of adrenocortical function in advanced AIDS patients. *Endocr J*. 2002; 49(6):641–647. [PubMed: 12625414]
- Kamau JW, Kuria W, et al. Psychiatric morbidity among HIV-infected children and adolescents in a resource-poor Kenyan urban community. *AIDS Care*. 2012; 24(7):836–842. [PubMed: 22292795]
- Kang JI, Chung HC, et al. FKBP5 polymorphisms as vulnerability to anxiety and depression in patients with advanced gastric cancer: a controlled and prospective study. *Psychoneuroendocrinology*. 2012; 37(9):1569–1576. [PubMed: 22459275]
- Kant GJ, Eggleston T, et al. Habituation to repeated stress is stressor specific. *Pharmacol Biochem Behav*. 1985; 22(4):631–634. [PubMed: 2986182]
- Keller-Wood ME, Dallman MF. Corticosteroid inhibition of ACTH secretion. *Endocr Rev*. 1984; 5(1):1–24. [PubMed: 6323158]
- Klein ZA, Romeo RD. Changes in hypothalamic-pituitary-adrenal stress responsiveness before and after puberty in rats. *Horm Behav*. 2013; 64(2):357–363. [PubMed: 23465865]
- Kruman II, Nath A, et al. HIV-1 protein Tat induces apoptosis of hippocampal neurons by a mechanism involving caspase activation, calcium overload, and oxidative stress. *Exp Neurol*. 1998; 154(2):276–288. [PubMed: 9878167]
- Lala R, Palomba E, et al. ACTH and cortisol secretions in children with perinatal HIV-1 infection. *Pediatr AIDS HIV Infect*. 1996; 7(4):243–245. [PubMed: 11361716]
- Lichtenstein B, Laska MK, et al. Chronic sorrow in the HIV-positive patient: issues of race, gender, and social support. *AIDS Patient Care STDS*. 2002; 16(1):27–38. [PubMed: 11839216]
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻(Delta Delta C(T)) Method. *Methods*. 2001; 25(4):402–408. [PubMed: 11846609]
- Lopes M, Olfson M, et al. Gender, HIV status, and psychiatric disorders: results from the National Epidemiologic Survey on Alcohol and Related Conditions. *J Clin Psychiatry*. 2012; 73(3):384–391. [PubMed: 22053858]
- McCormick CM, Roberts D, et al. Long-lasting, sex- and age-specific effects of social stressors on corticosterone responses to restraint and on locomotor responses to psychostimulants in rats. *Horm Behav*. 2005; 48(1):64–74. [PubMed: 15919386]
- McKlveen JM, Myers B, et al. Role of prefrontal cortex glucocorticoid receptors in stress and emotion. *Biol Psychiatry*. 2013; 74(9):672–679. [PubMed: 23683655]
- McNally JG, Muller WG, et al. The glucocorticoid receptor: rapid exchange with regulatory sites in living cells. *Science*. 2000; 287(5456):1262–1265. [PubMed: 10678832]
- Mellins CA, Elkington KS, et al. Prevalence and change in psychiatric disorders among perinatally HIV-infected and HIV-exposed youth. *AIDS Care*. 2012; 24(8):953–962. [PubMed: 22519762]
- Membreno L, Irony I, et al. Adrenocortical function in acquired immunodeficiency syndrome. *J Clin Endocrinol Metab*. 1987; 65(3):482–487. [PubMed: 3040796]
- Menke A, Klengel T, et al. Genetic variation in FKBP5 associated with the extent of stress hormone dysregulation in major depression. *Genes Brain Behav*. 2013; 12(3):289–296. [PubMed: 23406438]
- Minelli A, Maffioletti E, et al. Role of allelic variants of FK506-binding protein 51 (FKBP5) gene in the development of anxiety disorders. *Depress Anxiety*. 2013; 30(12):1170–1176. [PubMed: 23861224]
- Myers DA, McDonald TJ, et al. Effect of implantation of dexamethasone adjacent to the paraventricular nucleus on messenger ribonucleic acid for corticotropin-releasing hormone and proopiomelanocortin during late gestation in fetal sheep. *Endocrinology*. 1992; 130(4):2167–2172. [PubMed: 1547733]
- National Research Council. Guide for the Care and Use of Laboratory Animals: Eighth Edition. The National Academies Press; Washington, DC: 2011.
- Nemeth CL, Glasper ER, et al. Meloxicam Blocks Neuroinflammation, but Not Depressive-Like Behaviors, in HIV-1 Transgenic Female Rats. *PLoS One*. 2014; 9(10):e108399. [PubMed: 25271421]

- Niitsu T, Fabbri C, et al. Pharmacogenetics in major depression: a comprehensive meta-analysis. *Prog Neuropsychopharmacol Biol Psychiatry*. 2013; 45:183–194. [PubMed: 23733030]
- Panagiotakopoulos L, Neigh N. Development of the HPA axis: Where and when do sex differences manifest? *Front Neuroendocrinol*. 2014
- Peng J, Vigorito M, et al. The HIV-1 transgenic rat as a model for HIV-1 infected individuals on HAART. *J Neuroimmunol*. 2010; 218(1-2):94–101. [PubMed: 19913921]
- Perhach JL Jr, Barry H. Stress responses of rats to acute body or neck restraint. *Physiol Behav* (3rd). 1970; 5(4):443–448. [PubMed: 5535495]
- Raffi F, Brisseau JM, et al. Endocrine function in 98 HIV-infected patients: a prospective study. *AIDS*. 1991; 5(6):729–733. [PubMed: 1883545]
- Reid W, Sadowska M, et al. An HIV-1 transgenic rat that develops HIV-related pathology and immunologic dysfunction. *Proc Natl Acad Sci U S A*. 2001; 98(16):9271–9276. [PubMed: 11481487]
- Rivier C, Brownstein M, et al. In vivo corticotropin-releasing factor-induced secretion of adrenocorticotropin, beta-endorphin, and corticosterone. *Endocrinology*. 1982; 110(1):272–278. [PubMed: 6274623]
- Romeo RD, Kaplowitz ET, et al. The influence of puberty on stress reactivity and forebrain glucocorticoid receptor levels in inbred and outbred strains of male and female mice. *Psychoneuroendocrinology*. 2013; 38(4):592–596. [PubMed: 22917621]
- Sarginson JE, Lazzeroni LC, et al. FKBP5 polymorphisms and antidepressant response in geriatric depression. *Am J Med Genet B Neuropsychiatr Genet*. 2010; 153B(2):554–560. [PubMed: 19676097]
- Scharf SH, Liebl C, et al. Expression and regulation of the Fkbp5 gene in the adult mouse brain. *PLoS One*. 2011; 6(2):e16883. [PubMed: 21347384]
- 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]
- Schweinsburg BC, Scott JC, et al. Altered prefronto-striato-parietal network response to mental rotation in HIV. *J Neurovirol*. 2012; 18(1):74–79. [PubMed: 22271019]
- Shinozaki G, Jowsey S, et al. Relationship between FKBP5 polymorphisms and depression symptoms among kidney transplant recipients. *Depress Anxiety*. 2011; 28(12):1111–1118. [PubMed: 22134958]
- Smith CJ, Ryom L, et al. Trends in underlying causes of death in people with HIV from 1999 to 2011 (D:A:D): a multicohort collaboration. *Lancet*. 2014; 384(9939):241–248. [PubMed: 25042234]
- Smith R, Chernoff M, et al. Impact of HIV severity on cognitive and adaptive functioning during childhood and adolescence. *Pediatr Infect Dis J*. 2012; 31(6):592–598. [PubMed: 22592486]
- Suh J, Sinclair E, et al. Progressive increase in central nervous system immune activation in untreated primary HIV-1 infection. *J Neuroinflammation*. 2014; 11(1):199. [PubMed: 25465205]
- Tatro ET, Everall IP, et al. Modulation of glucocorticoid receptor nuclear translocation in neurons by immunophilins FKBP51 and FKBP52: implications for major depressive disorder. *Brain Res*. 2009; 1286:1–12. [PubMed: 19545546]
- Tatro ET, Everall IP, et al. Differential expression of immunophilins FKBP51 and FKBP52 in the frontal cortex of HIV-infected patients with major depressive disorder. *J Neuroimmune Pharmacol*. 2009; 4(2):218–226. [PubMed: 19199039]
- Tatro ET, Nguyen TB, et al. Correlation of major depressive disorder symptoms with FKBP5 but not FKBP4 expression in human immunodeficiency virus-infected individuals. *J Neurovirol*. 2010; 16(5):399–404. [PubMed: 20726698]
- Touma C, Gassen NC, et al. FK506 binding protein 5 shapes stress responsiveness: modulation of neuroendocrine reactivity and coping behavior. *Biol Psychiatry*. 2011; 70(10):928–936. [PubMed: 21907973]
- Tritos N, Kitraki E, et al. Neurotransmitter modulation of glucocorticoid receptor mRNA levels in the rat hippocampus. *Neuroendocrinology*. 1999; 69(5):324–330. [PubMed: 10343173]
- Verges B, Chavanet P, et al. Adrenal function in HIV infected patients. *Acta Endocrinol (Copenh)*. 1989; 121(5):633–637. [PubMed: 2555993]

- Wayman WN, Dodiya HB, et al. Enduring cortical alterations after a single in-vivo treatment of HIV-1 Tat. *Neuroreport*. 2012; 23(14):825–829. [PubMed: 22828409]
- Wohnik GM, Ruegg J, et al. FK506-binding proteins 51 and 52 differentially regulate dynein interaction and nuclear translocation of the glucocorticoid receptor in mammalian cells. *J Biol Chem*. 2005; 280(6):4609–4616. [PubMed: 15591061]
- Wohl AR, Galvan FH, et al. Social support, stress and social network characteristics among HIV-positive Latino and African American women and men who have sex with men. *AIDS Behav*. 2010; 14(5):1149–1158. [PubMed: 20107888]

Highlights

- HIV-1 proteins accelerate HPA axis habituation to repeated restraint.
- HIV-1 proteins accelerate stress-induced reductions in FKBP5 expression in the HC.
- HIV-1 proteins cause chronic stress-induced reductions in FKBP4 expression in the PFC.

Figure 1. Glucocorticoid receptor in its free and bound states

Stimulatory inputs are depicted in green arrows and inhibitory signals in dashed red lines. A: Glucocorticoid Receptor monomer in its ligand-free state in the cytoplasm. B: Homodimer formation in the presence of glucocorticoid within the ligand binding domain and the relationship of the complex with co-chaperones; interaction with dynein allows for translocation of the GR-chaperone heterocomplex into the nucleus, where transcription can be affected in genes containing Glucocorticoid Response Elements. P23: Prostaglandin E Synthase; FKBP: Immunophilin FK506 Binding Protein; hsp: Heat Shock Protein; BAG 1: BCL-2 Associated Athanogene 1; PPID: Peptidyl-Propyl Isomerase D; SRC-1: Steroid Receptor Coactivator-1.

Figure 2. Corticosterone concentrations for WT (solid black bars) and HIV-1 TG (light gray bars) animals. Data shown represent mean $\pm$ SEM. Figure symbol: (*) indicates a statistically significant difference between annotated groups ($p < 0.05$).

Figure 3. Gene expression levels in the pre-frontal cortex

Changes in GR (A), FKBP5 (B), and FKBP4 (C) expression at control, immediately following acute stress, and following repeated stress exposures for wildtype (black) and transgenic (grey) animals in the PFC. Gene expression is normalized to the change of the geometric mean of b2m, rpl13a, beta actin, Hprt1, and ldha. Data shown represent mean fold change $2^{-CT} \pm SEM$ against WT control. Figure symbols: (*) = $p < 0.05$ Vs WT control; (#) = $p < 0.05$ Vs HIV-1 control; (b) = $p < 0.05$ Vs HIV-1 acute stress; (&) = $p < 0.05$ Vs WT repeat stress.

Figure 4. Gene expression levels in the hippocampus

Changes in GR (A), FKBP5 (B), and FKBP4 (C) expression at control, immediately following acute stress, and following repeated stress exposures for wildtype (black) and transgenic (grey) animals in the HC. Gene expression in the HC is normalized to change of *hprt1*. Data shown represent mean fold change $2^{-\Delta\Delta CT} \pm \text{SEM}$ against WT control. Figure symbols: (*) = $p < 0.05$ Vs WT control; (#) = $p < 0.05$ Vs HIV-1 control; (a) = $p < 0.05$ Vs WT stress.