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# Glucocorticoid receptor translocation and expression of relevant genes in the hippocampus of adolescent and adult male rats

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ABSTRACT

We investigated whether pre-pubertal (postnatal day [P] 35) and post-pubertal adolescent (P45) and adult (P75) male rats differed in stressor-induced hormonal responses and in glucocorticoid receptor (GR) translocation because it has been proposed that negative feedback is maturing in adolescence and may be a basis for the prolonged activation of the HPA axis in adolescents compared with adults. The three age groups did not differ at baseline in plasma corticosterone or progesterone concentrations, and P35 had lower concentrations of testosterone than did both P45 and P75 rats, which did not differ. After 30 min of restraint stress, plasma concentrations of corticosterone and progesterone increased to a greater extent in the adolescents than in the adults. Whereas restraint stress increased concentrations of testosterone in adult males, concentrations decreased in adolescents. In all three age groups, restraint stress reduced GR expression in the cytosol and increased expression in the nucleus within the hippocampus, and the increase in nuclear GR was greater in pre-pubertal adolescents compared with adults. In a separate set of rats we investigated age differences in hippocampal mRNA expression of corticosteroid receptors (*MR* and *GR*) and of chaperones (*FKBP5, FKBP4, BAG-1*), which are known to modulate their activity, at baseline and after restraint stress. Restraint stress decreased the expression of *GR* and increased the expression of *FKBP5* mRNA, and age was not a significant factor. Higher expression of *FKBP4* mRNA was found at P35 than at P75. Most research of HPA function in adolescent rats has involved pre-pubertal rats; the present findings indicate that despite their increase in gonadal function, responses to stressors in P45 rats are more like those of pre-pubertal than adult rats. The greater stressor-induced GR translocation in pre-pubertal adolescents parallels their greater release of corticosterone in response to stressors, which may contribute to the enhanced sensitivity of adolescent rats to the effects of chronic stress exposures compared with adults.



## 1. Introduction

The release of glucocorticoids (primarily corticosterone in rodents) is regulated by the hypothalamic-pituitary-adrenal (HPA) axis, which help individuals to meet the challenges of their environment by modulating glucose metabolism, cardiovascular function, immune function, and psychological processes (Sapolsky et al., 2000; Groeneweg et al., 2011). Actions of glucocorticoids are mediated predominantly by two intracellular receptors (mineralocorticoid and glucocorticoid receptors; MR and GR), which act as transcriptional regulators and differ in their distribution in the brain and in their affinities for glucocorticoids; at basal concentrations, unlike MR, GR are mostly unoccupied (Reul and de Kloet, 1985). Therefore, GR are more sensitive to stressor-induced increases in glucocorticoid concentrations and are believed to mediate HPA feedback, whereby elevations in circulating glucocorticoid concentrations suppress further HPA activation, preventing over-exposure (Groeneweg et al., 2011; Herman et al., 2012).

Adolescence is a transitional stage of development that involves significant changes in the functioning of the HPA axis. In rodents, it is well-documented that pre-pubertal adolescents have greater and more protracted corticosterone responses to stressors (e.g., restraint, ether exposure, foot shock) compared with responses found in early life (P1–14 days of age) and in adulthood (>P60 days of age) (Klein and Romeo, 2013; Green and McCormick, 2016). The pronounced release of corticosterone in pre-pubertal adolescent rats may be related to ongoing maturation at any level of the HPA

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axis. For example, the adrenals of pre-pubertal adolescents weigh more than those of adults after normalizing to total body weight, release more corticosterone in response to stressors, and differ in their response to exogenous adrenocorticotropic hormone (ACTH) (Romeo et al., 2004a, 2005, 2014; Romeo, 2010; Foilb et al., 2011; Dziedzic et al., 2014; Hall and Romeo, 2014). Further, after exposure to a stressor, pre-pubertal rats have greater circulating concentrations of ACTH compared with adults (Romeo et al., 2006b; Foilb et al., 2011; Dziedzic et al., 2014; Hall and Romeo, 2014) and greater and/or more prolonged activation of the paraventricular nucleus (PVN) of the hypothalamus, as evidenced by increased expression of immediate early genes and their protein products (e.g., Fos and Egr-1) relative to adults (Viau et al., 2005; Lui et al., 2012; Hodges et al., 2014), particularly in cells that are immunoreactive for corticotropin releasing factor (CRF) (Romeo et al., 2006a). There are also differences in gene expression of CRF and arginine vasopressin (AVP) in the PVN of pre-pubertal and adult rats, although the findings are somewhat inconsistent (Viau et al., 2005; Romeo et al., 2007).

The exaggerated HPA reactivity that is evident in pre-pubertal males likely is unrelated to developmental changes in hypothalamic-pituitary-gonadal (HPG) function (onset of puberty occurs approximately at postnatal day [P] 34 in female rats and P42 in males) and in circulating gonadal hormones, which are known to affect the stress response in adults; estradiol increases and testosterone decreases HPA reactivity (Goel et al., 2014; Green and McCormick, 2016). Pre-pubertal males continued to show a greater and more prolonged release of ACTH and corticosterone compared with adults when males of both ages were gonadectomized and administered testosterone to mimic adult-typical concentrations (Romeo et al., 2004a). The difference between pre-pubertal and adult females also persisted when both age groups were ovariectomized, suggesting that age differences in HPA function are not mediated by changes in ovarian hormones (Romeo et al., 2004b). Lastly, although post-pubertal adolescents (>∼P42 and <P60) have not been investigated to the same extent as pre-pubertal adolescents, the available evidence suggests that HPA function is still maturing during this time and into late adolescence (∼P50–P60) (Foilb et al., 2011; Hodges and McCormick, 2015).

The exaggerated HPA reactivity and slower recovery in pre-pubertal males may be because HPA negative feedback is immature; administration of cortisol or the GR agonist dexamethasone was less effective at suppressing the hormonal response to stressors in pre-pubertal adolescents compared with in adults, which suggests ongoing maturation of HPA feedback during adolescence (Goldman et al., 1973; Vazquez and Akil, 1993). No differences were found, however, between adolescents and adults, in GR protein and gene expression in regions involved in HPA negative feedback (e.g., PVN, pituitary, hippocampus, and medial prefrontal cortex) in rodents (Vazquez, 1998; Romeo et al., 2008, 2013; Dziedzic et al., 2014). Nevertheless, there may be age differences in the affinity or activity of GR that cannot be seen by measuring overall expression. A related possibility is that changes during the adolescent period in the expression of co-chaperones involved in modulating GR activity are involved in age-differences in HPA function. For example, FK506 binding protein 51 (Fkbp51, encoded by the *FKBP5* gene) acts as a suppressor of GR activity by inhibiting the receptor's ability to bind its ligand and translocate to the nucleus to affect gene expression (Binder, 2009). BAG-1 is thought to inhibit GR activity by interfering with the receptor's assembly, by reducing receptor translocation, and by binding to the receptor's hinge region and impeding DNA binding (Schmidt et al., 2003). In contrast, Fkbp52 (encoded by the *FKBP4* gene) can be swapped in for Fkbp51, which promotes translocation into the nuclear compartment by recruiting the motor protein dynein (Davies et al., 2002). Furthermore many of the hormones that regulate chaperone expression (Hubler and Scammell, 2004; Binder, 2009; Malviya et al., 2013) increase in concentration during adolescence.

In Experiment 1 we investigated age and stressor effects on hormone concentrations (corticosterone, testosterone, and progesterone) and on GR translocation in the hippocampus, a region that richly expresses corticosteroid receptors and is involved in negative feedback (Reul and de Kloet, 1985; Herman and Cullinan, 1997). Based on previous findings, we predicted that pre-pubertal adolescent males would have greater corticosterone and progesterone release in response to an acute stressor than would adults (Romeo et al., 2005, 2006b). Because pre-pubertal rats are postulated to have dampened feedback relative to adults (Goldman et al., 1973; Vazquez and Akil, 1993), we also tested the hypothesis that prolonged corticosterone release in pre-pubertal adolescent rats involved reduced GR translocation from the cytoplasm to the nucleus after restraint stress. In Experiment 2 we investigated developmental and stressor-induced changes in the expression of genes that code for corticosteroid receptors and their chaperone proteins within the hippocampus (i.e., *NR3C1, NR3C2, FKBP5, FKBP4, BAG-1*). In addition to investigating pre-pubertal adolescents (P35) and adults (P75), we investigated post-pubertal adolescents (P45) to gain insight into an age group for which less is known about HPA function to better understand the transition into adulthood.

## 2. Methods

### 2.1. Experiment 1

#### 2.1.1. Animals

For Experiment 1a, male Long-Evans rats were obtained at P30 ($n = 32$; 80–115 g), P40 ($n = 32$; 180–220 grams), and P70 ($n = 32$; 300–385 g) from Charles River (St. Constant, Quebec) and housed in same-aged pairs. For Experiment 1b, a second batch of males arrived at P30 ($n = 16$; 100–130 g), P40 ($n = 16$; 175–215 g), and P70 ($n = 16$; 300–355 g). Animals were given free access to food and water and kept on a 12 h light-dark cycle (lights on at 09:00). All procedures were approved by the Brock University Animal Care and Use Committee and were in keeping with the National Institute of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) as well as the Canadian Council on Animal Care guidelines.

#### 2.1.2. Acute stressor procedure and sample collection

The acute stressor procedure and sample collection began four to six days after the animals arrived at the facility, and thus were (±1 day) P35, P45, or P75 at collection. On each collection day, all age groups and time-points were represented. Within ∼2–5 h after lights on, rats were decapitated either directly from the home cage (baseline) or immediately after 30 min of restraint stress (post-restraint) in Plexiglas® restrainers (Experiment 1a), or after 30 min of recovery after the restraint stress (Experiment 1b), for the collection of trunk blood and brains. This range in time of day was chosen for the experiments so that samples were collected during the phase of the light cycle when basal plasma corticosterone concentrations and the percentage of bound GR are low; these conditions would better allow investigation of the effects of stress on GR translocation. Trunk blood was collected into ice-chilled glass tubes containing EDTA and centrifuged at 3000 RCF for 15 min. Plasma was collected and stored at −20° C until hormone assays were conducted. Brains were quickly extracted, sliced into 1 mm thick sections on ice, and frozen on dry ice before stored at −80° C until further processed.