Review
Changes in hypothalamic–pituitary–adrenal stress responsiveness before and after puberty in rats
Zoe A. Klein1, Russell D. Romeo*
Department of Psychology and Neuroscience and Behavior Program, Barnard College of Columbia University, New York, NY 10027, USA

ABSTRACT
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Many endocrine changes are associated with pubertal and adolescent development. One such change is the responsiveness of the hypothalamic–pituitary–adrenal (HPA) axis to physical and/or psychological stressors. Recent human and non-human animal studies have shown that hormonal stress reactivity increases significantly throughout puberty and adolescence. Specifically, exposure to various stressors results in greater adrenocorticotrophic hormone (ACTH) and glucocorticoid responses in peripubertal compared to adult animals. This review will focus on how stress reactivity changes throughout puberty and adolescence, as well as potential mechanisms that mediate these changes in stress responsiveness. Though the implications of these pubertal shifts in stress responsiveness are not fully understood, the significant increase in stress-related mental and physical dysfunctions during this stage of development highlights the importance of studying pubertal and adolescent maturation of HPA function and its reactivity to stress.

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Introduction
Pubertal and adolescent stages of development mark the transition from a state of dependence on caregivers to a state of independence and reproductive competency (Spear, 2010). Though puberty and adolescence are often associated with developmental gains in numerous physiological and cognitive domains, these periods of maturation can also be accompanied by the onset of various psychological and physiological disorders, such as anxiety, depression, schizophrenia, and drug abuse (Andersen, 2003; Conger and Petersen, 1984; Costello et al., 2003; Patton and Viner, 2007; Spear, 2000). It is unclear what factors mediate the increase in these dysfunctions, but stress exposure during puberty and adolescence has been postulated to contribute to these vulnerabilities in both human and non-human animals (Dahl and Gunnar, 2009; Romeo and McEwen, 2006; Turner and Lloyd, 2004). Interestingly, substantial changes in stress reactivity have been noted to occur during this period of development (Dahl and Gunnar, 2009; McCormick et al., 2010; Romeo, 2010b), and thus may contribute to these stress-related vulnerabilities. The present review will discuss some of the ways in which pubertal and adolescent development shapes hormonal stress reactivity and potential mechanisms that may modulate these changes in stress responsiveness.

* Corresponding author. Fax: +1 212 854 3601.
E-mail address: rromeo@barnard.edu (R.D. Romeo).
1 Present address: Yale University School of Medicine, Program in Neuroscience, Neurodegeneration, and Repair, New Haven, CT 06536, USA.
The hormonal stress response and the hypothalamic–pituitary–adrenal axis

The hypothalamic–pituitary–adrenal (HPA) axis is the primary neuroendocrine axis responsible for mediating the hormonal response to a physiological and/or psychological stressor (Herman et al., 2003; Sapolsky et al., 2000). The response is initiated by a group of cells in the paraventricular nucleus of the hypothalamus (PVN) that secrete corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP), which in turn stimulate the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary. ACTH then leads to the release of the glucocorticoids (cortisol in primates and corticosterone in many rodent species) from the adrenal cortex (Fig. 1). The HPA axis is controlled through a negative feedback loop such that the glucocorticoids acting mainly through glucocorticoid receptors in the anterior pituitary, PVN, and other extra-hypothalamic brain regions (e.g., hippocampus, prefrontal cortex), reduce CRH, AVP, and ACTH production, thus decreasing further glucocorticoid secretion (Herman et al., 2003; Fig. 1).

In the short term, glucocorticoid release enables the organism to cope with the demands imposed by the stressful event, and in part, contributes to the organism’s ability to return to homeostasis. However, if this response is chronically engaged, or not terminated in a timely fashion, long term exposure to the glucocorticoids can damage many organ systems, including the nervous, reproductive, and immune systems (McEwen, 2007). Thus, identifying factors that modulate HPA reactivity can have important implications for understanding an organism’s physiological and neurobehavioral function in response to acute or chronic stress. Though many factors influence HPA reactivity and the dynamics of the hormonal stress response, such as sex (Becker et al., 2007), time of day (Lightman and Conway-Campbell, 2010), and previous experiences (Grissom and Bhatnagar, 2009), we will focus on the role that development plays, particularly that of pubertal and adolescent development.

Pubertal- and adolescent-related changes in hypothalamic–pituitary–adrenal reactivity

During pubertal and adolescent stages of maturation, the types of stressors encountered by an organism, and the frequency at which those stressors occur, change dramatically (Gest et al., 1999). In addition, an organism’s hormonal response to stress also changes significantly during this time. For instance, a number of recent studies have shown that stress-induced HPA axis reactivity is higher during puberty and adolescence compared to neonatal or adult stages of development (Dahl and Gunnar, 2009; McCormick and Mathews, 2007; McCormick et al., 2010; Romeo, 2010a,b). Specifically, studies in rats indicate that prepubertal males and females exposed to an acute stressor such as restraint (Romeo et al., 2004a,b, 2006b), ether inhalation (Vazquez and Akil, 1993), or foot shock (Goldman et al., 1973) demonstrate protracted hormonal responses compared to adults (Fig. 2). It should be noted that plasma corticotropin-binding globulin (CBG) levels are similar in juvenile and adult males (Romeo et al., 2006a), and thus these prolonged corticosterone responses prior to puberty do not appear to be buffered by increased CBG levels in prepubertal animals. Taken together, these studies suggest that upon termination of the stressor, prepubertal animals are exposed to bioavailable corticosterone for a longer duration than adults.

The physiological implications of this extended hormonal stress response prior to puberty are unclear, but the important role of corticosterone in energy mobilization (Pecoraro et al., 2006; Sapolsky et al., 2000) may point to different metabolic demands in prepubertal and adult animals during stress exposure. In support of this notion, prepubertal male rats do show higher post-stress glucose levels than adults (Romeo et al., 2007a). However, the exact contribution of these age-specific hormonal responses to metabolism and energetics is currently unknown. Though beyond the scope of the present review, it is also important to call attention to the potential behavioral consequences of these pubertal changes in hormonal stress reactivity, ranging from effects on cognition, drug use, and emotionality (reviewed in; McCormick et al., 2010).

Given these rather significant stress-induced changes in HPA reactivity following an acute stressor in animals tested before and after pubertal maturation, we conducted a study to determine whether the changes in stress responsiveness are gradual or relatively more abrupt during the course of puberty and adolescence. We found that levels of both ACTH and corticosterone following acute stress show rather abrupt changes to their adult-like response patterns (Foibl et al., 2011). Specifically, the
ACTH response shifts between 50 and 60 days of age (Fig. 3A), while the change in the corticosterone response occurs between 30 and 40 days of age (Foilb et al., 2011; Fig. 3B). These data indicate that shifts in hormonal stress responsiveness occur throughout adolescent maturation and that each hormone shows a distinct developmental profile. Moreover, the dissociation between ACTH and corticosterone levels, particularly in late adolescent and early adult stages, suggests that there may be age-dependent changes in adrenocortical sensitivity to ACTH. Experiments are currently investigating this possibility.

Though the studies mentioned above indicate robust age-related changes in stress reactivity, it is still unclear what factors mediate these changes. One study reported that prepubertal male rats show less glucocorticoid-dependent negative feedback than adults following a stressor (Goldman et al., 1973), which may account for the slower return to baseline hormone levels following the termination of the stressor. Despite these potential differences in negative feedback, little or no change in the level of glucocorticoid receptors in the neural-pituitary network responsible for negative feedback has been found in either rats or mice before and after puberty (Romeo et al., 2008, in press; Vazquez, 1998). Others have observed higher levels of neural activation, as indexed by FOS expression, in the PVN of prepubertal compared to adult male rats (Lui et al., 2012; Romeo et al., 2006a; Viau et al., 2005), suggesting greater hypothalamic drive to the pituitary prior to puberty. Moreover, basal expression of CRH in the PVN appears to be higher in male rats prior to puberty (Romeo et al., 2007b), though the opposite trend has been noted in females (Viau et al., 2005). Thus, the protracted stress-induced hormonal response in prepubertal males may be due to a combination of less negative feedback on, and more positive activation of, the HPA axis. It is also possible that there are age-dependent shifts in the sensitivity of the pituitary and/or adrenal glands to CRH, AVP, and ACTH, as well as changes in the ability of the pituitary and adrenal glands to synthesize ACTH and corticosterone, respectively (Foilb et al., 2011). Taken together, it appears that the mechanisms that mediate the pubertal- and adolescent-related changes in stress reactivity involve each node along the HPA axis and may differ between the sexes.

**The influence of the hypothalamic–pituitary–gonadal axis on the hypothalamic–pituitary–adrenal axis**

In addition to pubertal-related changes in HPA axis physiology, substantial alterations in many other neuroendocrine axes are observed during this developmental stage. Perhaps the most conspicuous and well-studied change in endocrine function during this time is the major increase in the production and secretion of the hormones regulated by the hypothalamic–pituitary–gonadal (HPG) axis, namely testosterone, estradiol, and progesterone (Ojeda and Terasawa, 2002). Interestingly, these HPG-related hormones are known to have significant influences on the responsiveness of the HPA axis to stress in adult males and females (Viau, 2002; Williamson et al., 2005). For instance, testosterone tends to reduce stress reactivity in males, such that castrated males show greater stress-induced hormonal responsiveness than intact or testosterone-treated males (Handa et al., 1994; McCormick et al., 2002; Viau and Meaney, 1996). The opposite effect is observed with estradiol and progesterone in females in that ovariectomized females display less stress responsiveness compared to females treated with exogenous estradiol and progesterone (Carey et al., 1985; McCormick et al., 2002; Redeil et al., 1994). Furthermore, females in the estrus/diestrus stage of their estrous cycle (i.e., when estrogen and progesterone levels are relatively low) show lower levels of stress reactivity than females in proestrus (i.e., when estrogen and progesterone levels are relatively high; Viau and Meaney, 1991).

Given that gonadal hormone levels are substantially different in prepubertal and adult animals, we conducted experiments in both male and female rats to determine whether the pubertal change in gonadal hormones was at least in part responsible for the shifts in stress reactivity before and after puberty. Surprisingly, we found that in the presence of adult-like testosterone levels prepubertal males continued to show protracted stress-induced ACTH and corticosterone responses compared to adults (Romeo et al., 2004a; Fig. 4). The pubertal-related change in stress reactivity also persisted in ovariectomized prepubertal and adult females (Romeo et al., 2004b), such that in the absence of ovarian hormones, prepubertal females display significantly protracted stress-induced corticosterone responses compared to adults (Fig. 5). Thus, differences in gonadal hormone levels experienced by animals before and after puberty do not appear to account for the significant developmental shifts in stress responsiveness. It should be noted, however, that testosterone has been implicated in the changes in hormonal reactivity of mid-pubertal males (i.e., 40 days of age; Gomez et al., 2004), suggesting that after pubertal onset gonadal hormones may play a role in these age-related differences in stress responsiveness. The role of ovarian hormones on stress reactivity of mid-pubertal females has yet to be explored.

Though it appears that gonadal hormones have little influence on the acute activation of the HPA axis prepubertally, the possibility remains that exposure to the pubertal rise in gonadal hormones to some extent organizes the function of the HPA axis. It is important to note that puberty, in addition to the perinatal period, can serve as a period of hormone-dependent organization of the central nervous system (Romeo, 2003; Schulz et al., 2009). Moreover, perinatal exposure to gonadal hormones has been shown to organize adult sex differences in stress reactivity (McCormick et al., 1998), indicating that the HPA axis is sensitive to gonadal hormone-dependent organization during critical periods of development.

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**Fig. 3.** Mean (± SEM) plasma adrenocorticotropic hormone (ACTH, A) and corticosterone (B) concentrations in 30, 40, 50, 60, and 70 day old male rats before, during, and after a 30 min session of restraint (black bar under x-axis). Asterisks in panel A indicate a significant difference from the 60 and 70 day old animals at that time point, while in panel B asterisks indicate that 30 day old animals are significantly different from all other ages at that time point. Adapted from Foilb et al. (2011).
In order to investigate these questions further, we performed a preliminary set of experiments to determine whether the pubertal rise in gonadal hormones further organizes the HPA axis, resulting in the pattern of hormonal stress responsiveness observed in adulthood. Using a between-subjects experimental design, we assessed hormonal stress reactivity in intact prepubertal and adult males as well as males castrated or sham-castrated prior to or after puberty and sampled later in adulthood. All animals were exposed to a 30 min session of restraint stress and blood samples were taken before, during, or after the stress session. Plasma corticosterone levels were measured via radioimmunoassay. As expected, we found the prolonged corticosterone response in intact prepubertal compared to adult males (Fig. 6A). More importantly, however, we found that animals that were castrated prior to puberty and sampled later in early adulthood had a similar corticosterone recovery pattern as the sham-castrated animals (Fig. 6B). Thus, the rate at which the hormonal stress response returns to baseline following termination of restraint appears to be more dependent on chronological age than experiencing the pubertal rise in gonadal hormones. We did find that animals castrated prior to puberty showed a significantly lower stress-induced peak corticosterone response compared to their sham-castrated counterparts. This is opposite to the pattern observed in animals castrated after puberty, in which castrated adults displayed slightly, but significantly, higher corticosterone levels than intact adults sampled immediately after the stressor was terminated (Fig. 6C). These results in the animals manipulated in adulthood are consistent with previously published reports on androgens dampening the hormonal stress response of adult males (Fig. 6A) which would cause any transient rise in testosterone following stress exposure (Amario and Castellanos, 1984; Foilb et al., 2011; Romeo et al., 2004a; Siegel et al., 1981). Interestingly, even though prepubertal males have relatively low baseline levels of testosterone, exposure to acute stressors actually reduces these low levels of testosterone even further (Foilb et al., 2011; Romeo et al., 2004a). This reverse pattern of stress-induced testosterone secretion in prepubertal and adult animals may be due to the heightened testosterone-mediated negative feedback on the prepubertal HPG axis (Negró-Vilar et al., 1973), which would cause any transient rise in testosterone levels following stress in younger animals to be rapidly inhibited.

In adult females, acute stress has been shown to elevate estradiol and progesterone levels both transiently (Romeo et al., 2004b; Shors et al., 1999) and for up to 24 h after the stressor has been terminated (Shors et al., 1999). However, unlike the significant stress-induced decrease in testosterone secretion in prepubertal males, prepubertal females show little change in ovarian estradiol or progesterone following restraint stress. In contrast, the HPG axis in adult females shows a significant surge in estradiol and progesterone levels following stress exposure (Amario and Castellanos, 1984; Foilb et al., 2011; Romeo et al., 2004a; Siegel et al., 1981). Interestingly, even though prepubertal males have relatively low baseline levels of testosterone, exposure to acute stressors actually reduces these low levels of testosterone even further (Foilb et al., 2011; Romeo et al., 2004a). This reverse pattern of stress-induced testosterone secretion in prepubertal and adult animals may be due to the heightened testosterone-mediated negative feedback on the prepubertal HPG axis (Negró-Vilar et al., 1973), which would cause any transient rise in testosterone levels following stress in younger animals to be rapidly inhibited.

As the section above indicates, the HPG axis has significant effects on stress responsiveness. However, it is important to note that this relationship is bi-directional in that stress can significantly influence the production and secretion of gonadal hormones. Importantly, these effects of stress on HPG reactivity show a certain degree of dependence on pubertal development.

In the context of acute stress, adult males demonstrate a significant surge in testosterone levels following stress exposure (Amario and Castellanos, 1984; Foilb et al., 2011; Romeo et al., 2004a; Siegel et al., 1981). Interestingly, even though prepubertal males have relatively low baseline levels of testosterone, exposure to acute stressors actually reduces these low levels of testosterone even further (Foilb et al., 2011; Romeo et al., 2004a). This reverse pattern of stress-induced testosterone secretion in prepubertal and adult animals may be due to the heightened testosterone-mediated negative feedback on the prepubertal HPG axis (Negró-Vilar et al., 1973), which would cause any transient rise in testosterone levels following stress in younger animals to be rapidly inhibited.

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Pubertal- and experience-dependent changes in HPA reactivity

Along with age, another potent modulator of HPA reactivity is previous experience with stressors. In adults, for instance, exposure to the same stressor (i.e., homotypic stress) can result in a habituated hormonal response compared to adults experiencing that stressor for the first time (Bhatnagar et al., 2002; Doremus-Fitzwater et al., 2009; Fernandes et al., 2002; Girotti et al., 2006; Harris et al., 2004; Helmreich et al., 1997; Magarinos and McEwen, 1995; Marti and Armario, 1997; Romeo et al., 2006a). Conversely, if an adult experiences homotypic stress and is then confronted with a novel stressor (i.e., heterotypic stress), it will often exhibit a sensitized hormonal response compared to an animal experiencing that novel stressor alone (Bhatnagar and Dallman, 1998; Fernandes et al., 2002; Ma et al., 1999). Importantly, the ways in which experience modifies hormonal stress responsiveness are dependent on the pubertal development of the animal.

In a recent study, we exposed prepubertal and adult male rats to acute stress (restraint), homotypic stress (repeated restraint) or heterotypic stress (repeated cold exposure followed by restraint) and assessed their hormonal reactivity. We found that in response to both acute and heterotypic stresses, prepubertal males exhibit prolonged corticosterone responses compared to adults (Lui et al., 2012). We also found that while adults habituate to homotypic stress, the opposite response was observed in animals prior to puberty. Specifically, prepubertal male rats had higher ACTH and corticosterone responses after homotypic stress than adults (Doremus-Fitzwater et al., 2009; Lui et al., 2012; Romeo et al., 2006a). Remarkably, prepubertal female rats show similar habituated corticosterone responses to a homotypic stressor as adult females (Doremus-Fitzwater et al., 2009), suggesting possible sex differences in experience-induced changes in HPA reactivity prior to puberty. Though the mechanisms that mediate these age- and sex-dependent changes are currently unknown, these data clearly indicate that pubertal development and previous stress experience significantly interact to shape HPA reactivity, and perhaps do so in a sex specific manner. Future research will need to clarify the role, if any, that gonadal hormones may play in this experience-dependent plasticity and whether these unique changes in HPA responsiveness in prepubertal animals endure into adulthood.

Conclusions

In summary, the data reviewed above indicates that there are substantial prepubertal- and adolescent-related changes in the stress reactivity of the HPA axis. Specific aspects of these changes appear to be dependent on the organism’s current or past gonadal hormone milieu, chronological age, and experience with stress. Though we are still far from a complete understanding of how and why these changes occur, the stress-related vulnerabilities often associated with puberty and adolescence highlight the importance of studying HPA maturation during these crucial stages of development.

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