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Adolescent and adult male rats habituate to repeated isolation, but only adolescents sensitize to partner unfamiliarity

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ABSTRACT

We investigated whether adolescent male rats show less habituation of corticosterone release than adult male rats to acute vs repeated (16) daily one hour episodes of isolation stress, as well as the role of partner familiarity during recovery on social behavior, plasma corticosterone, and Zif268 expression in brain regions. Adolescents spent more time in social contact than did adults during the initial days of the repeated stress procedures, but both adolescents and adults that returned to an unfamiliar peer after isolation had higher social activity than rats returned to a familiar peer ($p = 0.002$) or undisturbed control rats ($p < 0.001$). Both ages showed evidence of habituation, with reduced corticosterone response to repeated than acute isolation ($p = 0.01$). Adolescents, however, showed sensitized corticosterone release to repeated compared with an acute pairing with an unfamiliar peer during recovery ($p = 0.03$), a difference not found in adults. Consistent with habituation of corticosterone release, the repeated isolation groups had lower Zif268 immunoreactive cell counts in the paraventricular nucleus ($p < 0.001$) and in the arcuate nucleus ($p = 0.002$) than did the acute groups, and adolescents had higher Zif268 immunoreactive cell counts in the paraventricular nucleus than did adults during the recovery period ($p < 0.001$), irrespective of stress history and partner familiarity. Partner familiarity had only modest effects on Zif268 immunoreactivity, and experimental effects on plasma testosterone concentrations were only in adults. The results highlight social and endocrine factors that may underlie the greater vulnerability of the adolescent period of development.

Introduction

The hypothalamic–pituitary–adrenal (HPA) axis is a means by which an organism coordinates its response to stressors, both psychological and physical. When a stressor is perceived or anticipated, the paraventricular nucleus of the hypothalamus initiates the release of secretagogues that in turn lead to the release of ACTH from the pituitary. ACTH then increases the release of glucocorticoids (primarily corticosterone in rodents) by acting on the adrenal cortex (Herman et al., 2012). Glucocorticoid release helps an organism adapt to, and recover from, the effects of a stressor through influences on the metabolic, cardiovascular, immune, and neurological systems (De Bosscher et al., 2008; McEwen et al., 2012). Nevertheless, prolonged exposure to glucocorticoids in response to repeated (or chronic) stressors may alter endocrine and neural functioning and impair an organism's responses to future stressors (reviewed in Lupien et al., 2009). The ability to recover from the effects of chronic stressors, however, depends on the stage of development of an organism (Eiland and Romeo, 2013; Hollis et al., 2013).

There is growing evidence of a differential susceptibility of adolescents compared with adults to the negative consequences of stressors (reviewed in Green and McCormick, 2013; McCormick and Green, 2013). For example, a reduction in hippocampal volume and deficits in spatial memory were found in adolescent rats three weeks after exposures to chronic stress (Isgor et al., 2004). In contrast, effects of chronic stress exposures in adulthood on spatial memory and dendritic arborisation in the adult hippocampus dissipated within days of termination of the stress (Conrad et al., 1999; Sousa et al., 2000). Further, the pattern of the effects of stressors sometimes differs for the two age groups, as recently found in the basolateral amygdala after exposure to chronic social instability in adolescent and adult rats (Tsai et al., 2014). Developmental maturation of HPA function and of the central nervous system both have been proposed as bases for the age differences in the consequences of exposure to stressors.

Several studies have reported that adolescent rats have a more prolonged release of corticosterone than do adult rats in response to acute stressors such as intermittent foot shock (Goldman et al., 1973), ether inhalation (Vázquez and Akil, 1993), two hour exposure to a novel environment (Novak et al., 2007), and restraint (Bingham et al.,

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2011; Cruz et al., 2008; Dziedzic et al., 2014; Foilb et al., 2011; Gomez et al., 2002; Hall and Romeo, 2014; Lui et al., 2012; Romeo et al., 2004a, 2004b, 2006a, 2006b, 2014; Vázquez and Akil, 1993). In contrast, other studies have reported higher or more prolonged release of corticosterone to acute stressors in adult rats than in adolescent rats (e.g. nicotine, Cao et al., 2010; lipopolysaccharide, Goble et al., 2011; confinement to an elevated platform, McCormick et al., 2008; injection of ethanol, Willey et al., 2012), and others have found no differences between adolescent and adult rats in corticosterone release in response to an acute stressor (novelty stress, Goldman et al., 1973; one hour isolation, Hodges et al., 2014; forced swim, Mathews et al., 2008; tetrahydrocannabinol, Schramm-Sapyta et al., 2007). The basis for differential HPA function between adolescents and adults in response to acute stressors remains unknown, although the type of stressor is likely an important factor.

Adolescents and adults do not differ in glucocorticoid receptor (GR) or mineralocorticoid receptor binding capacities (Vázquez, 1998; Vázquez and Akil, 1993), in GR mRNA expression (Romeo et al., 2008), or in GR protein immunoreactive cell counts (Dziedzic et al., 2014) in brain regions that regulate the stress response (e.g., the hippocampus, medial prefrontal cortex, paraventricular nucleus). Although earlier studies found no difference between adolescents and adults in sensitivity of the adrenal gland to ACTH (Goldman et al., 1973; Vázquez and Akil, 1993), a recent study suggests that adrenal sensitivity to ACTH may be greater in adolescent than in adult rats after acute restraint, and that the heightened sensitivity of adolescents may involve a greater expression of adrenal melanocortin 2 receptor accessory protein (Mrap) mRNA in adolescent than in adult rats (Romeo et al., 2014). In addition, several studies have reported age differences in the brain regions activated by stressors. For example, pre-pubertal adolescents had greater Fos expression (an index of neuronal activation) in the paraventricular nucleus than did adults after acute 30 min of restraint (Lui et al., 2012; Romeo et al., 2006a; Viau et al., 2005). Moreover, rats in early adolescence (postnatal day 28) had greater Fos expression than did adults after 2 h in a novel environment in several brain regions (paraventricular nucleus of the hypothalamus, medial amygdala, cingulate gyrus, lateral septum, paraventricular nucleus of the thalamus, and centromedial nucleus of the thalamus) (Novak et al., 2007). Other studies, however, found no difference between adolescent and adult rats in Fos expression in the paraventricular nucleus after either 15 min or 2 h of restraint (Kellogg et al., 1998). Using a different marker of neural activation, Zif268 (protein product of the immediate-early gene zif268), higher immunoreactive cell counts were found in the paraventricular nucleus of the hypothalamus during recovery from 1 h of isolation stress in adolescents compared with adults (Hodges et al., 2014). Thus, a basis for differences between adolescents and adults in HPA function may involve extra-hypothalamic neural regions that continue to mature in adolescence (reviewed in Spear, 2000).

Fewer studies have investigated HPA function in response to repeated stressors in adolescents compared with adults. Adolescent rats had a higher concentration of corticosterone compared with adult rats in response to a seventh exposure to 30 min of restraint, although no difference was found after the first restraint session (Lui et al., 2012; Romeo et al., 2006a). Similar age differences (adolescent > adults after repeated stress) were found in response to five exposures to cat odor (Wright et al., 2012) or to five days of restraint stress (Doremus-Fitzwater et al., 2009). In addition, studies have also reported greater Fos expression in the paraventricular nucleus after the seventh exposure to 30 min of restraint in adolescent rats than in adult rats (Lui et al., 2012; Romeo et al., 2006a). These results suggest that the HPA response of adolescents may be differentially affected by repeated stressors compared with that of adults.

Stressors that involve social manipulations may be particularly relevant for age differences in HPA function, considering the developmental stage variation in social behavior. In both humans and rodents, adolescence is a period of increased novelty-seeking and increased social interactions with peers compared to adulthood (reviewed in Spear, 2000). For example, whereas adolescent rats initiated more play behaviors with conspecifics, adult rats displayed less social play (Klein et al., 2010) and more agonistic behaviors (Meaney and Stewart, 1981) towards conspecifics. Moreover, socially-deprived adolescent rats engaged in more social interactions (Varlinskaya and Spear, 2008) and had a greater preference for the side of a cage that was previously paired with a conspecific (Douglas et al., 2004) than did similarly socially-deprived adults. In addition, our repeated social instability stress procedure (SS: 16 days of daily repeated isolation for 1 h and return to an unfamiliar cage partner) produced immediate and long-lasting decrements in memory of conditioned fear (Morrissey et al., 2011) and altered behavioral responses to psychostimulants (McCormick et al., 2005) when experienced in adolescence, but not when experienced in adulthood, which attests to the heightened vulnerability of the adolescent period.

We have investigated some aspects of HPA function in adolescent rats exposed to the social instability stress (SS) procedure, but we have not compared adolescent exposure with adult exposure. We found that, irrespective of whether returned repeatedly to unfamiliar or familiar cage partners after daily isolations, there was evidence of habituation to repeated isolation; corticosterone concentrations were lower on postnatal day 45 (mid-adolescence) immediately after the 16th isolation compared with age-matched controls undergoing a first isolation (McCormick et al., 2007). Further, those that had been repeatedly paired with unfamiliar cage partners after isolation showed a significant increase in CRH mRNA in the central nucleus of the amygdala after one hour isolation as did those undergoing a first isolation, whereas those always recovering from repeated isolation with a familiar partner did not (McCormick et al., 2007). The recovery phase was not investigated after the 16th episode of isolation, however, which might uncover additional effects of partner (un)familiarity. When we compared adolescents and adults to an acute isolation, however, the two ages did not differ in corticosterone concentrations after isolation, and both ages had higher corticosterone concentrations when paired with an unfamiliar partner during the recovery period than when with a familiar partner (Hodges et al., 2014). Nevertheless, age differences might emerge under conditions of repeated stress exposures.

Here we directly compare HPA function and neural activations (as measured by Zif268-immunoreactivity) in response to, and in recovery from, repeated (versus acute) isolation stress, as well as the influence of partner familiarity (social stability vs unfamiliarity/social instability) in both adolescents and adults. In addition to measuring plasma corticosterone concentrations, we also investigated changes in testosterone concentrations, which have been reported to increase in response to various stressors (e.g., reviewed in Chichinadze and Chichinadze, 2008; Foilb et al., 2011) and in response to social interactions (reviewed in Gleason et al., 2009). Lastly, we also monitored behavior in the home cage during recovery from isolation in both the early and in the later phases of the 16 day procedure to better characterize effects of the SS procedure at both ages. We predicted that partner familiarity would be a greater factor in adolescents than in adults across all measures, and that adults may show greater habituation to the repeated stress exposures than would adolescents.

Methods

Animals

Male Long–Evans rats (N = 144) were obtained from Charles River, St. Constant, Quebec, at 22 days of age. Rats were housed in pairs and maintained under a 12 h light–dark cycle (lights on at 08:00 h) with food and water available ad libitum. Use of animals in this experiment was approved by the Brock University Institutional Animal Care Committee (ACC) and was carried out in adherence to the Canadian Council of Animal Care guidelines.