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Differential neuroendocrine responses to chronic variable stress in adult Long Evans rats exposed to handling-maternal separation as neonates

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Summary Burgeoning evidence supports a preeminent role for early- and late-life stressors in the development of physio- and psychopathology. Handling-maternal separation (HMS) in neonatal Long Evans hooded rats leads to stable phenotypes ranging from resilient to vulnerable to later stressor exposure. Handling with 180 min of maternal separation yields a phenotype of stress hyper-responsiveness associated with facilitation of regional CRF neurocircuits and glucocorticoid resistance. This study assessed whether or not prolonged HMS (180 min/day, HMS180) on post-natal days 2–14 sensitizes the adult limbic hypothalamo–pituitary–adrenal (LHPA) axis to chronic variable stress (CS) compared to brief HMS (15 min/day, HMS15). We examined regional mRNA densities of corticotropin-releasing factor (CRF), its receptor CRF1, glucocorticoid receptor (GR), and mineralocorticoid receptor (MR); regional CRF1 and CRF2α binding, and pituitary–adrenal responses to an acute air-puff startle (APS) stressor in four groups: HMS15, nonstressed; HMS15, stressed; HMS180, nonstressed; HMS180, stressed. As expected we observed exaggerated pituitary–adrenal responses to APS, increased regional CRF mRNA density, decreased regional CRF1 binding, and decreased cortical GR mRNA density in nonstressed HMS180 vs. HMS15 animals. However, in contrast to our hypothesis, CS decreased pituitary–adrenal reactivity and central amygdala CRF mRNA density in HMS180 rats, while increasing cortical GR mRNA density and CRF1 binding. CS had no effect on the pituitary–adrenal response to APS in HMS15 rats, despite tripling hypothalamic paraventricular CRF mRNA density. The data suggest that many effects of prolonged HMS are reversible in adulthood by CS, while the neuroendocrine adaptations imbued by brief HMS are sufficiently stable to restrain pituitary–adrenal stress responses even following CS.

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1. Introduction

Throughout life the continuous interaction of genetic and environmental factors shapes the neurocircuitry of the mammalian central nervous system, giving rise to individual differences in stress responsiveness that may become adaptive or maladaptive over time depending on circumstances. Recent modifications of the stress diathesis theory of depression suggest that adverse experiences early in life may sensitize specific neurocircuits to subsequent acute stressors, thereby increasing individual vulnerability to the onset of physio- and psycho-pathology (Brown et al., 1987; Anisman et al., 1998; Heim and Nemeroff, 2001). We proposed that one such neurocircuit is that containing corticotropin-releasing factor (CRF), a 41-amino acid peptide which coordinates the mammalian endocrine, behavioral, autonomic, and immunological responses to stress via the HPA axis and reciprocal limbic-brainstem neurocircuits (Owens and Nemeroff, 1991; Herman et al., 1996; Herman and Cullinan, 1997; Arborelius et al., 1999; van de Kar and Blair, 1999). Regional expression of CRF and its receptors CRF1 and CRF2α is sensitive to external stressors (Imaki et al., 1991; Lightman and Harbuz, 1993; Gomez et al., 1996; Albeck et al., 1997; Brown and Sawchenko, 1997; Bonaz and Rivest, 1998; Stout et al., 2000), including those encountered in early life (Eghbal-Ahmadi et al., 1997, 1998, 1999).

To explore the long-term consequences of early adverse experience on individual adult stress responsiveness, we have developed a rodent model of moderate handling-maternal separation (180 min/day; HMS180) during post-natal days (PND) 2–14 and have compared these animals to briefly handled (15 min/day, HMS15) rats that we consider an appropriate handling and cage-transfer comparison group (Ladd et al., 2000). As adults, these two rearing groups exhibit distinct and stable individual phenotypes in stress responsiveness (Sanchez et al., 2001). Compared to HMS15 animals, the HMS180 group exhibits increased basal tone of central CRFergic activity as well as sensitization of the HPA axis (Plotsky and Meaney, 1993; Ladd et al., 2000; Sanchez et al., 2001), which may be secondary to facilitation of ascending noradrenergic neurocircuits (Liu et al., 2000), dampened regional GABAergic tone (Caldji et al., 2000), and/or focal glucocorticoid resistance (Ladd et al., 2000).

While several investigators have reported the effects of chronic stress on CRF neurocircuits (Imaki et al., 1991; Lightman and Harbuz, 1993; Gomez et al., 1996; Albeck et al., 1997; Brown and Sawchenko, 1997; Bonaz and Rivest, 1998; Stout et al., 2000, 2002) and regional corticosteroid receptor expression (Herman and Spencer, 1998; Lopez et al., 1998; Kim et al., 1999; Paskitti et al., 2000) in normal adult rats, little is known about the influence of rearing conditions on individual adult responses to chronic stress. Given the propensity for stress-related neurocircuits to be sensitized by perceived insults (Plotsky and Meaney, 1993; Heim et al., 1997; Bruijnzeel et al., 1999; Ladd et al., 2000; Heim and Nemeroff, 2001; Weaver et al., 2004), we evaluated the hypothesis that early adverse experience sensitizes CRF and glucocorticoid responsive neurocircuits toward later stressors by examining CRF, glucocorticoid receptor (GR), mineralocorticoid receptor (MR), and CRF receptor expression in maternally separated rats exposed to chronic variable stress (CS).

2. Materials and methods

2.1. Animals

Animal studies were approved by the Emory University Institutional Care and Use Committee and conformed to the NIH Guidelines for the Care and Use of Laboratory Animals. Timed-pregnant Long Evans hooded rats arrived at the Emory University vivarium on gestation day 11–12 from Charles Rivier (Portage, MI). All dams were housed in transparent, polypropylene cages (20×25 cm) with 2 cm wood shavings and ad libitum access to food (Purina Lab Chow) and water in a room on a 12 h:12 h light:dark cycle (lights on at 07:00 h). Day of birth marked post-natal day (PND) 0. On PND 2, all pups were removed from their home cages, randomized, and culled to eight male pups per dam. Each litter was then exposed to one of two rearing conditions from PND 2 to 14, inclusive: (a) brief handling-maternal separation, in which animals were removed from the home cage daily for 15 min periods (HMS15 group) or (b) prolonged handling-maternal separation in which pups were removed from the home cage for 180 min daily (HMS180 group). There were four litters of each rearing condition, totaling 64 animals.

Prior to manipulation of the HMS15 and HMS180 pups, the foster mother was removed from her home cage and housed in a clean cage throughout the daily separation period. During the separation period, rat pups in the HMS180 and HMS15 groups were housed as individual litters in a temperature-controlled environment (incubator set at 30±0.5 °C