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DISTRIBUTION OF CORTICOTROPIN-RELEASING FACTOR RECEPTOR 1 IN THE DEVELOPING MOUSE FOREBRAIN: A NOVEL SEX DIFFERENCE REVEALED IN THE ROSTRAL PERIVENTRICULAR HYPOTHALAMUS

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Abstract—Corticotropin-releasing factor (CRF) signaling through CRF receptor 1 (CRFR1) regulates autonomic, endocrine and behavioral responses to stress and has been implicated in the pathophysiology of several disorders including anxiety, depression, and addiction. Using a validated CRFR1 reporter mouse line (bacterial artificial chromosome identified by green fluorescence protein (BAC GFP-CRFR1)), we investigated the distribution of CRFR1 in the developing mouse forebrain. Distribution of CRFR1 was investigated at postnatal days (P) 0, 4, and 21 in male and female mice. CRFR1 increased with age in several regions including the medial amygdala, arcuate nucleus, paraventricular hypothalamus, medial septum, CA1 hippocampal area, and the lateral habenula. Regions showing decreased CRFR1 expression with increased age include the intermediate portion of the periventricular hypothalamic nucleus, and CA3 hippocampal area. We report a sexually dimorphic expression of CRFR1 within the rostral portion of the anteroventral periventricular nucleus of the hypothalamus (AVPV/PeN), a region known to regulate ovulation, reproductive and maternal behaviors. Females had a greater number of CRFR1-GFP-ir cells at all time points in the AVPV/PeN and CRFR1-GFP-ir was nearly absent in males by P21. Overall, alterations in CRFR1-GFP-ir distribution based on age and sex may contribute to observed age- and sex-dependent differences in stress regulation.

Key words: hypothalamic–pituitary–adrenal axis, corticotropin-releasing factor, corticotropin-releasing factor receptor 1, anteroventral periventricular nucleus, sex difference.

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Abbreviations: BAC, bacterial artificial chromosome; BNST, bed nucleus of the stria terminalis; CRF, corticotropin-releasing factor; CRFR1, CRF receptor 1; GFP, green fluorescent protein; PBS, phosphate-buffered saline; PVN, paraventricular nucleus; TH, tyrosine hydroxylase.

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INTRODUCTION

Corticotropin-releasing factor (CRF) signaling through the G_s-coupled receptor CRF receptor 1 (CRFR1) regulates autonomic, endocrine and behavioral responses to stress (Perrin et al., 1993; Heinrichs et al., 1995; Smith et al., 1998; Subbannayya et al., 2013). Dysregulation of CRFR1 has specifically been linked to several psychiatric disorders including anxiety, depression, and addiction (Chrousos, 2009; Garcia-Carmona et al., 2015; da Silva et al., 2016; Holly et al., 2016; Schussler et al., 2016). Compared to CRF receptor 2 (CRFR2), CRFR1 is expressed at higher levels in the brain (Van Pett et al., 2000; Lein et al., 2007) and has a greater binding affinity to CRF (Perrin et al., 1995). CRFR1 mRNA is expressed throughout the mature rodent brain, and during rodent and human development (Van Pett et al., 2000; Korosi and Baram, 2008; Sandman and Glynn, 2009). Developmental studies on the distribution of CRFR1 are limited to the rat, however, and have primarily investigated the hippocampus, amygdala, cortex, and the paraventricular nucleus of the hypothalamus (PVN) (Avishai-Eliner et al., 1996).

Emerging studies indicate that during pre- and postnatal periods, signaling through CRFR1 exerts lasting effects on the development of several brain regions both during normal development and following early-life stress. Neonatal stress induces alterations in dendritic development, spinogenesis, and synapse formation in the mouse hippocampus and cerebral cortex and these effects are ameliorated by pharmacological antagonism of CRFR1 (Liao et al., 2014; Yang et al., 2015). Recent work by Garcia and colleagues (2014) investigating the role of CRFR1 in normal development of the mouse olfactory bulb suggests that CRF signaling through CRFR1 drives synapse and dendrite formation. Furthermore, early-life stress can produce lasting effects on expression of CRFR1 in several brain regions including the rat amygdala (Grundwald and Brunton, 2015).

CRF-producing cells are expressed in several regions throughout the brain, including the PVN, central amygdala, bed nucleus of the stria terminalis (BNST), Barrington's nucleus, cingulate cortex, hippocampus, accessory bulb of the nucleus accumbens, olfactory bulb, and periaqueductal gray of both mice and rats (Swanson et al., 1983; Aguilera et al., 2004; Kono et al., 2016). CRF has been described in the mouse PVN as

early as embryonic day 13.5 (E13.5) and undergoes dynamic fluctuations over perinatal development marked by decreased levels around the time of birth (Keegan et al., 1994). By contrast, in the amygdala there are low levels of CRF during the prenatal period with levels rising during the neonatal period (∼Postnatal day (P) 3) (Keegan et al., 1994). Other areas such as the cerebral cortex express no CRF mRNA until P3 (Keegan et al., 1994). Distribution of CRF receptors in adult rats and mice is vast; CRFR1-GFP-ir has been reported throughout the olfactory bulb, cerebral cortex, hypothalamus, hippocampus, amygdala, brainstem regions, and cerebellum (Van Pett et al., 2000; Justice et al., 2008). CRFR2 is also expressed throughout the brain with the highest levels found in the lateral septal nucleus, BNST, medial portion of the amygdala, and dorsal raphe nucleus of mice and rats (Chalmers et al., 1995; Van Pett et al., 2000; Aguilera et al., 2004). Less is known about CRF receptor distribution during development, particularly in the mouse. However, in the rat, large fluctuations in brain CRFR1 have been reported during the neonatal period (Avishai-Eliner et al., 1996). A more comprehensive description of CRFR1 distribution in the neonatal brain will enhance our understanding of the neural circuitry that regulates early-life stress. In humans, specific CRFR1 polymorphisms have been linked to the onset of adult depression following early-life adversity (Grabe et al., 2010; Laucht et al., 2013). This indicates that CRFR1 expression and function early in life may be critical to development of adult mood disorders.

Sex differences in rats have been reported with males having more CRF-expressing cells than females within the central amygdala (Karanikas et al., 2013). On the contrary, CRF-positive cells are more abundant in the female rat preoptic area and BNST (McDonald et al., 1994; Funabashi et al., 2004). Sex differences in CRFR1 have also been reported and depend upon the region of interest and species. Wealthington et al. (2014) reported greater CRFR1 binding in adult female compared to male rats within the nucleus accumbens, olfactory tubercle, piriform cortex, and the anterior cingulate, while Lim and colleagues (2005) found greater CRFR1 expression in the BNST of female voles. To our knowledge, potential sex differences in CRFR1 expression have not been explored in mice. Such sex differences in the distribution of CRF and its receptors may contribute to observed differences in a variety of stress-related behavioral and hormonal responses reported in both rats and mice (Handa et al., 1994; Jasnow et al., 2006; Zuloaga et al., 2008). Deletion of CRF receptors in mice results in differential effects on anxiety-like behavior in male and female mice (Bale et al., 2002), further supporting the importance of CRFR1 in regulating sex differences in anxiety. In humans, sex differences in distribution and function of CRF and CRFR1 expression may potentially contribute to the etiology of stress-related psychiatric disorders such as anxiety and depression (Bao et al., 2006; Valentino et al., 2012), both of which are more prevalent in women than men (Weissman et al., 1993; Kornstein et al., 2000; Kessler et al., 2005; Seney and Sibille, 2014).

In the current study, we investigated potential sex differences in, and the development of the CRFR1 system using bacterial artificial chromosome (BAC) transgenic mice reporting expression of CRFR1, with green fluorescent protein (GFP; BAC transgenic CRFR1-GFP mice; Justice et al., 2008). Our findings indicate that CRFR1 expression is dynamic throughout neonatal development, with sex- and age-dependent differences.

EXPERIMENTAL PROCEDURES

Animals

BAC transgenic CRFR1-GFP mice were used in this study (Justice et al., 2008). Male and female CRFR1-GFP mice were mated and brains of 30 offspring (n = 15 per sex) were collected at multiple time points for use in this study. 10 animals per age group (n = 5 per sex; P0, 4, 21) were used for the study, comprising the three developmental time points. Specific ages were selected based on previous reports that indicated major developmental changes in a variety of genes (including CRF) across comparable developmental time points (Cao and Patisaul; 2013; Keegan et al., 1994; Zuloaga et al., 2014). Mice were maintained under a 12/12 L/D cycle (lights on at 0700), with food and water available *ad libitum.* Mice were genotyped by PCR; using the nucleotide sequences: CCT ACG GCG TGC AGT GCT TCA GC forward and CGG CGA GCT GCA CGC TGC GTC CTC reverse EGFP350 primers. Actin470 was used as a control gene. All procedures were approved by the University at Albany Institutional Animal Care and Use Committee and were in accord with National Institutes of Health guidelines.

Perfusion and tissue processing

On the day of birth (postnatal day 0; P0) and P4, mice were cryoanesthetized by placing them on crushed ice. Mice were then intracardially perfused with 5 ml (P0) or 10 ml (P4) of 4% phosphate-buffered paraformaldehyde. P21 mice were overdosed with a 100 mg/kg ketamine/xylazine/acepromazine cocktail prior to perfusion with 15 ml of 4% paraformaldehyde. Brains were excised and stored in 4% paraformaldehyde for 24 h at 4 °C. Brains were then placed into a 30% sucrose solution, and stored at 4 °C. P0 brains were sectioned through the coronal plane using a cryostat (Microm HM505E, MICROM international GmbH, Walldorf, Germany) at 50 μm into two alternate series, P4 were sectioned at 40 μm into two series, and P21 were sectioned at 40 μm into three series. P0 tissue is fragile, and therefore, P0 brains were sectioned at 50 instead of 40 μm to preserve integrity. Tissue was stored in a cryopreservative solution at 4 °C until immunohistochemistry was performed.

Immunohistochemistry

For visualization of CRFR1-GFP-ir, sections were rinsed in phosphate-buffered saline (PBS; pH 7.6), incubated in 1% hydrogen peroxide and 0.4% Triton-X in PBS (PBS-