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DEVELOPMENTAL ALTERATIONS IN CNS STRESS-RELATED GENE EXPRESSION FOLLOWING POSTNATAL IMMUNE ACTIVATION

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Abstract—Early-life adversity is associated with dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis and increased susceptibility to later-life psychopathology. Specifically, there is mounting evidence suggesting that the immune system plays an important role in central nervous system (CNS) development and in the programing of behavior. The current study investigated how early-life immune challenge affects the development of CNS stress neurocircuitry by examining the gene expression profile of corticotropin-releasing hormone (CRH), CRH receptors, and the major corticosteroid receptors within the limbic-hypothalamic circuit of the developing rodent brain. Mice were administered a 0.05 mg/kg lipopolysaccharide (LPS) injection on postnatal day (P) 3 and 5 and gene expression was assessed using *in situ* hybridization from P14 to P28. Target genes investigated were CRH, CRH receptor-1 (CRHR1), CRH receptor-2, the mineralocorticoid receptor, and the glucocorticoid receptor (GR). Early LPS challenge resulted in a transient decrease in CRHR1 mRNA expression in the cornuammonis 1 (CA1) and CA3 regions of the hippocampus that were accompanied by increased hippocampal GR mRNA expression in the CA1 region between P14 and P21. This was followed by increased hypothalamic CRH expression in LPS-mice on P28. Our current findings suggest that early-life LPS challenge impacts the developmental trajectory of CNS stress neurocircuitry. These results lend insight into the molecular basis for the later development of stress-related behaviors as previously described in early immune challenge rodents.

Key words: HPA axis, stress circuitry; lipopolysaccharide; immune challenge; neurodevelopment; postnatal.

INTRODUCTION

Early development is a vulnerable time period during which wiring of the central nervous system (CNS) is fine-tuned and receptive to changes in environmental conditions. In rodents, early-life stressors introduced during the first week of life have been demonstrated to have long-term, permanent consequences on behavior and physiology (Sapolsky and Meaney, 1986; Anisman et al., 1998; Lippmann et al., 2007; Brunton and Russell, 2010; Llorente et al., 2011; O'Malley et al., 2011). In particular, the hypothalamic pituitary adrenal (HPA) axis, a key structure in the stress response, has been implicated in mediating the effects of early life stress and later life alterations in functioning (Catalani et al., 2011; Stiller et al., 2011). Clinically, inappropriate regulation of the HPA axis during development has been suggested to influence the later presentation of mental health symptoms (Essex et al., 2011).

Rapid activation, as well as termination, of the HPA axis is essential to CNS functioning (Turnbull et al., 1998). The parvocellular cells of the hypothalamic paraventricular nuclei (PVN) receive input from limbic and brainstem circuits and are activated by psychological and physical stressors. Upon activation, corticotropin-releasing hormone (CRH) is secreted from the median eminence into the hypophyseal portal circulation to reach the anterior pituitary. This stimulates pro-opiomelanocortin (POMC)-producing cells of the anterior lobe to release adrenocorticotropic-releasing hormone (ACTH), an end product of POMC precursor molecule processing. ACTH is released into circulation and stimulates glucocorticoid secretion from the adrenal cortex (Turnbull et al., 1998). Failure to appropriately and effectively inhibit HPA axis activity may result in permanent effects on growth and differentiation of developing systems in the CNS. During chronic stress, glucocorticoids exert negative feedback effects on several levels of the stress axis: directly on the biosynthesis and release of CRH in the PVN and/or ACTH in the anterior pituitary, or indirectly by acting on brainstem catecholaminergic nuclei such as the locus coeruleus that input onto neurons in the PVN; this results in a decrease in CRH production in the PVN (Makino et al.,

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Abbreviations: 5-HT, serotonin; 5-HT2A, serotonin receptor 2A; 5-HT2C, serotonin receptor 2C; ACTH, adrenocorticotropic releasing hormone; BLA, basolateral amygdala; CA1, cornuammonis 1; CA3, cornuammonis 3; CeA, central amygdala; CNS, central nervous system; CORT, corticosterone; CPM, counts per minute; CRH, corticotropin-releasing hormone; CRHR1, corticotropin-releasing hormone receptor-1; CRHR2, corticotropin-releasing hormone receptor-2; DG, dentate gyrus; DPM, disintegrations per minute; GABA, gamma-aminobutyric acid; GR, glucocorticoid receptor; HPA, hypothalamic–pituitary–adrenal; IL, interleukin; LPS, lipopolysaccharide; MR, mineralocorticoid receptor; mRNA, messenger ribonucleic acid; P, postnatal; PBS, phosphate buffered saline; POMC, pro-opiomelanocortin; PVN, paraventricular nucleus; SAL, saline; SEM, standard error of the mean; SSC, saline-sodium citrate; TNF-α, tumor necrosis factor-α.

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2002). Additionally, the hippocampus acts to inhibit HPA activity via glucocorticoid binding to hippocampal mineralocorticoid (MR) and glucocorticoid receptors (GR) (Magarinos et al., 1987; Feldman and Weidenfeld, 1999).

Although the rodent peripheral HPA axis is relatively hyporesponsive to most mild stressors during early postnatal life (between postnatal days 4–14), the central stress axis retains reactivity to environmental stimuli (Dent et al., 1999). Importantly, the stress neurocircuit connecting limbic regions to the hypothalamus is functionally immature during early postnatal development and is sensitive to environmental programing. Early-life adversity may, therefore, interfere with the stress hyporesponsive period and act to reprogram CNS stress neurocircuitry, resulting in long-term HPA dysregulation. Indeed, it has been demonstrated that early life immune challenge with lipopolysaccharide (LPS), a component of the cell wall of gram-negative bacteria and a potent activator of the HPA axis, on postnatal day (P) 3 and 5 leads to long-term alterations to adult stress-related behaviors (Shanks et al., 1995, 2000; Breivik et al., 2002; Tenk et al., 2008). Yet the molecular mechanisms linking increased immune activity during the first postnatal week with long-term changes in stress-related behavior remain unknown. Furthermore, whereas the majority of early immune challenge studies have focused on molecular and behavioral alterations that occur well into adulthood(Shanks et al., 2000; Boisse et al., 2004, 2005; Spencer et al., 2005, 2006; Bilbo et al., 2008; Galic et al., 2008; Harre et al., 2008; Kohman et al., 2008; Bland et al., 2010; Walker et al., 2009), we focus our attention on changes that occur earlier in life and have recently demonstrated that early LPS challenge in mice altered the developmental trajectory of CNS serotonergic-related gene expression during postnatal development (Sidor et al., 2010). Given that serotonergic activity, itself, contributes to regulation of the central HPA axis and modulated HPA activity (Lowry, 2002; Leonard, 2006; Holmes, 2008), the current study extends our previous findings by investigating how immune activation during early postnatal development affects the expression profile of genes important for HPA regulation at the level of the CNS including CRH, corticotropin-releasing hormone (CRHR) 1, CRHR2, MR and GR. This was accomplished by measuring gene expression at various time points during postnatal development (P14, P17, P21, P28) within the limbic-hypothalamic stress circuit in order to identify the onset of CNS HPA dysregulation. In addition, HPA activation in response to immune challenge was determined by measuring plasma corticosterone levels. Such results will provide a greater understanding of the neurobiological mechanisms involved in immune system-CNS crosstalk during development and how this contributes to long-lasting changes in emotionality.

EXPERIMENTAL PROCEDURES

Animals

Female and male CD-1 mice (8–10 weeks, Charles River) were bred in-house and male and female offspring were used in these experiments (n = 11 litters). Postnatal day (P) 0 denotes day of birth. Litters were culled to a maximum of 12 on P1. Mice were weaned on P21, separated based on sex, and housed in standard cages (max. 5/cage). Mice were maintained on a 12 h:12 h light:dark cycle (lights on at 07:00) with food and water provided *ad libitum*. All procedures were approved by the McMaster University Animal Research Ethics Board and carried out in accordance with the guidelines described in the Guide to the Care and Use of Experimental Animals (Canadian Council on Animal Care, 1993).

Experimental procedure

On P3 and P5, whole litters were given an intraperitoneal injection of either 0.05 mg/kg LPS (*Escherichia coli* LPS; Sigma, Oakville, ON, Canada) in 50 µl/g or an equal volume of saline (SAL; 0.9%) between 7:00 and 9:00 h (n = 5 litters for SAL; n = 6 litters for LPS). LPS dose was based on previous early immune challenge literature (Shanks et al., 1995; Walker et al., 2010, 2011). Dams were removed from the home cage and returned once all pups had received an injection; maternal separation did not exceed 10 min. Mice were weighed daily between P2 and P7 and weekly thereafter (see Table 1).

Corticosterone (CORT) analysis. Nine litters of mice were used in this arm of the study (Cohort 2). Blood was collected 6 and 24 h following LPS or SAL injection. Blood from 2 to 3 pups per time point was combined (P3–6 h, P3–24 h, P5–6 h, P5–24 h) in cold EDTA-tubes and centrifuged for 15 min at 3000 RPM. Plasma was aliquoted and frozen at −70 °C until use. CORT was measured in duplicate using a standard radioimmunoassay kit from MP Biomedicals.

Gene expression analysis. A separate cohort of 48 mice was used in this arm of the study (six per treatment per time point: P14, P17, P21 and P28). At each time point, pups from 4 to 6 different litters were included. Groups were balanced for sex such that an equal number of males and females were used (n = 3/sex/treatment/time point). The study was not powered, however, to examine potential sex differences in a statistically meaningful way.

At P14, P17, P21 and P28, brains were rapidly removed following decapitation, frozen in −60 °C isopentane, and stored at −70 °C until cryostat sectioning. A series of 12 µm coronal sections were collected through the central amygdala ([CeA] Bregma −0.70 to −0.94 mm), paraventricular nucleus ([PVN] Bregma −0.70 to −0.94 mm), and dorsal hippocampus (Bregma 1.46–1.94 mm) according to the stereotaxic atlas of Paxinos and Franklin (2001). For all regions collected, reference sections

Table 1. Body weight of experimental mice

	Postnatal (P) age	Weight (g)*	
		SAL	LPS
Cohort 1	P2	2.26 ± 0.09	2.34 ± 0.12
	P3	2.68 ± 0.08	2.61 ± 0.16
	P4	3.34 ± 0.12	3.30 ± 0.20
	P5	3.84 ± 0.06	3.74 ± 0.18
	P6	4.59 ± 0.14	4.49 ± 0.23
	P7	5.19 ± 0.15	4.95 ± 0.31
	P14	8.0 ± 0.37	8.17 ± 0.47
	P21	13.0 ± 0.91	14.7 ± 0.84
	P28	21.67 ± 1.1	21.6 ± 1.03
Cohort 2	P2	2.25 ± 0.020	2.25 ± 0.026
	P3	2.73 ± 0.029	2.65 ± 0.27
	P4	3.49 ± 0.039	3.12 ± 0.054
	P5	4.44 ± 0.056	3.87 ± 0.05
	P6	5.30 ± 0.094	4.71 ± 0.11

* Data provided represents mean ± S.E.M.