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Long-term dysregulation of brain corticotrophin and glucocorticoid receptors and stress reactivity by single early-life pain experience in male and female rats

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Summary Inflammatory pain experienced on the day of birth (postnatal day 0: PD0) significantly dampens behavioral responses to stress- and anxiety-provoking stimuli in adult rats. However, to date, the mechanisms by which early life pain permanently alters adult stress responses remain unknown. The present studies examined the impact of inflammatory pain, experienced on the day of birth, on adult expression of receptors or proteins implicated in the activation and termination of the stress response, including corticotrophin releasing factor receptors (CRFR1 and CRFR2) and glucocorticoid receptor (GR). Using competitive receptor autoradiography, we show that Sprague Dawley male and female rat pups administered 1% carrageenan into the intraplantar surface of the hindpaw on the day of birth have significantly decreased CRFR1 binding in the basolateral amygdala and midbrain periaqueductal gray in adulthood. In contrast, CRFR2 binding, which is associated with stress termination, was significantly increased in the lateral septum and cortical amygdala. GR expression, measured with in situ hybridization and immunohistochemistry, was significantly increased in the paraventricular nucleus of the hypothalamus and significantly decreased in the hippocampus of neonatally injured adults. In parallel, acute stress-induced corticosterone release was significantly attenuated and returned to baseline more rapidly in adults injured on PD0 in comparison to controls. Collectively,

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these data show that early life pain alters neural circuits that regulate responses to and neuroendocrine recovery from stress, and suggest that pain experienced by infants in the Neonatal Intensive Care Unit may permanently alter future responses to anxiety- and stress-provoking stimuli.
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1. Introduction

Approximately 12% of live births in the United States occur before 37 gestational weeks and are considered premature (http://www.marchofdimes.com/peristats/). These infants spend on average 25 days in the Neonatal Intensive Care Unit (NICU) where they endure 10—18 painful and inflammatory procedures per day, including heel lance, endotracheal intubation, respiratory and gastric suctioning and surgery (Barker and Rutter, 1995; Simons et al., 2003; Carbajal et al., 2008) (http://www.marchofdimes.com/peristats/). Despite strong evidence that pain and stress circuitry are established and functional in preterm infants (Anand et al., 1987; Grunau et al., 2005; Bartocci et al., 2006; Slater et al., 2006), 65% of these procedures are performed in the absence of analgesia (Barker and Rutter, 1995; Simons et al., 2003; Carbajal et al., 2008).

Clinical studies suggest early life pain has an immediate and long-term impact on responses to stress- and anxiety-provoking stimuli (Sullivan et al., 2012). Intrinsically, painful NICU procedures activate the stress response (Anand et al., 1987; Grunau et al., 2005, 2010). For example, preterm infants undergoing surgical procedures without analgesia have significantly higher concentrations of catecholamines and glucocorticoids (corticosterone: CORT) during and after surgery as compared with infants receiving analgesic treatment (Anand et al., 1987). While initially heart rate, facial reactivity and cortisol levels of preterm infants are high in response to procedural pain, they become significantly blunted as the number of skin breaking procedures increases (Grunau et al., 2005, 2010). Even in early childhood, cortisol release in response to painful stimuli remains blunted in former preterm infants (Grunau et al., 2007, 2010), and represents a known risk factor for adult psychopathologies such as depression and post-traumatic stress disorder (PTSD) (Chrousos, 2009).

We have previously reported that a single inflammatory insult (1% carrageenan; hind paw), administered on the day of birth (postnatal day 0: PD0), significantly dampens behavioral responses to stress-, anxiety-, and pain-provoking stimuli in adult rats (LaPrairie and Murphy, 2007, 2009; Victoria et al., 2013a). Our studies further show that these behavioral changes are due to alterations in the endogenous opioid system (LaPrairie and Murphy, 2007, 2009; Victoria et al., 2013a). Most recently, we reported that early life pain permanently upregulates enkephalin mRNA and protein in the central amygdala, lateral septum and midbrain periaqueductal gray, brain regions that are highly responsive to stress (Victoria et al., 2013a). Notably, the endogenous opioid system works in parallel with classic systems regulating hypothalamic pituitary adrenal (HPA) axis activity. For example, enkephalin and corticotrophin releasing factor (CRF), which is essential for activation of the HPA axis (Vale et al., 1981), are simultaneously released from the hypothalamus in response to stress (Lightman and Young, 1989). Further, CRF and CRF receptors (CRFR) co-localize with and co-express endogenous opioids throughout the brain (Rivalland et al., 2005; Mousa et al., 2007). The glucocorticoid receptor (GR) system, which terminates HPA activity (Dallman et al., 1987), regulates expression of both the endogenous opioid and CRFR systems (Lightman and Young, 1989). Given that early life pain alters the endogenous opioid system, which interacts with factors regulating the stress axis, the present study was conducted to test the hypothesis that a single inflammatory insult on the day of birth alters CRFR and GR systems in adulthood.

2. Methods

2.1. Animals

Pregnant Sprague—Dawley rat dams were obtained on gestational day 14 (GD14; Charles River, USA). Dams were housed individually under 12:12 h light:dark cycle with ad libitum access to food and water. On the day of birth (PD0), pups were sexed by examination of anogenital distance and subjected to neonatal treatment. All litters were reared identically, weaned on PD21 and housed with same sex littermates in groups of 2—3. Male and female rats were used in all experiments and tested on separate days. All experiments adhered to the guidelines of the Committee for Research and Ethical Issues of International Association for the Study of Pain, and were approved by the Georgia State University Animal Care and Use Committee. Behavior experiments were conducted during the light phase (09:00—12:30 h), animal order was randomized and the experimenter was blinded to neonatal treatment.

2.2. Neonatal treatment

Acute neonatal inflammatory injury was induced as in our previous studies (LaPrairie and Murphy, 2007, 2009; Victoria et al., 2013a). Within 24 h of birth on PD0, male and female rat pups received an injection of 5 μL carrageenan (CGN, 1% dissolved in saline; Sigma, USA) into the intraplantar surface of the right hind paw. This time point is developmentally comparable to 24 weeks of gestation in humans (Workman et al., 2013). Intraplantar CGN is a well-established model of early life inflammatory pain that results in local edema lasting approximately 24—72 h in pups (Lidow et al., 2001; Ren et al., 2004; LaPrairie and Murphy, 2007). Separate litters were handled as a control. Intraplantar saline was not administered as it results in an inflammatory response (<24 h). Pups were separated from their dam for no more than 20 min, maintained on a warm surface and returned to the home cage as a group. We have previously reported that this protocol does not alter maternal behavior (LaPrairie and Murphy, 2007, 2009; LaPrairie et al., 2008). In total, 26 litters