Psychoneuroendocrinology (2014) 41, 1—12

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/psyneuen

Altered nociceptive, endocrine, and dorsal horn neuron responses in rats following a neonatal immune challenge

Ihssane Zouikr [a], Melissa A. Tadros [b], Javad Barouei [a], Kenneth W. Beagley [c], Vicki L. Clifton [d], Robert J. Callister [b], Deborah M. Hodgson [a,*]

[a] *Laboratory of Neuroimmunology, School of Psychology, University of Newcastle, Newcastle, New South Wales, Australia*
[b] *School of Biomedical Sciences and Pharmacy, University of Newcastle, Newcastle, New South Wales, Australia*
[c] *Institute of Health Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland, Australia*
[d] *Robinson Institute, University of Adelaide, Adelaide, South Australia, Australia*

Received 29 August 2013; received in revised form 18 November 2013; accepted 18 November 2013

KEYWORDS
LPS;
HPA-axis;
Hypothalamus;
SDH neurons;
Formalin test;
Pain;
Corticosterone

Summary The neonatal period is characterized by significant plasticity where the immune, endocrine, and nociceptive systems undergo fine-tuning and maturation. Painful experiences during this period can result in long-term alterations in the neurocircuitry underlying nociception, including increased sensitivity to mechanical or thermal stimuli. Less is known about the impact of neonatal exposure to mild inflammatory stimuli, such as lipopolysaccharide (LPS), on subsequent inflammatory pain responses. Here we examine the impact of neonatal LPS exposure on inflammatory pain sensitivity and HPA axis activity during the first three postnatal weeks. Wistar rats were injected with LPS (0.05 mg/kg IP, *Salmonella enteritidis*) or saline on postnatal days (PNDs) 3 and 5 and later subjected to the formalin test at PNDs 7, 13, and 22. One hour after formalin injection, blood was collected to assess corticosterone responses. Transverse spinal cord slices were also prepared for whole-cell patch clamp recording from lumbar superficial dorsal horn neurons (SDH). Brains were obtained at PND 22 and the hypothalamus was isolated to measure glucocorticoid (GR) and mineralocorticoid receptor (MR) transcript expression using qRT-PCR. Behavioural analyses indicate that at PND 7, no significant differences were observed between saline- or LPS-challenged rats. At PND 13, LPS-challenged rats exhibited enhanced licking ($p < .01$), and at PND 22, increased flinching in response to formalin injection ($p < .05$). LPS-challenged rats also displayed increased plasma corticosterone at PND 7 and PND 22 ($p < .001$)

* Corresponding author at: Laboratory of Neuroimmunology, School of Psychology, University of Newcastle, University Drive, Callaghan, Newcastle, New South Wales 2308, Australia. Tel.: +61 0249216701; fax: +61 0249216980.
E-mail address: Deborah.Hodgson@newcastle.edu.au (D.M. Hodgson).

http://dx.doi.org/10.1016/j.psyneuen.2013.11.016

but not at PND 13 following formalin administration. Furthermore, at PND 22 neonatal LPS exposure induced decreased levels of GR mRNA and increased levels of MR mRNA in the hypothalamus. The intrinsic properties of SDH neurons were similar at PND 7 and PND 13. However, at PND 22, ipsilateral SDH neurons in LPS-challenged rats had a lower input resistance compared to their saline-challenged counterparts ($p < .05$). These data suggest neonatal LPS exposure produces developmentally regulated changes in formalin-induced behavioural responses, corticosterone levels, and dorsal horn neuron properties following noxious stimulation later in life. These findings highlight the importance of immune activation during the neonatal period in shaping pain sensitivity later in life. This programming involves both spinal cord neurons and the HPA axis.

1. Introduction

It is now well accepted that the immune, endocrine and nervous systems communicate in a tightly regulated manner (Czura and Tracey, 2005; Hodyl et al., 2007; Sternberg, 2006). The hypothalamic-pituitary-adrenal (HPA) axis is a key component in this communication and its activation results in altered plasma corticosterone levels and altered pain responses, most notably in response to a painful inflammatory insult, such as the formalin test (Aloisi et al., 1998; Sorg et al., 2001). Elevated plasma corticosterone levels are observed in both neonatal (Butkevich et al., 2013) and preadolescent (Butkevich et al., 2009a) rats after formalin injection into the hindpaw, suggesting inflammatory pain results in rapid activation of the HPA axis.

Interestingly, HPA axis function in adults can be modulated by early life events (for review see Matthews, 2002). One example of this ''programming'' of the HPA axis by early life events is potentiation of stress responses and reduced natural killer cell activity in adult rats after neonatal exposure to lipopolysaccharide (LPS) (Hodgson et al., 2001). Neonatal exposure to LPS also increases basal corticosterone secretion and lymphocyte sensitivity in adult animals (Shanks et al., 2000). Furthermore, rats exposed to LPS during early postnatal development display thermal and mechanical hyperalgesia and allodynia when tested as adults (Boisse et al., 2005). These data suggest neonatal LPS exposure alters neuroimmunoendocrine communication and nociceptive behaviours.

While numerous studies have examined the effects of neonatal LPS exposure on responses to noxious thermal and mechanical stimuli in adults, few have examined the effects of neonatal LPS on responses to inflammatory pain stimuli, such as the formalin test, during the neonatal and preadolescent period. This is surprising as gestational stress enhances nociceptive behaviours and elevates plasma corticosterone levels in response to the formalin test in neonatal (PND 7) rats (Butkevich et al., 2013), suggesting early life events programme the HPA axis and inflammatory pain responses at least in the first week after birth. Responses to the formalin test also appear to vary during early postnatal development, with infant rats being susceptible to much lower concentrations of formalin than adults (Gagliese and Melzack, 1999; Zouikr et al., 2013). Thus, the effect of neonatal exposure to LPS on the development of inflammatory pain response appears to be complex and is not well understood.

Like components of the HPA axis, the development of spinal cord dorsal horn circuits is also known to be vulnerable to early life events. For example, substantial changes in synaptic function are observed in adult superficial dorsal horn (SDH) neurons following neonatal injury (Baccei, 2010). It appears the neonatal period represents a critical time point when major perturbations can disrupt the maturation of SDH neurons and their connections (Baccei and Fitzgerald, 2005; Walsh et al., 2009). Surprisingly, there have been no investigations of the long-term effects of neonatal exposure to LPS on both the HPA axis and dorsal horn activity even though we know formalin injection in adult animals increases metabolic activity in the spinal cord (Aloisi et al., 1993), and alters dorsal horn signalling (Dickenson and Sullivan, 1987; McCall et al., 1996).

Clearly early life events are important for programming both the HPA axis and nociceptive pathways. In order to test how neonatal inflammation impacts the development of components of the HPA axis and inflammatory pain responses we exposed neonatal rats to LPS and assessed the effects of a neonatal immune challenge on behavioural, endocrine and neural responses to inflammatory pain stimuli at three time points during the first three weeks after birth. Our data suggest neonatal LPS exposure results in developmentally regulated changes in formalin-induced behavioural responses, corticosterone levels, and dorsal horn neuronal properties following noxious stimulation later in life.

2. Materials and methods

2.1. Experimental procedures

Eight experimentally naïve female Wistar rats were obtained from the University of Newcastle Animal House and allowed one week acclimatization prior to mating in a vivarium. Mating resulted in 86 offspring for this study. The male was removed from the harem after two weeks and dams were housed individually in custom designed polycarbonate-perspex home boxes (43.5 cm × 28 cm × 12.5 cm; Mascot Wire Works, Sydney, Australia). At PND 3 and 5 (PND 1 as day of birth), pups were briefly removed from their home boxes, weighed and injected (IP) with either LPS (*Salmonella enterica*, serotype *enteritidis*; Sigma—Aldrich Chemical Co., USA, dissolved in sterile pyrogen-free saline, 0.05 mg/kg) or an equal volume of saline (Livingstone International, Australia).