Published in final edited form as:
Front Neuroendocrinol. 2010 April ; 31(2): 193–202. doi:10.1016/j.yfrne.2010.02.001.

Long Term Impact of Neonatal Injury in Male and Female Rats: Sex Differences, Mechanisms and Clinical Implications

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Abstract

Over the last several decades, the relative contribution of early life events to individual disease susceptibility has been explored extensively. Only fairly recently, however, has it become evident that abnormal or excessive nociceptive activity experienced during the perinatal period may permanently alter the normal development of the CNS and influence future responses to somatosensory input. Given the significant rise in the number of premature infants receiving high-technology intensive care over the last twenty years, ex-preterm neonates may be exceedingly vulnerable to the long-term effects of repeated invasive interventions. The present review summarizes available clinical and laboratory findings on the lasting impact of exposure to noxious stimulation during early development, with a focus on the structural and functional alterations in nociceptive circuits, and its sexually dimorphic impact.

Keywords

neonate; inflammation; pain; nociception; development; infant

Premature Birth and Neonatal Intensive Care

Advances in perinatal medical care over the last two decades have substantially increased the survival of infants born premature [1]. As part of this life-saving care, however, preterm neonates are exposed to multiple invasive procedures in the neonatal intensive care unit (NICU) which are frequently accompanied by local inflammation and tissue damage lasting for several hours to days [2]. Growing clinical and basic science data suggests that exposure to repeated tissue damaging interventions in neonates may induce lasting changes in the CNS and have profound consequences for subsequent nociceptive processing [3;4;5;6;7;8;9;10].

Premature birth, defined as birth prior to 37 weeks gestation, occurs at alarmingly high rates worldwide. According to the World Health Organization, 16.5% of all infants are born premature, with over 500,000 preterm babies born each year in the United States alone [11]. Indeed, the rates of premature births in the United States have been escalating steadily to nearly 35% of all live births within the last two decades [11]. While the underlying causes of prematurity are diverse and not completely understood, several factors are known to contribute to the increased prevalence of preterm births, including assisted reproductive techniques [11],

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tobacco, alcohol and illicit drug use during pregnancy [12], as well as high maternal blood pressure, diabetes and obesity [13].

Intrinsic to their care in the NICU, preterm infants undergo on average 14 noxious (painful and/or tissue damaging) procedures per day including repeated heel lances, endotracheal intubation, surgery, and respiratory and gastric suctioning [14;15]. Mounting evidence indicates that nociceptive circuitry is both established and functional during late gestation, and that premature infants are indeed capable of mounting developmentally specific and distinct responses to noxious and non-noxious stimuli [16;17]. Moreover, cortical activation in response to acute noxious stimulation in preterm neonates at 25 weeks has been reported, suggesting the potential for higher-level processing of pain [18;19].

The Lasting Impact of Neonatal Noxious Stimulation

Pain is unique amongst sensory modalities. While olfactory, auditory, and tactile stimulation are plentiful after birth, the newborn mammalian CNS is rarely exposed to nociceptive input. During the last two decades, however, this situation has changed dramatically due to the wide application of intensive care interventions in high-risk preterm neonates [7;20]. As the neonatal period is a sensitive window for experience-induced plasticity due to the ongoing maturation of nociceptive systems [21], accumulating evidence from clinical and animal research studies indicates that exposure to noxious stimulation, experienced early in life, can leave a legacy of altered somatosensory processing [8;22;23;24;25;26;27].

Long-Term Effects of Early Noxious Insult on Developing Nociceptive Systems-Clinical Studies

Early pioneering studies on the lasting impact of early life noxious stimulation in human infants reported that heel lance elicits decreased facial and enhanced cardiovascular responses, indicative of an increased threshold for pain, in preterm infants with prior NICU experience compared to age matched full-term infants [28]. Subsequent studies revealed that a higher frequency of invasive procedures in preterm infants is significantly associated with dampened nociceptive responses at 32 weeks of age compared to controls [29]. Moreover, decreased facial responsiveness to immunization at 4 and 8 months [30], and blunted nociceptive sensitivity have been reported in 18 month old former preterm neonates compared to full term peers [24]. Former NICU toddlers are also rated by parents as less pain sensitive compared to term-born controls, with a higher frequency of procedural pain exposure associated with more dampened nociceptive responsiveness to noxious stimulation at 18 months of age [24]. Furthermore, a recent study reported that former extremely preterm children display a generalized decrease in thermal but not mechanical nociceptive sensitivity during pre-adolescence, suggesting lasting centrally mediated alterations in nociceptive pathways [31].

In contrast to the aforementioned reduced nociceptive responsiveness following superficial (i.e. *acute* and *inflammatory)* types of neonatal noxious stimulation, deep somatic and visceral noxious stimulation (i.e. *early surgery* and *tissue damage*) in infancy leads to prolonged sensitization of nociceptive responses. Hypersensitivity to tissue damage is observed in human infants, in that a decrease in sensory thresholds is observed for days or weeks in the presence of local or deep visceral tissue injury [32;33]. Furthermore in premature infants, the withdrawal reflex threshold in an area of local tissue damage following *repeated* heel lances is half the value of that on the intact contralateral heel for several months following the initial insult [34;35]. Interestingly, this response is not restricted to the site of injury, as former NICU infants also display secondary hyperalgesia (sensitivity in surrounding areas of undamaged tissue) in the intact, contralateral limb [36]. Similarly, infants that experienced surgery within the first three months of life display enhanced hypersensitivity to subsequent surgery performed in the same dermatome that persists for more than one year [37]. This hypersensitivity is also not

restricted to the site of tissue damage, as neonates demonstrate greater sensitivity to mechanical stimulation both in the area of incision and on the contralateral side of the body following corrective unilateral abdominal surgery [38]. Moreover, term-born males that experienced un-anesthetized neonatal circumcision respond more intensely to routine inoculation at 4-6 months in comparison to uncircumcised infants; this effect is partially attenuated by pre-treatment with a local anesthetic [39].

Interestingly, alterations in nociception do not appear to be transient in nature, whereby both full- and preterm infants with prior NICU experience display an increased threshold for acute thermal stimuli (i.e. decreased sensitivity), but enhanced perceptual sensitization to a prolonged heat stimulus (i.e. hyper-sensitivity) up to 14 years of age [31;40]. Former preterm adolescents also display significantly greater tenderness in response to pressure [41], are more prone to lasting clinical somatization [40], and report earlier onset of pediatric migraine [42] compared to full-term peers. Indeed, 10-year old children with former NICU experience also rate pictures of medical events as more intense than pictures of psychosocial pain events, unlike term-born children [43]. (See Table 1)

Long-Term Effects of Early Noxious Insult on Developing Nociceptive Systems-Experimental Animal Studies

There is considerable parallel evidence in non-human animal models that neonatal noxious stimulation induces persistent alterations in somatosensory structure and function that last into adult life [8;27;44]. Data collected to date suggest, however, that the type of noxious stimulation (acute: lasting minutes to days, versus tonic: lasting weeks to months) is critical to the long-term impact. Early pioneering studies reported that chronic neonatal inflammation induced by unilateral intraplantar application of Complete Freund's adjuvant results in enhanced nociceptive sensitivity, as well as increased primary afferent nerve fiber innervation of the spinal cord that extend into adulthood [27]. Similarly, local hindpaw skin wounds induced during the first week of life result in long-lasting cutaneous hypersensitivity, expanded dorsal horn receptive fields, and profound sprouting of local sensory nerve terminals in adulthood [27;44]. This hyperinnervation is associated with a long-lasting decrease in mechanical threshold in the wounded region, as well as a substantial up-regulation of growth factors including NGF and BDNF [45;46;47]. Moreover, *repeated* intraplantar carrageenan administration over the first three postnatal weeks results in enhanced nociceptive sensitivity [26]. Thermal hyperalgesia following exposure to *repetitive* needle pricks, and lasting visceral hyperalgesia associated with neonatal chronic chemical irritation of the colon have also been reported [22;48]. Finally, a persistent neonatal lipopolysaccharide immune challenge produces long-lasting hypersensitivity to mechanical and thermal stimuli in adulthood [49].

In contrast, a generalized *decrease* in nociceptive sensitivity as a consequence of *acute* or *superficial* stimulation such as foot shock and intraplantar formalin injections have been demonstrated [6;50]. Likewise, a long-term global elevation of nociceptive thresholds in response to noxious thermal and mechanical stimulation following short-lasting local neonatal inflammation with intraplantar carrageenan has been reported [8]. Remarkably, this hypoalgesia is not only present in the neonatally injured hindpaw but is also present in the intact contralateral paw. The degree of hypoalgesia produced by the P0 insult is not trivial; paw withdrawal latencies increase by more than 40% in adult animals that were injured on the day of birth in comparison to control animals. Injury-induced hypoalgesia is observed in both adolescence (P40) and adulthood (P60), and is significantly greater in neonatally injured females compared to injured males [25]. Alternatively, a few studies using similar paradigms have failed to report any long-term effects on sensory thresholds [51;52]. This may be due to variability in the concentration of the inflammatory agents used, as well as additional contributing factors that are still not well-understood. The abovementioned hypoalgesic

response that we report is also associated with excessive hyperalgesia in the presence of on-going inflammation following a subsequent inflammatory insult in adulthood [8], with neonatally injured females again exhibiting significantly greater hyperalgesia in the inflamed paw than neonatally injured males. While an early study only reported this effect in the neonatally inflamed hindpaw [8], we observed enhanced hyperlagesia in both the neonatally injured and uninjured paws, which is consistent with previous studies reporting long-lasting sensitization of afferent neurons and hyperalgesia following neonatal insult [22;46;48]. These inconsistent results may be due to differences in the timing of neonatal injury, adult reinflammation and/or behavioral testing.

This increased *hyperalgesia* following re-inflammation in adulthood may appear disparate with the observed basal *hypoalgesia*. Anatomical studies, however, suggest that neonatal inflammatory insult results in alterations in primary afferent innervation of the dorsal horn [53], which may account for our observed hyperalgesia. In particular, neonatal inflammatory insult increases primary afferent innervation in the L3-L5 spinal cord, as reflected by increased expression of both CGRP and substance P immunoreactivity (unpublished observations). Parallel changes are not observed in CGRP expression in the thoracic spinal cord of injured animals, indicating that these changes are site-specific. Similar findings of an increase in substance P levels in laminae I and II of the dorsal horn have been reported following chronic inflammation in rodents [54]. As both CGRP and substance P are pro-nociceptive, enhanced dorsal horn release of these peptides due to increased primary afferent input would be associated with an enhanced response to noxious stimulation, and may provide the biological basis for the observed increased hyperalgesia following intraplantar CFA in adulthood. The dual findings of baseline hypoalgesia and enhanced hyperalgesia following a subsequent insult are also surprisingly consistent with previous reports in former premature children. Grunau and colleagues found that ex-preterm neonates are rated by parents as less reactive to everyday bumps and scrapes; however parents rate that these children experience medical procedural pain as more intense [24;55]. Similarly, adolescents with prior NICU experience display an increased threshold for acute thermal stimuli (i.e. decreased sensitivity), but enhanced perceptual sensitization to a prolonged heat stimulus (i.e. hyper-sensitivity) [40]. Taken together, these data suggest that early life exposure to acute versus persistent noxious stimulation may differentially affect developing nociceptive circuitry, thereby producing distinct long-term effects. (See Table 2)

Interestingly, the aforementioned studies suggest that the long-term impact of neonatal noxious insult mirrors the developmental consequences of early life stress. Compelling evidence in experimental animal models has revealed that stressful experiences during the perinatal period result in profound and permanent consequences on the behavioral and neuroendocrine responses to stress stimuli in adulthood. Specifically, exposure to a potent stressor such as repeated maternal separation results in lasting hyperactivation of the hypothalamic pituitary adrenal (HPA) axis, while brief bouts of handling (i.e. mild stressor in rodents) during the perinatal period result in a stress hypo-responsive phenotype [56;57;58]. Thus, given that the lasting impact of early life stress on the HPA axis is dependent upon the degree (mild versus severe) of perinatal stress exposure, the type of noxious stimulation (acute versus tonic), similarly, appears to be critical to the long-term bivalent effects of neonatal insult on baseline nociceptive thresholds.

Critical Period

The ability of early life experience to alter the organization of the CNS and subsequent behavior is a major focus of neuroscientific research. Previous research in both human and non-human animal models suggests that there are periods during nervous system development within which perturbations have long-lasting, if not permanent consequences. This is in contrast to the

relatively transient effects associated with the same perturbations at times outside these periods [59;60]. Work in our laboratory has indicated that there is indeed a critical period for the long-term consequences of neonatal inflammatory insult on adult sensory thresholds. Animals that experienced unilateral neonatal hindpaw inflammation on both postnatal days zero and eight (P0 and P8) display a significant decrease in sensitivity to noxious stimuli (hypoalgesia) in adulthood, compared to animals injured at two weeks of age (P14) [25]. Together, these results suggest that the impact of neonatal inflammation is dependent upon a sensitive period, and that noxious insult occurring outside of this critical window does not permanently alter thermal sensory thresholds. These results are consistent with previous animal studies that have also reported that neonatal injury permanently alters visceral and somatic sensory processing, however, only when induced during the first week of life [8;10].

Sex Differences In Response to Neonatal Noxious Stimulation

Given the sizable body of literature that indicates that males and females experience pain differently [61;62;63], it is surprising that the majority of previous studies examining the impact of neonatal noxious insult have been conducted exclusively in male rodents [8;26;27]. Our laboratory has hypothesized that sexually dimorphic organizational hormones may contribute to significant sex differences in response to noxious inflammatory insult [64;65;66;67]. Sex steroid hormones such as estrogens and androgens modulate prenatal and postnatal functional development and have potent influences on pain thresholds in male and female rats [68;69]. Prenatally, males experience a significant surge of testicular testosterone that is centrally aromatized to estradiol and ultimately results in the masculinization of the male brain [64;65;66;67]. In females, the ovaries are quiescent and intracerebral estradiol remains low at birth [64;65;66;67]. Similar differences in hormone levels may also be present in peripheral tissues as well. Given that estrogens have been shown to exert neuromodulatory and neuroprotective effects following acute and chronic central injuries, increased perinatal central estradiol in males may contribute to lasting sexually dimorphic responses to early life noxious stimulation [64;70;71].

To our knowledge, we reported for the first time that neonatal inflammatory insult was indeed sexually dimorphic, with females displaying significantly greater basal hypoalgesia in adulthood in comparison to males. The paw withdrawal latency of females injured with 1% CGN was more than 3 seconds longer in both the inflamed and intact hindpaws compared to injured males [25]. Moreover, we showed that female rats injured at P14, when estradiol concentrations are comparable in males and females, displayed equivalent levels of baseline hypoalgesia as injured males. This further suggests that sex differences in the neonatal neuroendocrine environment contribute to the observed sexually dimorphic impact of neonatal inflammatory insult [25]. Estrogen also influences the expression of a number of pro-inflammatory as well as pro-nociceptive agents that may contribute to sex differences in nociceptive responses. For example, prostaglandins (which are pro-inflammatory) are released peripherally in response to injury, and estrogen has been shown to modulate both prostaglandin and COX-1 and COX-2 expression in peripheral tissues [72]. In adults, estrogens modulate vascular tone in a tissue specific manner (vasodilation, vasoconstriction), which may lead to differences in inflammation-induced edema [71]. Peripheral injury also results in increased BDNF that is thought to promote neuronal survival and healing. As estradiol increases BDNF expression centrally, this may also attenuate the adverse effect of peripheral injury [73].

In addition, activational gonadal hormones can alter the processing of nociceptive information. Sex-steroids influence endogenous opioid systems [74;75], as well as the activity of other neuromodulators involved in nociceptive processing; including substance P, gamma-aminobutyric acid (GABA), glutamate, dopamine, serotonin and norepinephrine [76;77]. Moreover, gonadal hormones have been shown to have a marked influence on estrous cycle

effects on nociceptive and analgesic sensitivity in rodents [78;79], as well as menstrual cycle variability in chronic pain conditions such as migraine headache [80], temporomandibular disorders [81], and fibromyalgia [82].

Thus, several mechanisms may contribute to a sexually dimorphic effect of neonatal noxious insult, including sex differences in the neuroendocrine environment at the time of injury and/or at the time of testing. While there are no reported sex differences in response to early life noxious stimulation in premature infants, primarily because of the small sample sizes that are unable detect sexual dimorphic effects, the aforementioned studies suggest that the lasting impact of procedural pain experienced in the NICU may indeed be sexually dimorphic. In addition, all of the previous experimental rodent studies that have examined the lasting consequences of neonatal noxious insult on developing nociceptive circuits have been conducted exclusively in males. Hence, the inclusion of female subjects in basic and clinical research studies examining this topic is warranted, as premature females may be at considerably increased risk for long-term consequences of early life trauma.

Increased Endogenous Opioid Tone: A Potential Mechanism for the Neonatal Injury-Induced Deficits in Nociceptive Responsiveness

While the impact of neonatal noxious stimulation on developing nociceptive circuitry and subsequent pain processing and perception has been the focus of a significant amount of research within the last decade, clinical and experimental studies have failed to elucidate the mechanisms underlying the reported lasting alterations in nociceptive responsiveness. The periaqueductal gray (PAG), and its descending projections to the rostral ventromedial medulla (RVM) and the spinal cord dorsal horn, constitute a primary anatomical circuit for the descending modulation of pain [83]. The PAG is rich in nerve terminals and fibers containing endogenous opioids [84], and opioid receptors are localized throughout the rostral-caudal axis of the PAG [85]. Interestingly, while in rats the anatomical connections for nociceptive modulation are present at birth, descending inhibitory controls are functionally immature throughout the first postnatal weeks [86;87]. The delayed maturation of descending inhibition may therefore contribute to the increased vulnerability of the immature somatosensory system to excessive afferent input, whereby exposure to neonatal noxious stimulation during a critical window may alter the functional integrity of endogenous descending inhibitory systems. Indeed, our laboratory hypothesized that neonatal injury during this critical developmental period (P0-P8) [25] results in increased afferent drive to CNS sites responsive to noxious input (eg. PAG). This increased drive results in the activation of endogenous pain inhibitory circuits and the subsequent release of endogenous opioid peptides. As the inflammation associated with intraplantar carrageenan is persistent (lasting approximately 24-48 hours), the release of endogenous opioids is sustained, and this continuous opioid release, during a time of increased developmental plasticity, is subsequently maintained into adulthood. This is supported by mounting behavioral data [8;25]. Specifically, the observable hypoalgesia following neonatal injury is limb non-specific (present in both the forepaws and hindpaws) and global in nature (somatic and visceral) [10;25]. Therefore, it appears to involve multiple segmental levels of the spinal cord, various dermatomes, and occurs bilaterally. Indeed these results are not easily explained by the induced unilateral neonatal insult that impacts few ipsilateral spinal levels. Consequently, the hypoalgesia appears better explained by alterations in descending nociceptive circuitry, such as that arising from the PAG, that produce global, limb-non-specific analgesia along the entire axis when activated.

Parallel studies have reported that increased afferent drive during the developmental critical period results in the reorganization of somatosensory circuits in adulthood, and interestingly, changes in endogenous pain modulation in humans as a consequence of neonatal pain has been

previously proposed to account for the long term changes in pain sensitivity observed in NICU infants exposed to frequent noxious interventions [88].

We have recently reported a significant increase in PAG opioid peptide expression (beta-endorphin and met/leu-enkephalin) as a consequence of neonatal injury [89]. It was notable that alterations in the endorphin and enkephalinergic systems were very well correlated across similar regions and levels of the PAG, suggesting that the two opioidergic systems act in parallel in response to neonatal inflammation and nociception. While comparable studies cannot be conducted in neonates, previous studies in adult rats have also reported that hindpaw inflammation results in upregulated biosynthesis of pro-dynorphin and pro-enkephalin in dorsal horn neurons [90;91], and a significant increase in endogenous opioid peptide release within the PAG [92]. Noxious stimulation-induced changes in opioid peptide expression are also paralleled by an increase in mRNA expression [90]. As stated above, our working hypothesis is that neonatal inflammation results in the release of endogenous opioid peptides within the PAG as a mechanism of decreasing nociception. Surprisingly, a parallel decrease in opioid receptor expression was also noted. Previous studies have similarly reported a long-term increase in endogenous opioid peptide along with a concomitant decrease in mu and delta opioid receptor density in the lateral hypothalamus of offspring following gestational stress [93;94].

Alternatively, however, opioid receptors are rapidly internalized following ligand binding, which would also result in a concomitant decrease in opioid receptor availability. Taken together, these studies suggest that early life stressors can confer long-lasting changes in supraspinal opioidergic circuits that are reflected by changes in peptide and receptor expression. Interestingly, we reported a significantly greater increase in met-enkephalin observed in neonatally injured females compared to males. This differential change in peptide expression may contribute to the increased hypoalgesia observed in females [25]. No significant sex difference in met-enkephalin immunoreactivity was present at baseline, indicating that the observed sex differences were injury-induced. In parallel behavioral studies, we found that intra-PAG administration of the opioid antagonist naloxone significantly reduced neonatal injury-induced hypoalgesia, further implicating the PAG as the primary site whereby neonatal injury permanently alters somatosensory processing [89].

While these results strongly suggest that persistent alterations in baseline nociceptive thresholds associated with neonatal inflammatory insult are mediated by a central increase in endogenous opioid tone, additional mechanisms may also contribute. Previous studies have demonstrated that early life insult also results in increased serotonergic receptor expression in the PAG [95], as well as upregulated GABA, serotonin, opioid, neuropeptide Y, tachykinin and interleukin systems at the spinal level [96]. Therefore, a multitude of alterations at the levels of the brain and spinal cord may contribute to the observable behavioral alterations in adulthood following neonatal noxious insult.

Additionally, noxious neonatal experiences lead not only to decreased nociceptive sensitivity in adulthood, but also to significant alterations in the behavioral and neuroendocrine responses to stress [6;22;97;98;99;100]. Blunted emotionality, decreased anxiety, and reduced basal and stress induced plasma corticotropin releasing factor (CRF) and adrenocorticotropin hormone (ACTH) are displayed in adult rats following short-lasting, local inflammation experienced during the first week of life [6;95]. Premature infants with extensive NICU care also exhibit low basal levels of stress hormones at 3 months of age compared to their full-term counterparts [101]. Thus, alterations in the developing hypothalamic-pituitary-adrenal axis may also contribute to the long-term basal hypoalgesia following neonatal hindpaw inflammation. Indeed, our preliminary data suggests that neonatal noxious stimulation produces a generalized reduction in reactivity to non-life threatening aversive environmental stimuli due to parallel

alterations in supraspinal nociceptive and stress modulatory circuits [8;95]. For example, neonatally injured male and female rats tested in adulthood display reduced anxiety in the open field and elevated plus maze, as indicated by an increase in time spent in the open areas. By contrast, in the forced swim test, injured animals display significantly shorter latencies to immobility suggesting a hyper-response to a strong physiological stressor (unpublished observations). Furthermore, corticosterone (CORT) levels were significantly blunted in injured females at baseline and following restraint stress. Anseloni et al [85] similarly reported that neonatally-injured animals displayed reduced anxiety behavior, however found increased latency to immobility to the forced swim test. The reasons for the discrepant results are not known, but may include differences in the type of injury paradigm employed, or in the age at the time of injury. Regardless, together these studies indicate that neonatal injury alters adulthood stress and anxiety-related behaviors in a sexually dimorphic manner, and contributes to mounting evidence that neonatal trauma in the absence of analgesics has long-term polysystemic adverse effects.

Clinical Implications for Changes in Endogenous Opioid Tone

Our studies have shown that persistent pain experienced early in life results in decreased mu and delta receptor availability, suggesting a persistent activation of opioid receptors due to enhanced release of endogenous opioid peptides. This decrease in receptor availability is supported by our previous findings demonstrating a significant rightward shift in the morphine dose-response curve in adult animals that were injured neonatally [102]. Similar results have been reported in children, in that the number of invasive procedures experienced in the neonatal intensive care unit are inversely correlated with morphine effectiveness [29]. Despite the current knowledge that neonates are responsive to noxious stimuli, the majority of routine procedures, including repeated heel-lances, endotracheal intubations and minor surgeries are performed in the absence of analgesics [15]. Indeed recent studies have reported that neonates receiving NICU treatment experience an average of 14 noxious procedures per day, with fewer than 35% receiving appropriate analgesic therapy [15]. The findings that neonatal injury results in long-term changes in opioid tone and hypoalgesia have serious implications for future pain management in neonates.

Effects Of Analgesia On Developing Nociceptive Circuitry

Despite the current knowledge that preterm infants are responsive to noxious stimulation [17;103;104] and the accumulating evidence that invasive procedures can have lasting effects on developing nociceptive circuitry, neonatal pain remains an under-recognized and under-treated condition in the NICU [15;105;106]. Indeed, many life-saving intensive care interventions are performed in the absence of analgesics [15;105;106;107].

Conflicting evidence exists as to the clinical benefits of opioid analgesia in premature infants undergoing invasive procedures in the NICU [29;108;109;110;111]. For example, altered pain responses in former preterm neonates can be predicted by the number of previous painful procedures and are normalized by the early use of morphine as an analgesic [29]. In addition, post-operative morphine analgesia in preterm and full-term infants reduces behavioral and hormonal stress responses [112;113;114] and is associated with decreased mortality [115; 116]. Furthermore, 45 month-old children that experienced operations following pre-emptive analgesia during early life respond to immunization pain in a similar manner as non-operated age-matched controls [117]. Lastly, the long-term outcomes at 5-6 years of age of formerly preterm children who were exposed to morphine in the neonatal period indicate no adverse effects of morphine on intelligence, motor function, cognitive development or other behavioral outcomes [29;30;110;118].

Alternatively, morphine is not recommended as a standard of care for acute pain resulting from invasive procedures in ventilated preterm newborns [119;120]. Further conflicting evidence exists on the long-term effects of early opiate exposure on later cognitive and motor abilities in preterm infants. Specifically, higher morphine exposure is associated with poorer motor development at 8 months, but not 18 months of age [109]. Similarly, neonatal morphine analgesia contributes to subtle neurobehavioral differences, including altered motor function, in preterm infants at 36 weeks [111]. Parallel evidence in rodents suggests delays in motor development following early morphine exposure [121], and a recent study demonstrated marked learning impairments in passive avoidance and forced swim tasks in adulthood following neonatal exposure to opiates [122].

Clearly, the aforementioned studies imply the importance of additional research to evaluate both the short-term and long-term effects of morphine analgesia on the neurobehavioral outcomes of prematurity, specifically the impact of neonatal opiate exposure on motor and cognitive development. Further studies examining the effects of pre-emptive analgesics in the NICU are challenging, however, as the humane care of infants requires physicians to treat those perceived to be in distress. As such, the gaps in our knowledge of the long-term risks and benefits of analgesic therapy in newborns would greatly benefit from experimental animal models, as very few studies have examined whether opioid analgesics can be used to prevent the long-term sequelae associated with neonatal noxious insult [100].

We have previously demonstrated that pre-emptive morphine administration blocks neonatal injury induced thermal and mechanical hypoalgesia in both the injured and uninjured paws in adolescence (P40) and adulthood (P60) [99]. Moreover, morphine attenuation of the hypoalgesia was reported to be comparable in males and females [90]. These results are consistent with previous studies in rodents that report daily morphine administration prior to intraplantar formalin during the first week of life significantly reduces the long-term effects of repetitive pain [123]. Previous studies in humans have also reported that morphine therapy ameliorates the effects of early repetitive noxious stimuli in extremely low birth weight infants at 4 months of age [29]. Similarly, children who had minor neonatal operations and received pre-emptive analgesia responded to immunization pain in a similar manner as non-operated age-matched controls [117]. The ability of pre-emptive morphine to block the hypoalgesia may indeed occur through direct modulation of primary afferent drive into the spinal cord, thereby inhibiting the central relay of inflammatory pain and preventing the subsequent increase in descending endogenous opioid tone [99]. However, the effects of morphine in these studies may partly be a consequence of modification of the inflammatory response and/or the stress response to neonatal inflammatory insult [124;125].

Neonatal morphine has also been shown to significantly attenuate CFA- induced hyperalgesia and increased the rate of recovery, such that both males and females recover 7 days faster than saline treated injured controls [99]. As previously stated, increased primary afferent innervation of the spinal cord dorsal horn following neonatal inflammatory insult may account for our observed hyperalgesia in adulthood [25]. Administration of morphine at the time of injury would be expected to inhibit this increase in primary afferent input, thereby preventing the entire cascade of behavioral, physiological and anatomical deficits associated with neonatal inflammation. In regard to recovery, clinical reports demonstrate that at 32 weeks of age, preterm infants experience a reduced rate of recovery to skin breaking procedures [126], and exhibit subtle differences in ability to recover from finger lance at 4 months compared to full term controls [30]. There are no reports on the impact of pre-emptive morphine on recovery rates in premature neonates; however, these data suggest that morphine analgesia may in fact significantly increase the rate of recovery following procedural pain in NICU infants.

While neonatal morphine administration does not significantly alter morphine's antinociceptive effects in adulthood in males or females (i.e. no significant shift in ED50 values), interestingly, a significant rightward shift in ED50 is noted in neonatally injured animals that do *not* receive neonatal morphine [99]. These results have serious clinical implications. Previous studies have reported that pre-term infants that experience surgery during the first three months of life have significantly higher peri- and post-operative analgesic requirements in response to surgery in the same or different dermatome compared to control infants [37;114]. Similarly, mice exposed to chronic noxious stimulation display increased tail flick latencies compared to control animals, and a significant two-fold increase in the ED50 of morphine in response to abdominal constriction [127]. As noxious stimulation during the neonatal period leads to increased activation of opioid systems in a manner analogous to the repeated application of exogenous opiates, these studies suggest that neonatal injury produces cross-tolerance to the analgesic effects of morphine thereby decreasing the subsequent effectiveness of morphine [128;129;130]. Again interestingly, exposure to *morphine* neonatally does not result in a significant shift in ED50 values. Therefore, it appears that opioid cross tolerance may be associated with neonatal injury-induced chronic exposure to *endogenous* opioids resulting from a potentiation of the descending inhibitory circuit, and not a result of exposure to morphine on P0. Alternatively, neonatal stress associated with maternal separation and repeated handling has also been suggested to reduce opioid analgesia [99;131]. This suggests that alterations in opioid analgesia may reflect a combined effect of neonatal nociceptive experience as well as early life stress, and may involve altered responsiveness of endogenous analgesia circuits as well as the hypothalamic-pituitary-adrenal axis [4].

Final Remarks

Although research into the long-term consequences of noxious stimulation during the neonatal period have spanned over two decades, our understanding of neonatal pain is literally still in its infancy. The studies presented in this review have established that exposure to neonatal noxious insult is associated with a lasting alteration in both basal nociceptive sensitivity and response to a subsequent injury in adulthood (See Figure 1). Moreover, the impact of neonatal injury appears to be significantly exacerbated in females in comparison to males. The clear presence of a sex difference in the response to early insult may indeed contribute to the higher prevalence, severity and duration of pain syndromes observed in women than men. Furthermore, the profound alterations of nociceptive thresholds following neonatal inflammation may be mediated by an experience-induced facilitated activation of descending nociceptive pathways, characterized by dynamic physiological and anatomical modification and modulation of opioidergic systems in the PAG. Finally, pre-emptive analgesia has been shown to ameliorate the long-term effects of neonatal injury on adult nociception, which provides compelling justification for the use of analgesics prior to the initiation of noxious procedures performed on neonates. Collectively, these studies present valuable information about the long-term consequences of neonatal noxious stimulation, which may ultimately lead to improved understanding and treatment of the lasting effects of repeated invasive interventions in premature infants in the NICU.

Acknowledgments

This work was supported by National Institute of Health grants DA16272 and AR49555 awarded to Anne Z. Murphy, the Center for Behavioral Neuroscience NSF: IBN 9876754, and the Georgia State University Brains and Behavior Program.

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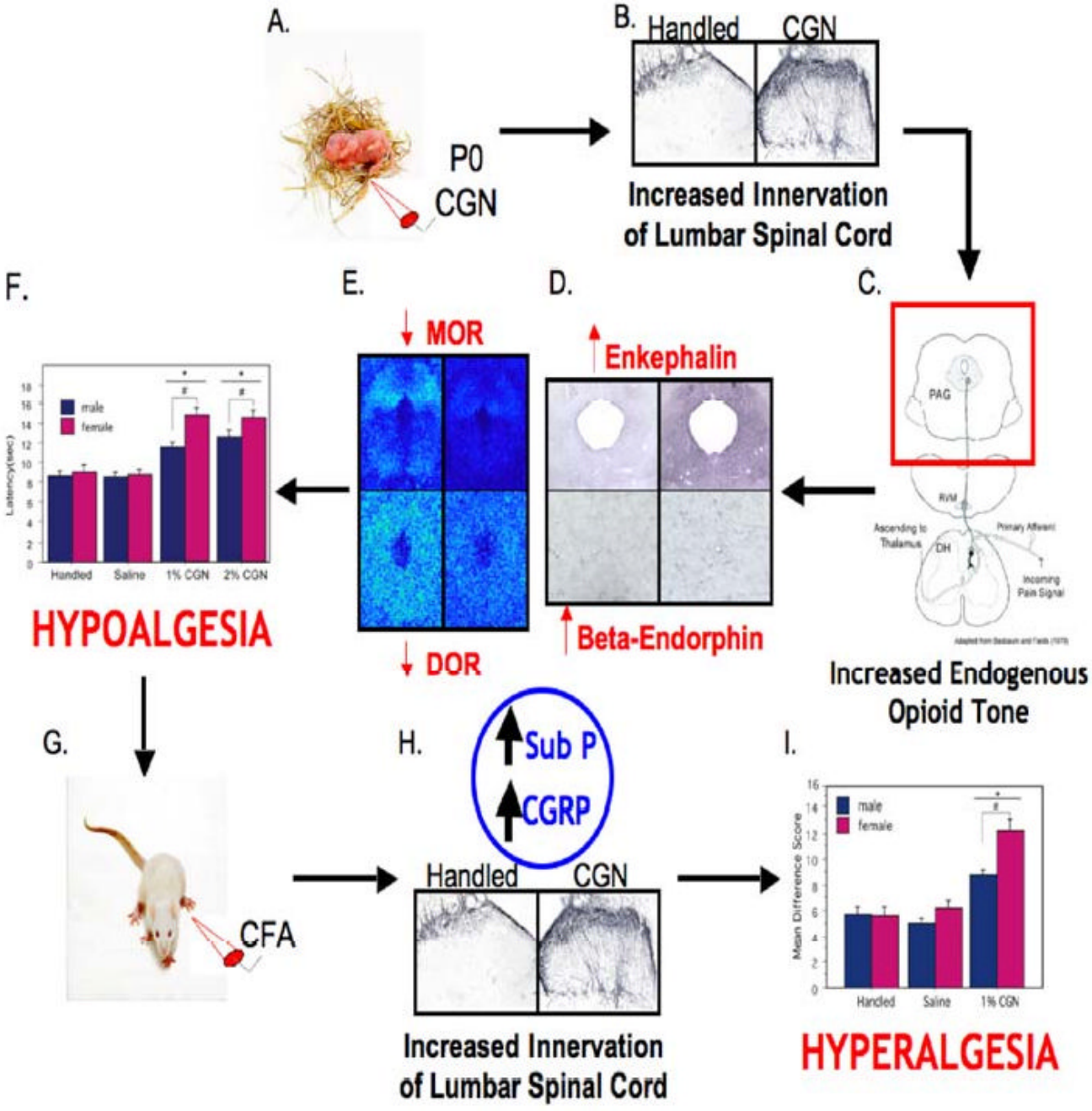

Figure 1.

The Lasting Impact of Neonatal Inflammatory Insult: A Summary. (A) Intraplantar carrageenan (CGN) on the day of birth (P0) results in (B: left-handled; right-1% CGN) a lasting increase in primary afferent innervation of the dorsal horn of the lumbar spinal cord, ultimately leading to (C) an increase in endogenous opioid tone which is characterized by (D: top left-met enkephalin handled; top right-met enkephalin 1% CGN; bottom right-beta endorphin 1% CGN; bottom left-beta endorphin handled) a significant increase in enkephalin and beta endorphin immunoreactivity and (E: top left-MOR handled; top right-MOR 1% CGN; bottom right-DOR 1% CGN; bottom left-DOR handled) a significant decrease in mu and delta opioid receptor binding in the PAG. This increase in opioid tone contributes to the (F) observed

hypoalgesia at baseline testing. (G) In the presence of a subsequent major noxious insult in adulthood, (H: left-handled; right-1% CGN) neonatally injured animals have increased release of pro-nociceptive peptides (i.e. CGRP and substance P) compared to handled animals, resulting in (I) enhanced hyperalgesia following intraplantar CFA.

Front Neuroendocrinol. Author manuscript; available in PMC 2011 April 1.

Table 1

Neonatal Pain: Human Studies

Term	NICU	Age At Testing	Type Testing	Results	Citation
preterm <28 GA	yes	32 wks PCA	CORT, facial & cardiac reactivity to blood collection	dec. CORT, dec. facial reacitvty	4
preterm & fullterm	yes	P1-P14	painful procedures & anagesia administered	ave NICU infant 14 painful procedures/day & <35% receive anagesia	15
gestation	no	GW18-37	plasma NE to intrahepatic vein insertion	inc. NE in response to vein insertion	17
preterm 28-36 GA	yes	24-48 hrs old	Near Infrared Spectroscopy (NIRS) to venipuncture	inc. blood flow in SC, M>F	18
preterm 25-45 GA	yes	P1-P7	NIRS to heel lance	inc. cerebral oxygenation in SC at 25 weeks GA	19
preterm	yes	>5 years old	cognitive and behavioral	preterm children reduced cognitive scores and inc. ADHD	23
preterm	yes	18 mo CA	parental pain ratings	ELBW preterm toddlers dec pain sensitivity	24
preterm	yes	PW1-8	facial and heart rate response to heel lance	dec. facial & HR response over 8 week period	28
preterm	yes	32 wks PCA	facial and heart rate response to heel lance	inc. NICU pain results in dec facial & HR response	29
preterm	yes	4 mo CA	facial and heart rate response to finger lance	ELBW infants display dec. para & inc. symp responses	30
preterm	yes	28-49 wks PCA	cutaneous flexion reflex	inc. sensitivity with limb tissue damage	31
fullterm	no	<1 year old	mechanical abdominal skin reflex	dec. mechanical reflex thresold at wound site	32
preterm 25-34 GA	yes	27-39 wks PCA	cutaneous flexion reflex	infants <29 wks PCA have low thresholds, inc. with PCA	34
preterm & fullterm	yes	27-42 wks PCA	cutaneous flexion reflex	repeated mechanical stimulation lowers thresholds in infants <35 wks	35
fullterm	no	<3 years old	morphine & pain to surgery	inc. morphine, inc. pain following operations	36
fullterm	no	30-95 wks PCA	mechanical abdominal skin reflex	dec. thresholds in abdomen of infants with UH	37
fullterm	no	4-6 mo	pain ratings to routine innoculation	inc. pain in circumcized males to innoculation	38
preterm & fullterm	yes	11-14 years old	withdrawl thresholds to thermal stimuli	infants with NICU exp show inc. thresholds for acute thermal stimuli	39
preterm & fullterm	yes	12-18 years old	tenderness thresholds by dolorimeter	preterm children more tender points & lower tender thresholds, F>M	40
preterm & fullterm	yes	3, 4.5 years old	somatization scores	ELBw preterm children higher clinical somatization	41
preterm & fullterm	yes	8-15 years old	migraine suffering	former NICU children have earlier onset of pediatric migraines	42
preterm & fullterm	yes	8-10 years old	Pediatric Pain Inventory-rating painful events	NICU children rate medical more intense than psychosocial pain	43
preterm <33 GA	yes	3,6,8,18 mo	salivary cortisol	NICU infants low basal cortisol at 3 mo	98
fullterm	no	45 mo	immunization pain	early surgical exp with analgesia does not affect iimmunization pain	114
preterm <26 GA	yes	7-11 years old	sensory testing-thermal & mechanical	dec. sensitivity thermal not mechanical stimuli	125
preterm & fullterm	yes	9-12 years old	sensory testing-thermal & mechanical	dec. sensitivity to thermal & mechanical stimuli	126

KEY:

CORT-cortisol

GA-gestational age

PCA-post conceptional age

wks-weeks

ave-average

exp-experience

GW-gestational week

NE-norepinephrine

SC-somatosensory cortex

mo-months

CA-corrected age

P-postnatal day

PW-postnatal week

HR-heart rate

NICU-neontal intensive care unit

ELBW-extremely low birth weight

para-parasympathetic

symp-sympathetic

UH-unilateral hydronephrosis

Table 2

Neonatal Pain: Experimental Animal Studies

Species	Model	Age At Injury	Age At Testing	Results	Citation
rat	repeated intaplantar formalin	P1-P7	P74-P95	inc. HPL, M>F	6
rat	intraplantar CGN	P3	P41,P63,P125	inc. PWL to mech & therm stimuli (hindpaws)	8
rat	intraplantar CFA	P1	P56	inc. nerve terminal fields lamina II in DH	9
rat	intraplantar CGN	P3	P60	inc. CRD thresholds	10
rat	repeated heelsticks	P0-P7	P16,P22,P65	dec. HPL & inc. preference for alcohol	22
rat	intraplantar CGN & Adult CFA	P0	P40, P60	bsln inc. PWL (hindpaws), inc. hyperalgesia to CFA; F>M	25
rat	intraplantar CGN & Adult CFA	P0	P60	bsln inc. PWL, inc. hyperalgesia to CFA	26
rat	intraplantar CFA	P0,P3	P60	unilateral inc. primary afferents in laminae I & II in DH	27
rat	hindpaw skin wounds	P1	P19, P40	inc. DH receptive fields	44
rat	hindpaw skin wounding	P1	P1-P4	inc. NGF in hindpaw skin	45
rat	hindpaw skin wounding	P0,P7	P84	hyperinnervation of hindpaw, dec PWL to mech stimuli	46
rat	chemical irritation-colon	P8-P21	P60	visceral hyperalgesia	48
rat	daily footshock	P1-P21	P90	inc. HPL, inc. morphine analgesia	49
mouse	laparotomy	P1	P80	inc. TFL & HPL, dec. pain response to acidic acid	50
rat	intraplantar CGN	P0	P60	inc. primary afferents in DH (L3-L5) spinal cord	51
rat	hot plate (2× daily)	P1-P15	P90	inc. acitve avoidance learning	94
mouse	repeated heelsticks	P8-P14	P30	inc. anxiety in EPM	95
rat	intraplantar CGN	P3	P50	dec. anxiety in EPM & inc. stress coping in FST	97
rat	morphine + intraplantar CGN	P0	P60	dec. morphine analgesia	99
rat	morphine + intraplantar formalin	P0-P7	P1-P20	dec. hyperlagesia	116
rat	intraperitoneal lipopolysaccharide	P14	P56-P84	dec. PWL to mech & therm stimuli	127
rat	intraplantar CGN	P0	P60	inc. BE & ENK, dec. MOR and DOR in PAG	128

KEY:

TFL-tail flick latency

inc-increased

dec-decreased

CGN-carrageenan

PWL-paw withdrawal latency

mech-mechanical

therm-thermal

CFA-Complete Freund's Adjuvant

bsln-baseline

F-female

M-male

DR-dose response

CRD-colorectal distention

HPL-hotplate latency

NGF-nerve growth factor

EPM-elevated plus maze

FST-forced swim task

BE-beta-endorphin

ENK-enkephalin

MOR- mu opioid receptor

DOR-delta opiood receptor

PAG-periaqueductal gray