2010

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

Jamie L. LaPrairie

Anne Z. Murphy PhD
Georgia State University, amurphy@gsu.edu

Recommended Citation

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

and Clinical Implications

Jamie L. LaPrairie, Anne Z. Murphy*

Neuroscience Institute
Georgia State University
38 Peachtree Center Ave, 806 GCB
Atlanta, GA 30303

Correspondence should be addressed to:
Anne Z. Murphy, Ph.D.
Neuroscience Institute
Georgia State University
38 Peachtree Center Ave
Atlanta, GA 30303
Phone: 404.413.5332
Fax: 404.413.
amurphy@gsu.edu

Number of Figures/Tables: 3
Number of Pages: 32

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.

Key Words: 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], 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.

Literature Cited

[1] R.E. Grunau, L. Holsti, and J.W. Peters, Long-term consequences of pain in human neonates. Semin Fetal Neonatal Med 11 (2006) 268-75.
[2] K.J. Anand, Clinical importance of pain and stress in preterm neonates. Biol Neonate 73 (1998) 1-9.
[3] K.J. Anand, Pain, plasticity, and premature birth: a prescription for permanent suffering? Nat Med 6 (2000) 971-3.
[4] R.E. Grunau, L. Holsti, D.W. Haley, T. Oberlander, J. Weinberg, A. Solimano, M.F. Whitfield, C. Fitzgerald, and W. Yu, Neonatal procedural pain exposure predicts lower cortisol and behavioral reactivity in preterm infants in the NICU. Pain 113 (2005) 293-300.
[5] M.F. Whitfield, and R.E. Grunau, Behavior, pain perception, and the extremely low-birth weight survivor. Clin Perinatol 27 (2000) 363-79.
[6] A.T. Bhutta, C. Rovnaghi, P.M. Simpson, J.M. Gossett, F.M. Scalzo, and K.J. Anand, Interactions of inflammatory pain and morphine in infant rats: long-term behavioral effects. Physiol Behav 73 (2001) 51-8.
[7] M.S. Lidow, Long-term effects of neonatal pain on nociceptive systems. Pain 99 (2002) 377-83.
[8] K. Ren, V. Anseloni, S.P. Zou, E.B. Wade, S.I. Novikova, M. Ennis, R.J. Traub, M.S. Gold, R. Dubner, and M.S. Lidow, Characterization of basal and re-inflammation-associated long-term alteration in pain responsivity following short-lasting neonatal local inflammatory insult. Pain 110 (2004) 588-96.
[9] S.M. Walker, J. Meredith-Middleton, C. Cooke-Yarborough, and M. Fitzgerald, Neonatal inflammation and primary afferent terminal plasticity in the rat dorsal horn. Pain 105 (2003) 185-95.
[10] G. Wang, Y. Ji, M.S. Lidow, and R.J. Traub, Neonatal hind paw injury alters processing of visceral and somatic nociceptive stimuli in the adult rat. J Pain 5 (2004) 440-9.
[11] J.A. Martin, B.E. Hamilton, P.D. Sutton, S.J. Ventura, F. Menacker, and S. Kirmeyer, Births: final data for 2004. Natl Vital Stat Rep 55 (2006) 1-101.
[12] P.H. Shiono, M.A. Klebanoff, R.P. Nugent, M.F. Cotch, D.G. Wilkins, D.E. Rollins, J.C. Carey, and R.E. Behrman, The impact of cocaine and marijuana use on low birth weight and preterm birth: a multicenter study. Am J Obstet Gynecol 172 (1995) 19-27.
[13] T.J. Mathews, and M.F. MacDorman, Infant mortality statistics from the 2003 period linked birth/infant death data set. Natl Vital Stat Rep 54 (2006) 1-29.
[14] D.P. Barker, and N. Rutter, Exposure to invasive procedures in neonatal intensive care unit admissions. Arch Dis Child Fetal Neonatal Ed 72 (1995) F47-8.
[15] S.H. Simons, M. van Dijk, K.S. Anand, D. Roofthooft, R.A. van Lingen, and D. Tibboel, Do we still hurt newborn babies? A prospective study of procedural pain and analgesia in neonates. Arch Pediatr Adolesc Med 157 (2003) 1058-64.
[16] K.J. Anand, and D.B. Carr, The neuroanatomy, neurophysiology, and neurochemistry of pain, stress, and analgesia in newborns and children. Pediatr Clin North Am 36 (1989) 795-822.
[17] X. Giannakoulopoulos, J. Teixeira, N. Fisk, and V. Glover, Human fetal and maternal noradrenaline responses to invasive procedures. Pediatr Res 45 (1999) 494-9.

[18] M. Bartocci, L.L. Bergqvist, H. Lagercrantz, and K.J. Anand, Pain activates cortical areas in the preterm newborn brain. Pain 122 (2006) 109-17.

[19] R. Slater, A. Cantarella, S. Gallella, A. Worley, S. Boyd, J. Meek, and M. Fitzgerald, Cortical pain responses in human infants. J Neurosci 26 (2006) 3662-6.

[20] M. Fitzgerald, Painful beginnings. Pain 110 (2004) 508-9.

[21] M. Fitzgerald, and E. Jennings, The postnatal development of spinal sensory processing. Proc Natl Acad Sci U S A 96 (1999) 7719-22.

[22] K.J. Anand, V. Coskun, K.V. Thrivikraman, C.B. Nemeroff, and P.M. Plotsky, Long-term behavioral effects of repetitive pain in neonatal rat pups. Physiol Behav 66 (1999) 627-37.

[23] A.T. Bhutta, M.A. Cleves, P.H. Casey, M.M. Cradock, and K.J. Anand, Cognitive and behavioral outcomes of school-aged children who were born preterm: a meta-analysis. Jama 288 (2002) 728-37.

[24] R.V. Grunau, M.F. Whitfield, and J.H. Petrie, Pain sensitivity and temperament in extremely low-birth-weight premature toddlers and preterm and full-term controls. Pain 58 (1994) 341-6.

[25] J.L. LaPrairie, and A.Z. Murphy, Female rats are more vulnerable to the long-term consequences of neonatal inflammatory injury. Pain 132 Suppl 1 (2007) S124-33.

[26] M.S. Lidow, Z.M. Song, and K. Ren, Long-term effects of short-lasting early local inflammatory insult. Neuroreport 12 (2001) 399-403.

[27] M.A. Ruda, Q.D. Ling, A.G. Hohmann, Y.B. Peng, and T. Tachibana, Altered nociceptive neuronal circuits after neonatal peripheral inflammation. Science 289 (2000) 628-31.

[28] C.C. Johnston, B. Stevens, F. Yang, and L. Horton, Developmental changes in response to heelstick in preterm infants: a prospective cohort study. Dev Med Child Neurol 38 (1996) 438-45.

[29] R.E. Grunau, T.F. Oberlander, M.F. Whitfield, C. Fitzgerald, and S.K. Lee, Demographic and therapeutic determinants of pain reactivity in very low birth weight neonates at 32 Weeks' postconceptional Age. Pediatrics 107 (2001) 105-12.

[30] T.F. Oberlander, R.E. Grunau, M.F. Whitfield, C. Fitzgerald, S. Pitfield, and J.P. Saul, Biobehavioral pain responses in former extremely low birth weight infants at four months' corrected age. Pediatrics 105 (2000) e6.

[31] S.M. Walker, L.S. Franck, M. Fitzgerald, J. Myles, J. Stocks, and N. Marlow, Long-term impact of neonatal intensive care and surgery on somatosensory perception in children born extremely preterm. Pain 141 (2009) 79-87.

[32] K. Andrews, and M. Fitzgerald, Cutaneous flexion reflex in human neonates: a quantitative study of threshold and stimulus-response characteristics after single and repeated stimuli. Dev Med Child Neurol 41 (1999) 696-703.

[33] K. Andrews, and M. Fitzgerald, Wound sensitivity as a measure of analgesic effects following surgery in human neonates and infants. Pain 99 (2002) 185-95.

[34] M. Fitzgerald, C. Millard, and N. MacIntosh, Hyperalgesia in premature infants. Lancet 1 (1988) 292.

[35] M. Fitzgerald, A. Shaw, and N. MacIntosh, Postnatal development of the cutaneous flexor reflex: comparative study of preterm infants and newborn rat pups. Dev Med Child Neurol 30 (1988) 520-6.

[36] K. Andrews, and M. Fitzgerald, The cutaneous withdrawal reflex in human neonates: sensitization, receptive fields, and the effects of contralateral stimulation. Pain 56 (1994) 95-101.

[37] J.W. Peters, R. Schouw, K.J. Anand, M. van Dijk, H.J. Duivenvoorden, and D. Tibboel, Does neonatal surgery lead to increased pain sensitivity in later childhood? Pain 114 (2005) 444-54.

[38] K.A. Andrews, D. Desai, H.K. Dhillon, D.T. Wilcox, and M. Fitzgerald, Abdominal sensitivity in the first year of life: comparison of infants with and without prenatally diagnosed unilateral hydronephrosis. Pain 100 (2002) 35-46.

[39] A. Taddio, M. Goldbach, M. Ipp, B. Stevens, and G. Koren, Effect of neonatal circumcision on pain responses during vaccination in boys. Lancet 345 (1995) 291-2.

[40] C. Hermann, J. Hohmeister, S. Demirakca, K. Zohsel, and H. Flor, Long-term alteration of pain sensitivity in school-aged children with early pain experiences. Pain 125 (2006) 278-85.

[41] D. Buskila, L. Neumann, E. Zmora, M. Feldman, A. Bolotin, and J. Press, Pain sensitivity in prematurely born adolescents. Arch Pediatr Adolesc Med 157 (2003) 1079-82.

[42] S.B. Maneyapanda, and A. Venkatasubramanian, Relationship between significant perinatal events and migraine severity. Pediatrics 116 (2005) e555-8.

[43] R.E. Grunau, M.F. Whitfield, and J. Petrie, Children's judgements about pain at age 8-10 years: do extremely low birthweight (< or = 1000 g) children differ from full birthweight peers? J Child Psychol Psychiatry 39 (1998) 587-94.

[44] C. Torsney, and M. Fitzgerald, Spinal dorsal horn cell receptive field size is increased in adult rats following neonatal hindpaw skin injury. J Physiol 550 (2003) 255-61.

[45] J. Constantinou, M.L. Reynolds, C.J. Woolf, B. Safieh-Garabedian, and M. Fitzgerald, Nerve growth factor levels in developing rat skin: upregulation following skin wounding. Neuroreport 5 (1994) 2281-4.

[46] M.L. Reynolds, and M. Fitzgerald, Long-term sensory hyperinnervation following neonatal skin wounds. J Comp Neurol 358 (1995) 487-98.

[47] D.J. Whitby, and M.W. Ferguson, Immunohistochemical localization of growth factors in fetal wound healing. Dev Biol 147 (1991) 207-15.

[48] E.D. Al-Chaer, M. Kawasaki, and P.J. Pasricha, A new model of chronic visceral hypersensitivity in adult rats induced by colon irritation during postnatal development. Gastroenterology 119 (2000) 1276-85.

[49] L. Boisse, S.J. Spencer, A. Mouihate, N. Vergnolle, and Q.J. Pittman, Neonatal immune challenge alters nociception in the adult rat. Pain 119 (2005) 133-41.

[50] C. Shimada, S. Kurumiya, Y. Noguchi, and M. Umemoto, The effect of neonatal exposure to chronic footshock on pain-responsiveness and sensitivity to morphine after maturation in the rat. Behav Brain Res 36 (1990) 105-11.

[51] A.G. Hohmann, M.H. Neely, J. Pina, and A.G. Nackley, Neonatal chronic hind paw inflammation alters sensitization to intradermal capsaicin in adult rats: a behavioral and immunocytochemical study. J Pain 6 (2005) 798-808.

[52] C.D. Walker, K. Kudreikis, A. Sherrard, and C.C. Johnston, Repeated neonatal pain influences maternal behavior, but not stress responsiveness in rat offspring. Brain Res Dev Brain Res 140 (2003) 253-61.

[53] J. LaPrairie, and A. Murphy, Neonatal injury diffrentially affects male and female sensory thresholds and response to re-injury in adulthood Soc Neuroscience Abst (2005).

[54] P. Honor, P.M. Menning, S.D. Rogers, M.L. Nichols, A.I. Basbaum, J.M. Besson, and P.W. Mantyh, Spinal substance P receptor expression and internalization in acute, short-term, and long-term inflammatory pain states. J Neurosci 19 (1999) 7670-8.

[55] R.E. Grunau, T. Oberlander, L. Holsti, and M.F. Whitfield, Bedside application of the Neonatal Facial Coding System in pain assessment of premature neonates. Pain 76 (1998) 277-86.

[56] C. Caldji, B. Tannenbaum, S. Sharma, D. Francis, P.M. Plotsky, and M.J. Meaney, Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. Proc Natl Acad Sci U S A 95 (1998) 5335-40.

[57] D. Liu, J. Diorio, B. Tannenbaum, C. Caldji, D. Francis, A. Freedman, S. Sharma, D. Pearson, P.M. Plotsky, and M.J. Meaney, Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. Science 277 (1997) 1659-62.

[58] P.M. Plotsky, and M.J. Meaney, Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. Brain Res Mol Brain Res 18 (1993) 195-200.

[59] B. Bishop, Neural plasticity: Part 2. Postnatal maturation and function-induced plasticity. Phys Ther 62 (1982) 1132-43.

[60] T. Rabinowicz, G.M. de Courten-Myers, J.M. Petetot, G. Xi, and E. de los Reyes, Human cortex development: estimates of neuronal numbers indicate major loss late during gestation. J Neuropathol Exp Neurol 55 (1996) 320-8.

[61] K.J. Berkley, Sex differences in pain. Behav Brain Sci 20 (1997) 371-80; discussion 435-513.

[62] K.J. Berkley, A. Robbins, and Y. Sato, Afferent fibers supplying the uterus in the rat. J Neurophysiol 59 (1988) 142-63.

[63] A.M. Unruh, Gender variations in clinical pain experience. Pain 65 (1996) 123-67.

[64] S.K. Amateau, J.J. Alt, C.L. Stamps, and M.M. McCarthy, Brain estradiol content in newborn rats: sex differences, regional heterogeneity, and possible de novo synthesis by the female telencephalon. Endocrinology 145 (2004) 2906-17.

[65] J. Balthazart, and G.F. Ball, Is brain estradiol a hormone or a neurotransmitter? Trends Neurosci 29 (2006) 241-9.

[66] C.A. Cornil, G.F. Ball, and J. Balthazart, Functional significance of the rapid regulation of brain estrogen action: where do the estrogens come from? Brain Res 1126 (2006) 2-26.

[67] J. Weisz, and I.L. Ward, Plasma testosterone and progesterone titers of pregnant rats, their male and female fetuses, and neonatal offspring. Endocrinology 106 (1980) 306-16.

[68] A.M. Aloisi, I. Ceccarelli, and P. Fiorenzani, Gonadectomy affects hormonal and behavioral responses to repetitive nociceptive stimulation in male rats. Ann N Y Acad Sci 1007 (2003) 232-7.

[69] N.J. Liu, and A.R. Gintzler, Prolonged ovarian sex steroid treatment of male rats produces antinociception: identification of sex-based divergent analgesic mechanisms. Pain 85 (2000) 273-81.

[70] L.M. Garcia-Segura, I. Azcoitia, and L.L. DonCarlos, Neuroprotection by estradiol. Prog Neurobiol 63 (2001) 29-60.

[71] A. Maggi, P. Ciana, S. Belcredito, and E. Vegeto, Estrogens in the nervous system: mechanisms and nonreproductive functions. Annu Rev Physiol 66 (2004) 291-313.

[72] Y. Zhang, A. Shaffer, J. Portanova, K. Seibert, and P.C. Isakson, Inhibition of cyclooxygenase-2 rapidly reverses inflammatory hyperalgesia and prostaglandin E2 production. J Pharmacol Exp Ther 283 (1997) 1069-75.

[73] A.L. Allen, and K.E. McCarson, Estrogen increases nociception-evoked brain-derived neurotrophic factor gene expression in the female rat. Neuroendocrinology 81 (2005) 193-9.

[74] L.A. Berglund, H. Derendorf, and J.W. Simpkins, Desensitization of brain opiate receptor mechanisms by gonadal steroid treatments that stimulate luteinizing hormone secretion. Endocrinology 122 (1988) 2718-26.

[75] Y.R. Smith, J.K. Zubieta, M.G. del Carmen, R.F. Dannals, H.T. Ravert, H.A. Zacur, and J.J. Frost, Brain opioid receptor measurements by positron emission tomography in normal cycling women: relationship to luteinizing hormone pulsatility and gonadal steroid hormones. J Clin Endocrinol Metab 83 (1998) 4498-505.

[76] P. Duval, V. Lenoir, S. Moussaoui, C. Garret, and B. Kerdelhue, Substance P and neurokinin A variations throughout the rat estrous cycle; comparison with ovariectomized and male rats: I. Plasma, hypothalamus, anterior and posterior pituitary. J Neurosci Res 45 (1996) 598-609.

[77] S.S. Smith, Female sex steroid hormones: from receptors to networks to performance-- actions on the sensorimotor system. Prog Neurobiol 44 (1994) 55-86.

[78] R.M. Craft, J.S. Mogil, and A.M. Aloisi, Sex differences in pain and analgesia: the role of gonadal hormones. Eur J Pain 8 (2004) 397-411.

[79] R.B. Fillingim, and T.J. Ness, Sex-related hormonal influences on pain and analgesic responses. Neurosci Biobehav Rev 24 (2000) 485-501.

[80] E.A. MacGregor, Menstruation, sex hormones, and migraine. Neurol Clin 15 (1997) 125-41.

[81] M.P. Warren, and J.L. Fried, Temporomandibular disorders and hormones in women. Cells Tissues Organs 169 (2001) 187-92.

[82] U.M. Anderberg, Z. Liu, L. Berglund, and F. Nyberg, Plasma levels on nociceptin in female fibromyalgia syndrome patients. Z Rheumatol 57 Suppl 2 (1998) 77-80.

[83] A.I. Basbaum, and H.L. Fields, Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. Annu Rev Neurosci 7 (1984) 309-38.

[84] D.B. Reichling, G.C. Kwiat, and A.I. Basbaum, Anatomy, physiology and pharmacology of the periaqueductal gray contribution to antinociceptive controls. Prog Brain Res 77 (1988) 31-46.

[85] A. Mansour, C.A. Fox, S. Burke, H. Akil, and S.J. Watson, Immunohistochemical localization of the cloned mu opioid receptor in the rat CNS. J Chem Neuroanat 8 (1995) 283-305.

[86] T. Boucher, E. Jennings, and M. Fitzgerald, The onset of diffuse noxious inhibitory controls in postnatal rat pups: a C-Fos study. Neurosci Lett 257 (1998) 9-12.

[87] M. Fitzgerald, and M. Koltzenburg, The functional development of descending inhibitory pathways in the dorsolateral funiculus of the newborn rat spinal cord. Brain Res 389 (1986) 261-70.

[88] P. Goffaux, S. Lafrenaye, M. Morin, H. Patural, G. Demers, and S. Marchand, Preterm births: Can neonatal pain alter the development of endogenous gating systems? Eur J Pain (2008).

[89] J.L. Laprairie, and A.Z. Murphy, Neonatal injury alters adult pain sensitivity by increasing opioid tone in the periaqueductal gray. Front Behav Neurosci 3 (2009) 31.

[90] M.J. Iadarola, J. Douglass, O. Civelli, and J.R. Naranjo, Differential activation of spinal cord dynorphin and enkephalin neurons during hyperalgesia: evidence using cDNA hybridization. Brain Res 455 (1988) 205-12.

[91] K. Noguchi, Y. Morita, H. Kiyama, M. Sato, K. Ono, and M. Tohyama, Preproenkephalin gene expression in the rat spinal cord after noxious stimuli. Brain Res Mol Brain Res 5 (1989) 227-34.

[92] F.G. Williams, M.A. Mullet, and A.J. Beitz, Basal release of Met-enkephalin and neurotensin in the ventrolateral periaqueductal gray matter of the rat: a microdialysis study of antinociceptive circuits. Brain Res 690 (1995) 207-16.

[93] M.D. Sanchez, M.V. Milanes, A. Pazos, A. Diaz, and M.L. Laorden, Autoradiographic evidence of mu-opioid receptors down-regulation after prenatal stress in offspring rat brain. Brain Res Dev Brain Res 94 (1996) 14-21.

[94] M.D. Sanchez, M.V. Milanes, A. Pazos, A. Diaz, and M.L. Laorden, Autoradiographic evidence of delta-opioid receptor downregulation after prenatal stress in offspring rat brain. Pharmacology 60 (2000) 13-8.

[95] V.C. Anseloni, F. He, S.I. Novikova, M. Turnbach Robbins, I.A. Lidow, M. Ennis, and M.S. Lidow, Alterations in stress-associated behaviors and neurochemical markers in adult rats after neonatal short-lasting local inflammatory insult. Neuroscience 131 (2005) 635-45.

[96] K. Ren, S.I. Novikova, F. He, R. Dubner, and M.S. Lidow, Neonatal local noxious insult affects gene expression in the spinal dorsal horn of adult rats. Mol Pain 1 (2005) 27.

[97] M. Bernardi, S. Genedani, and A. Bertolini, Behavioral activity and active avoidance learning and retention in rats neonatally exposed to painful stimuli. Physiol Behav 36 (1986) 553-5.

[98] H.M. Schellinck, L. Stanford, and M. Darrah, Repetitive acute pain in infancy increases anxiety but does not alter spatial learning ability in juvenile mice. Behav Brain Res 142 (2003) 157-65.

[99] W.F. Sternberg, and C.G. Ridgway, Effects of gestational stress and neonatal handling on pain, analgesia, and stress behavior of adult mice. Physiol Behav 78 (2003) 375-83.

[100] W.F. Sternberg, L. Scorr, L.D. Smith, C.G. Ridgway, and M. Stout, Long-term effects of neonatal surgery on adulthood pain behavior. Pain 113 (2005) 347-53.

[101] R.E. Grunau, D.W. Haley, M.F. Whitfield, J. Weinberg, W. Yu, and P. Thiessen, Altered basal cortisol levels at 3, 6, 8 and 18 months in infants born at extremely low gestational age. J Pediatr 150 (2007) 151-6.

[102] J.L. Laprairie, M.E. Johns, and A.Z. Murphy, Preemptive morphine analgesia attenuates the long-term consequences of neonatal inflammation in male and female rats. Pediatr Res 64 (2008) 625-30.

[103] R.P. Smith, R. Gitau, V. Glover, and N.M. Fisk, Pain and stress in the human fetus. Eur J Obstet Gynecol Reprod Biol 92 (2000) 161-5.

[104] S. Vanhatalo, and O. van Nieuwenhuizen, Fetal pain? Brain Dev 22 (2000) 145-50.

[105] D.J. Kahn, D.K. Richardson, J.E. Gray, F. Bednarek, L.P. Rubin, B. Shah, I.D. Frantz, 3rd, and D.M. Pursley, Variation among neonatal intensive care units in narcotic administration. Arch Pediatr Adolesc Med 152 (1998) 844-51.

[106] F.L. Porter, C.M. Wolf, J. Gold, D. Lotsoff, and J.P. Miller, Pain and pain management in newborn infants: a survey of physicians and nurses. Pediatrics 100 (1997) 626-32.

[107] C.C. Johnston, J.M. Collinge, S.J. Henderson, and K.J. Anand, A cross-sectional survey of pain and pharmacological analgesia in Canadian neonatal intensive care units. Clin J Pain 13 (1997) 308-12.

[108] K.J. Anand, R.W. Hall, N. Desai, B. Shephard, L.L. Bergqvist, T.E. Young, E.M. Boyle, R. Carbajal, V.K. Bhutani, M.B. Moore, S.S. Kronsberg, and B.A. Barton, Effects of morphine analgesia in ventilated preterm neonates: primary outcomes from the NEOPAIN randomised trial. Lancet 363 (2004) 1673-82.

[109] R.E. Grunau, M.F. Whitfield, J. Petrie-Thomas, A.R. Synnes, I.L. Cepeda, A. Keidar, M. Rogers, M. Mackay, P. Hubber-Richard, and D. Johannesen, Neonatal pain, parenting stress and interaction, in relation to cognitive and motor development at 8 and 18 months in preterm infants. Pain 143 (2009) 138-46.

[110] R. MacGregor, D. Evans, D. Sugden, T. Gaussen, and M. Levene, Outcome at 5-6 years of prematurely born children who received morphine as neonates. Arch Dis Child Fetal Neonatal Ed 79 (1998) F40-3.

[111] R. Rao, J.S. Sampers, S.S. Kronsberg, J.V. Brown, N.S. Desai, and K.J. Anand, Neurobehavior of preterm infants at 36 weeks postconception as a function of morphine analgesia. Am J Perinatol 24 (2007) 511-7.

[112] E.A. Farrington, G.A. McGuinness, G.F. Johnson, A. Erenberg, and R.D. Leff, Continuous intravenous morphine infusion in postoperative newborn infants. Am J Perinatol 10 (1993) 84-7.

[113] N.J. Bouwmeester, K.J. Anand, M. van Dijk, W.C. Hop, F. Boomsma, and D. Tibboel, Hormonal and metabolic stress responses after major surgery in children aged 0-3 years: a double-blind, randomized trial comparing the effects of continuous versus intermittent morphine. Br J Anaesth 87 (2001) 390-9.

[114] N.J. Bouwmeester, W.C. Hop, M. van Dijk, K.J. Anand, J.N. van den Anker, and D. Tibboel, Postoperative pain in the neonate: age-related differences in morphine requirements and metabolism. Intensive Care Med 29 (2003) 2009-15.

[115] K.J. Anand, and P.R. Hickey, Halothane-morphine compared with high-dose sufentanil for anesthesia and postoperative analgesia in neonatal cardiac surgery. N Engl J Med 326 (1992) 1-9.

[116] K.J. Anand, W.G. Sippell, and A. Aynsley-Green, Randomised trial of fentanyl anaesthesia in preterm babies undergoing surgery: effects on the stress response. Lancet 1 (1987) 62-6.

[117] J.W. Peters, H.M. Koot, J.B. de Boer, J. Passchier, J.M. Bueno-de-Mesquita, F.H. de Jong, H.J. Duivenvoorden, and D. Tibboel, Major surgery within the first 3 months of life and subsequent biobehavioral pain responses to immunization at later age: a case comparison study. Pediatrics 111 (2003) 129-35.

[118] J.C. Roze, S. Denizot, R. Carbajal, P.Y. Ancel, M. Kaminski, C. Arnaud, P. Truffert, S. Marret, J. Matis, G. Thiriez, G. Cambonie, M. Andre, B. Larroque, and G. Breart, Prolonged sedation and/or analgesia and 5-year neurodevelopment outcome in very preterm infants: results from the EPIPAGE cohort. Arch Pediatr Adolesc Med 162 (2008) 728-33.

[119] R. Bellu, K.A. de Waal, and R. Zanini, Opioids for neonates receiving mechanical ventilation. Cochrane Database Syst Rev (2008) CD004212.

[120] S.H. Simons, M. van Dijk, R.A. van Lingen, D. Roofthooft, H.J. Duivenvoorden, N. Jongeneel, C. Bunkers, E. Smink, K.J. Anand, J.N. van den Anker, and D. Tibboel, Routine morphine infusion in preterm newborns who received ventilatory support: a randomized controlled trial. JAMA 290 (2003) 2419-27.

[121] G.E. Handelmann, and D. Dow-Edwards, Modulation of brain development by morphine: effects on central motor systems and behavior. Peptides 6 Suppl 2 (1985) 29-34.

[122] R.J. McPherson, C. Gleason, M. Mascher-Denen, M. Chan, B. Kellert, and S.E. Juul, A new model of neonatal stress which produces lasting neurobehavioral effects in adult rats. Neonatology 92 (2007) 33-41.

[123] F.V. Abbott, and E.R. Guy, Effects of morphine, pentobarbital and amphetamine on formalin-induced behaviours in infant rats: sedation versus specific suppression of pain. Pain 62 (1995) 303-12.

[124] W.T. Adamson, R.T. Windh, S. Blackford, and C.M. Kuhn, Ontogeny of mu- and kappa-opiate receptor control of the hypothalamo-pituitary-adrenal axis in rats. Endocrinology 129 (1991) 959-64.

[125] C. Stein, M.J. Millan, T.S. Shippenberg, K. Peter, and A. Herz, Peripheral opioid receptors mediating antinociception in inflammation. Evidence for involvement of mu, delta and kappa receptors. J Pharmacol Exp Ther 248 (1989) 1269-75.

[126] S.J. Morison, L. Holsti, R.E. Grunau, M.F. Whitfield, T.F. Oberlander, H.W. Chan, and L. Williams, Are there developmentally distinct motor indicators of pain in preterm infants? Early Hum Dev 72 (2003) 131-46.

[127] M.J. Christie, P. Trisdikoon, and G.B. Chesher, Tolerance and cross tolerance with morphine resulting from physiological release of endogenous opiates. Life Sci 31 (1982) 839-45.

[128] B.T. Lett, V.L. Grant, M.T. Koh, and G. Flynn, Prior experience with wheel running produces cross-tolerance to the rewarding effect of morphine. Pharmacol Biochem Behav 72 (2002) 101-5.

[129] D.C. Stoller, L.J. Sim-Selley, and F.L. Smith, Role of kappa and delta opioid receptors in mediating morphine-induced antinociception in morphine-tolerant infant rats. Brain Res 1142 (2007) 28-36.

[130] D.C. Stoller, S.R. Thornton, and F.L. Smith, Loss of antinociceptive efficacy in rat pups infused with morphine from osmotic minipumps. Pharmacology 66 (2002) 11-8.

[131] S.V. Coutinho, P.M. Plotsky, M. Sablad, J.C. Miller, H. Zhou, A.I. Bayati, J.A. McRoberts, and E.A. Mayer, Neonatal maternal separation alters stress-induced responses to viscerosomatic nociceptive stimuli in rat. Am J Physiol Gastrointest Liver Physiol 282 (2002) G307-16.

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.