

## Impact of repeated procedural pain-related stress in infants born very preterm

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### Abstract

The majority of infants born very preterm (24–32 weeks gestational age) now survive, however, long-term neurodevelopmental and behavioral problems remain a concern. As part of their neonatal care very preterm infants undergo repeated painful procedures during a period of rapid brain development and programming of stress systems. Infants born this early have the nociceptive circuitry required to perceive pain, however, their sensory systems are functionally immature. An imbalance of excitatory versus inhibitory processes leads to increased nociceptive signaling in the central nervous system. Specific cell populations in the central nervous system of preterm neonates are particularly vulnerable to excitotoxicity, oxidative stress, and inflammation. Neonatal rat models have demonstrated that persistent or repeated pain increases apoptosis of neurons, and neonatal pain and stress lead to anxiety-like behaviors during adulthood. In humans, greater exposure to neonatal pain-related stress has been associated with altered brain microstructure and stress hormone levels, as well as with poorer cognitive, motor and behavioral neurodevelopment in infants and children born very preterm. Therefore, it is important that pain-related stress in preterm neonates is accurately identified, appropriately managed, and that pain management strategies are evaluated for protective or adverse effects in the long term.

### PAIN PROCESSING IN INFANTS BORN VERY PRETERM

Infants born preterm, especially those born between 24–32 weeks GA (very preterm) are exposed to repeated procedural pain-related stress, during a period of physiological vulnerability and rapid brain development, as part of their life-saving care in the NICU. Preterm infants have the nociceptive circuitry required to perceive pain, however, this system is functionally immature (1, 2). Cutaneous receptive fields are large in the neonate, and

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peripheral sensory fibres are sensitive to tissue injury and have reduced peak firing frequencies (2–5). Axon terminals temporarily overlap in lamina II of the spinal cord with low-threshold tactile inputs, making it more difficult for neonates to discriminate between noxious and non-noxious stimuli (6, 7). Thus, prior to 35 weeks gestation infants demonstrate central sensitization to repeated procedures (3, 8–12). This transition has been confirmed by electrophysiological (EEG) recordings, since responses to heel lance were dispersed neuronal bursts in very preterm neonates, in contrast to the modality-specific, localized, evoked potentials seen at ~36 weeks postmenstrual age (13). Moreover, changes in the EEG recordings of preterm infants correspond to the disappearance of the radial glia and increase in complexity of the cerebral cortex (14, 15). However, descending modulation of nociceptive activity in the dorsal horn of the spinal cord develops later, beyond term equivalent age (16, 17). Therefore, given that infants born very preterm have reduced localization and specification to noxious stimuli, become sensitized to repeated noxious stimuli and lack descending inhibitory control, identifying, relieving, and preventing pain are very important aspects of NICU care.

## **RESPONSES TO PAINFUL PROCEDURES IN INFANTS BORN VERY PRETERM**

Neonatal pain assessment instruments code a variety of behavioral and physiological responses (e.g. facial actions, body movements, cry, heart rate, respiratory rate, blood pressure, and oxygen saturation) in order to quantify pain in nonverbal patients (18, 19). However, these indicators are not specific to pain, and may also represent agitation or distress. Responses to neonatal pain also vary based on GA, sleep-wake state, illness severity, as well as recency and duration of previous exposures to pain and non-invasive interventions (9–11, 20–21). Therefore, clinicians are faced with the difficult task of discriminating and appropriately managing pain in infants born preterm. Dampened behavioral and physiological responses to pain do not necessarily represent absence of nociceptive processing in the CNS (22, 23). Pharmacological care is not ideal for routine pain management (24), and while non-pharmacologic management is recommended as a first step, often invasive procedures in the NICU are still performed without support (25). Unmanaged pain may have substantive effects on the developing brain and stress systems of premature neonates, however, pain management remains a challenge.

## **REPEATED NEONATAL PAIN-RELATED STRESS IN INFANTS BORN VERY PRETERM**

Stress hormones are glucocorticoids (cortisol in humans, corticosterone in rats) that regulate the transcription of genes throughout the body and the brain (26). Thus, prolonged activation of the HPA axis in physiologically immature neonates, can lead to long-term changes in hormonal (e.g. growth, glucocorticoid), physiological (e.g. metabolic, immune) and behavioral (anxiety, depression) systems (27–30). However, while very preterm infants are in the NICU, their cortisol levels are frequently lower than expected, considering the number procedures required during their hospitalization (31). This may represent an exhaustion of resources among these physiologically immature neonates, immaturity of the adrenal cortex,

or other factors in this medical context. Greater exposure to painful procedures in the NICU has been associated with the reprogramming of the stress hormone system. Grunau et al. (2005) found that greater cumulative neonatal procedural pain exposure was associated with lower cortisol responses to stress at 32 weeks postmenstrual age, independent of early illness severity and morphine exposure, and not accounted for by GA at birth (32). In contrast, in infants born at extremely low gestational age (ELGA: 24–28 weeks gestation) at 8 and 18 months corrected age (CA) cortisol levels were elevated, and the level was associated with exposure to higher numbers of skin-breaking procedures from birth to term equivalent age (33). Among infants born ELGA there is evidence for a shift from low basal cortisol levels at 3 months to relatively high levels at 8 and 18 months CA, which suggests a biological “resetting” of endocrine stress systems (34). Rat pups exposed to long periods of maternal separation early in life, have fewer hippocampal glucocorticoid receptors, higher corticotropin releasing factor, adrenocorticotropin and corticosterone production, during adulthood (30). Long-term changes to stress responses appear to be due to the fact that regions rich in glucocorticoid receptors (e.g. hippocampus, prefrontal cortex), are particularly vulnerable to the effects of ongoing stress (30, 35).

## NEONATAL PAIN-RELATED STRESS AND THE DEVELOPING BRAIN

Two cell populations are particularly vulnerable to injury in the premature brain: subplate neurons and preoligodendrocytes (36). Subplate neurons are among the first cells generated in the mammalian cerebral cortex, and are the first cortical neurons to receive excitatory synaptic inputs from thalamic axons, establishing a temporary link between thalamic axons and their final target in the cerebral cortex (37–39). Subplate neurons are particularly vulnerable to excitotoxic death, as was demonstrated by the selective ablation of subplate neurons after the administrations of glutamate agonist kainite into embryonic and postnatal kittens (40, 41), and in a model of hypoxic-ischemia in the neonatal rat (42). Glutamate n-methyl-D-aspartate (NMDA) receptors involved in the transmission of the pain signal are more active during early life because of the developmentally delayed expression of NR2A receptor subunits relative to NR2B. Therefore, repeated procedural pain may lead to excitotoxicity and apoptosis of the subplate neurons due the excessive release of glutamate and influxes of calcium (43–46). Neonatal pain-related stress may also impact preoligodendrocytes, which ensheath axons prior to differentiating into myelin-producing oligodendrocytes (36). The immaturity of these cells makes them particularly vulnerable to reactive oxygen, nitrogen species and cytokines secreted by microglia (47–51). Procedural pain induces both oxidative stress and inflammatory reactions (52, 53), and therefore may arrest the development of pre-myelinating cells.

Recently, studies have demonstrated for the first time that procedural pain/stress in very preterm infants is associated with abnormal brain development during neonatal intensive care, up to term-equivalent age (54, 55). These findings are supported by evidence from animal models that have demonstrated both inflammatory pain and repeated injections increase apoptosis in the neonatal rat brain (56, 57). Therefore, repeated exposure to procedural pain appears to impact neonatal brain development.

Importantly, associations between neonatal pain-related stress and brain development also appear to extend beyond the relationships observed in early life (54, 58). At 7 years of age, higher numbers of skin-breaking procedures in the NICU were associated with thinner cortical gray matter in 21 out of 66 cerebral regions assessed, predominately affecting the frontal and parietal lobes (59). Moreover, among infants born ELGA, greater exposure to neonatal pain-related stress was also associated with alterations in spontaneous neuromagnetic activity (60). Therefore, it appears that repeated exposure to neonatal procedural pain/stress is associated with long-term alterations to neuronal structure and function.

## **NEONATAL PAIN-RELATED STRESS AND NEURODEVELOPMENTAL OUTCOME**

Greater neonatal pain-related stress has been associated with lower cognitive and motor function at 8 and 18 months CA, and higher internalizing (anxious/depressed) behaviors at 18 months CA and at age 7 years (61–63). However, pain exposure may not in and of itself modify long-term outcomes of preterm children. Among children born ELGA, cumulative neonatal pain-related stress was associated with changes in spontaneous brain activity at school-age, and these alterations in brain oscillations were negatively correlated with visual-perceptual abilities (60). Therefore, it appears that the influence of neonatal pain-related stress on long-term cognitive outcomes may be through altered brain function among children born ELGA.

## **MANAGEMENT OF PAIN AND BRAIN PROTECTION IN THE NICU**

Pharmacological and environmental support strategies for pain management are frequently used in the NICU. Pharmacological management of pain, and possible long-term effects of analgesics, anesthetics and sedatives, are complex topics that have been recently reviewed elsewhere (64–67). Due to concerns regarding short-term and possible long-term effects of pharmacological agents, current recommendations are that opiates and sedatives be used sparingly in the NICU for non-surgical pain management of ventilated preterm neonates (68, 69). A number of environmental (non-pharmacologic) interventions are used for the management of routine acute procedural pain in the NICU (e.g. sucrose, swaddling, facilitated tucking, non-nutritive sucking, kangaroo care, breast feeding) (70, 71). Currently sucrose is the most widely used non-pharmacologic intervention for the treatment of minor procedures in preterm infants (72). However, while it is well-established that oral sucrose reduces behavioral responses and sometimes physiological responses (71), sucrose does not appear to dampen EEG response to pain (22). It is important for future studies to examine the extent to which various pain management strategies may be brain protective.

Parent support to promote sensitive and responsive interactions during hospitalization of preterm infants in the NICU appears to improve white matter maturation (73). Moreover, in ELGA infants, positive maternal interaction at 18 months CA was associated with lower cortisol levels (74). Positive parent interaction at 18 months CA appeared to ameliorate negative effects of neonatal pain on stress-sensitive behaviors (62). Therefore, while more research is needed to optimize pain management strategies in the NICU, it is encouraging

that initial studies suggest that sensitive and responsive caregiving appears to ameliorate some effects of neonatal-pain related stress on brain, stress and behavioral outcomes

## FUTURE PERSPECTIVES

Exposure to repeated neonatal pain-related stress is associated with altered brain development, function, and neurodevelopmental outcome in children born preterm. Therefore, it is of the utmost importance that pain-related stress in preterm neonates is accurately identified and appropriately managed. Pain management is required for humane care of infants; however, there are major gaps in knowledge as to which management interventions may be brain protective, and to what degree.

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