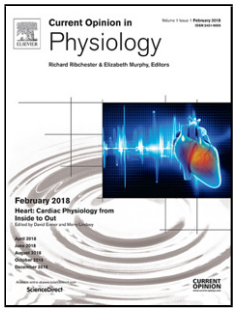

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Title: Early life pain – effects in the adult

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Abstract

Early life stress and injury can have long-term effects on nociceptive processing and the risk of persistent pain in later life. Neonates requiring prolonged intensive care, particularly those born extremely preterm, are at risk due both to immaturity at birth and exposure to tissue injury and pain from procedural interventions and surgery. This review will summarise clinical evaluations of pain experience and somatosensory function in preterm-born young adults; and highlight data from laboratory studies evaluating the potential for tissue injury in neonatal rodents to prime nociceptive processing and alter the response to subsequent injury in adulthood.

Keywords: pain; preterm; neonate; surgery; quantitative sensory testing; neuroimmune

Introduction

The Global Burden of Disease studies highlight the impact of chronic pain, both in terms of prevalence and years lived with disability [1,2]. There is increasing evidence that health outcomes in later life are influenced by early life stress and adversity [3-7], and epidemiological studies associate adversity and illness in childhood with chronic pain throughout the lifespan [8,9]. Identifying high-risk groups and evaluating the mechanisms by which early-life experience influences the transition to chronic pain [10] are essential for improving long-term outcome.

The developing nervous system is vulnerable to altered levels of activity that may disrupt normal developmental trajectories. Conversely, enhanced plasticity may be beneficial if neuroprotective factors and interventions can be identified [11]. Neonates born preterm are at particular risk, both in terms of physiological immaturity and the type and degree of exposures associated with neonatal intensive care [3]. Advances in clinical care have increased survival following birth at younger ages (eg. very preterm <32 weeks gestational age, VP; and extremely preterm <28weeks, EP)[12], but preterm birth remains a significant contributor to long-term disability [1,2]. This review will focus on recent clinical reports evaluating pain-related outcomes in adults born preterm, and laboratory studies identifying alterations in injury response in adulthood following neonatal tissue injury.

Persistent effects following neonatal pain: clinical cohorts

Pain exposure

Large numbers of repeated painful procedural interventions are required for monitoring and treatment during neonatal intensive care, and up to one-third of extremely preterm neonates also require surgery [13,14]. Noxious stimuli evoke changes in peripheral sensitivity, spinal reflex activity, and nociceptive-specific electroencephalography and near-infrared spectroscopy cortical responses that change with gestational age in preterm and term neonates [15,16]. Therefore, increased afferent input has the capacity to induce activity-dependent effects at multiple points within developing nociceptive pathways, and produce persistent changes in structure and/or function. Quantifying the overall allosteric load of pain exposure in preterm neonates, and differentiating effects of pain from other confounding factors, is difficult in clinical cohorts. The number of tissue-breaking procedures, requirement for surgery, and duration of mechanical ventilation or hospital stay are often used as proxy measures [14,17].

Pain report and experience in young adults

Evaluating associations between preterm birth, neonatal experience and the incidence of chronic pain in later life are hampered by differences in patient populations, definition of chronic pain, methodology and outcome [14,18-21](Table 1). In addition, differentiating persistent biological effects related to neonatal exposures, and subsequent modulation by psychosocial and environmental factors, becomes more difficult at older ages [22]. Epidemiological studies have the advantage of large sample sizes, but the sensitivity of the outcome and details of the type and impact of pain may be limited. Alternatively, more detailed evaluations in high-risk cohorts with more sensitive outcomes may be hampered by smaller sample size or loss to follow-up. Recent

reports in preterm born young adults tend to show greater differences in high-risk cohorts born at younger gestational ages (Table 1).

Persistent behavioral and emotional difficulties, altered relationships between risk and resilience for mood disorders and anxiety, and different levels of social support following preterm birth may influence the psychosocial aspects of pain experience [23,24](Figure 1). In EP young adults, anxiety was higher in preterm males and females, but sex had a greater impact on pain catastrophising than EP status [14]. Associations between extreme preterm birth and reduced cognitive function also extend into adulthood, albeit with inter-individual variability, and potential increased vulnerability in males [22,25]. Requirement for neonatal surgery also has an added impact on neurodevelopmental and cognitive outcome throughout childhood [13,22] and into early adulthood [14](Figure 1).

Somatosensory function

Quantitative sensory testing protocols incorporate a range of somatosensory modalities and intensities to evaluate small unmyelinated C-fibre, myelinated A-δ and A-β fibre function. Identifying different sensory profiles with increased and/or decreased sensitivity to thermal and mechanical stimuli improves phenotyping and may predict underlying mechanisms or treatment response [26]. Persistent somatosensory changes show some relationship to gestational age and degree of pain exposure. Generalized decreased sensitivity was more marked in VP versus term-born children following neonatal intensive care [27], and in EP children who also required neonatal surgery [3]. In VP young adults, thermal and pressure thresholds did not differ from term controls [28], whereas significant differences were seen in an EP cohort born at lower birth weight and gestational age who required longer hospital stay [14]. Despite reduced sensitivity to static stimuli (i.e. higher thresholds), more intense or prolonged stimuli may unmask increased sensitivity. A prolonged heat stimulus resulted in perceptual sensitization in VP-born children [27] but not adults [28]. Reduced prolonged cold tolerance has been reported in VP and EP young adults, particularly females [14,29,30], and those with additional neonatal exposures such as surgery [14] and necrotising enterocolitis [30].

As the prevalence of chronic pain and experimental pain sensitivity is increased in adult females [31], sex and/or gender should be considered. In EP young adults, a composite measure of generalized thermal sensitivity (time to HPT, CPT, and cold pressor tolerance) identified increased sensitivity in females, but decreased sensitivity in males, with greater change following neonatal surgery (Figure 1)[14,18]. Peripheral sensitivity adjacent to neonatal scars showed a different

pattern with brush allodynia and mechanical perceptual sensitization in both EP males and females [14].

Pain and brain structure following preterm birth

Preterm birth and early life stress alter brain structure and connectivity in sensory, cognitive and emotional networks [32,33]. Alterations in regions for integration of somatosensory input (e.g. thalamus), and central modulation of affective and behavioral responses (e.g. amygdala and frontal regions) have been related both to the degree of neonatal exposure, and to subsequent functional outcomes. Following VP/EP birth, adverse neonatal experience (higher skin breaking procedures, surgery, or illness severity) at earlier gestational ages was associated with slower thalamic growth, and poorer cognitive scores at 3 years of age [34]; lower thalamus and amygdala volumes with adverse effects on cognitive, visual-motor and behavioral outcomes at 8 years [35]; and increased frontal theta connectivity (resting state magnetoencephalography at 8 years) with poorer cognitive flexibility and behavioral regulation potentially reflecting disrupted maturation of top-down regulation [36]. In EP young adults, differences in thermal sensitivity were independent of cognitive scores. However, lower amygdala volume was associated with increased thermal sensitivity in EP females, but reduced sensitivity in EP males, suggesting that central affective circuits contribute to sex-dependent differences in experimental pain sensitivity [14]. Ongoing multimodal evaluation in longitudinal cohorts will improve awareness of early life experience effects on future pain, identify those at risk of persistent pain, and evaluate the most appropriate interventions to improve long-term outcome.

Persistent effects following tissue injury in neonatal rodents

Different aspects of early life tissue injury have been covered in recent reviews, including: age-dependent effects in specific injury models such as traumatic nerve injury [37]; persistent alterations in nociceptive processing following inflammation [6] and/or stress [4]; effects at different points along nociceptive pathways from the periphery and spinal cord [38] to brain networks [32]; potential epigenetic mechanisms [39,40]; and inter-related involvement of neuroimmune, neuroendocrine and stress systems [41-43].

To parallel data from clinical cohorts, the focus here is on injury during the first postnatal week in neonatal rodents, that have parallels with preterm human development [41]. Data demonstrating ‘priming’ of nociceptive networks by exposure to stimuli during the neonatal period that alter the response to a subsequent stimulus in adulthood are highlighted.

Immune challenge

Priming is a central function of the immune system function as early life exposures trigger enhanced responses to a subsequent challenge. Lipopolysaccharide (LPS) is a powerful activator of innate immune responses and neonatal intraperitoneal administration alters not only long-term response to a subsequent immune challenge, but also nociceptive thresholds [44] and injury response in later life [41]. Neonatal LPS enhances the response to subsequent hindpaw formalin with increases in behavioural hyperalgesia and excitability of local spinal networks [45], and increased cytokine release in the periphery and centrally (e.g. hippocampus)[41]. Intraperitoneal LPS in male and female rats on postnatal day 5 (P5) increased spinal microglial reactivity, and the non-specific microglial inhibitor minocycline decreased acute hyperalgesia and pro-inflammatory cytokine concentrations in the serum and spinal cord [46], but responses to later life injury were not assessed.

Hyperalgesic priming

Hindpaw injection of an inflammatory insult (eg. TNFα) induces long-lasting primary afferent nociceptor plasticity, with enhanced hyperalgesic responses to a subsequent stimulus (eg. PGE_2) in the same paw. Hyperalgesic priming is sexually dimorphic in adult rodents as PKCε-dependent mechanisms are not induced by TNFα, and are negatively regulated by estrogen, in females [47]. Primed hyperalgesic responses to PGE_2 were attenuated by microglial inhibition only in adult males [48]. Effects also vary during postnatal development: males showed more robust priming following TNFα at older than at younger ages (4-7 versus 1-3 weeks). Priming was induced by TNFα in juvenile females with low estrogen levels (1-4 weeks), but at older ages priming required co-administration of an estrogen receptor antagonist [49].

Surgical incision and tissue injury

Plantar hindpaw incision increases spinal reflex sensitivity at all ages, albeit with a shorter duration in younger animals [50]. To mirror clinical surgical injury both the skin and underlying plantaris muscle are incised, and muscle afferents may have a greater propensity than cutaneous afferents to increase excitability and long-term potentiation (LTP) in the spinal cord [51]. In juvenile rodents (P7-P11), excitatory cutaneous and muscle afferents converge on spinal lamina I projection neurons, but muscle afferents have increased probability of glutamate release, expression of Ca-permeable AMPA receptors, and potential for activity-dependent potentiation [52].

Neonatal incision has persistent effects on the balance of excitatory and inhibitory signalling in the dorsal horn and ascending pathways (Figure 2). The frequency of miniature excitatory post-synaptic

potentials (mEPSC) is increased, and reduced glycinergic and $GABA_A$ inhibition persists in adulthood [53]. Sensory drive onto adult projection neurons is enhanced, with widening of spike timing-dependent LTP, increased direct input from low threshold afferents, and reduced feed-forward inhibition [53]. While neonatal incision did not alter metabotropic $GABA_B$ receptor-mediated inhibition signalling in inhibitory interneurons, post-synaptic signalling in ascending projection neurons was enhanced, and may partially compensate for weaker $GABA_A$R-mediated inhibition [54].

As with hindpaw inflammation [3,6,55], neonatal incision has dual effects in adulthood that differ in time course and distribution: injury-induced changes in descending modulation and generalized hypoalgesia emerge after the fourth postnatal week; but enhanced responses to re-injury are evident from 1-2 weeks after the initial injury, and persist into adulthood [3](Figure 2). Effects are activity-dependent as blocking primary afferent input (sciatic nerve local anesthetic) at the time of neonatal incision normalizes adult sensory thresholds [56] and alterations in descending inhibition from the rostroventral medulla [57], and also prevents the enhanced hyperalgesic response to adult re-incision [56]. Recently, neonatal incision at different sites (ipsi- or contralateral hindpaw or forepaw or thigh) confirmed generalized hypoalgesia in adulthood; whereas re-incision hyperalgesia was segmentally restricted and maximal following incision in the same paw or ipsilateral hindlimb, while contralateral incision had no effect [50]. Although initial priming is dependent on primary afferent input, enhanced re-incision hyperalgesia is centrally mediated and not dependent on peripheral re-injury as a standardized afferent input (tibial nerve electrical stimulation) also evoked greater reflex sensitivity in adults with prior neonatal incision [58]. In addition, primed responses are not restricted to the same type of re-injury: neonatal hindpaw inflammation enhanced adult incision-induced hyperalgesia [59]; and repeated needle insertions in the paw during the first 1-2 postnatal weeks (to model repeated procedures in NICU) increased hyperalgesia following inflammation [60] or incision [61] in adulthood.

Sex-dependent responses to microglial inhibition

Sex-dependent differences following tissue injury [31] are increasingly identified; including differences in baseline thresholds in adolescence following neonatal incision [62] and in adult rodents following neonatal inflammation [63]. While different injury models produce hyperalgesia and increase microglial reactivity in both male and female adult rodents [31,64,65], spinal neuroimmune signalling is sexually dimorphic and microglial inhibitors reduce hyperalgesia in males only. Neonatal incision primes the spinal microglial response, and the enhanced hyperaglesic response to re-incision is reduced by intrathecal microglial inhibitors in males [58,66]. Microglial inhibitors at the time of neonatal incision also have long-term sex-dependent effects as adult re-

incision hyperalgesia is prevented in males, but not females [50]. In the developing brain, early-life stress and tissue injury can disrupt the normal sex-dependent developmental trajectories of microglia [67,68], or trigger long-term changes in phenotype, that alter reactivity to future immune or environmental challenges and influence neurodevelopmental and neurodegenerative outcomes [42,69]. In the spinal cord, long-term sex-dependent effects of microglial inhibition may relate to alterations in phenotype and/or alterations in microglial roles in the normal activity-dependent refinement of sensory system circuitry [70].

Neonatal pain and brain structure

Exposure to chronic early-life stress (e.g. maternal separation in rodents) alters the structure and function of cognitive and emotional brain networks [32]. Persistent effects of early tissue injury on brain structure are now being evaluated [71], and will provide opportunities for correlation with functional pain outcomes and identification of mechanisms underlying structural differences reported in preterm clinical cohorts. While repeated paw needle insertion from P1-P6 in mice did not alter regional brain volumes in adulthood, changes associated with repeated sucrose administration were exacerbated in regions relevant for pain signalling and the hippocampus [71] and associated with long-term impairment of spatial memory [72].

Conclusion

Awareness and interest in the impact of early life experience has significantly increased, and there is a need to consider sex as a biological variable in both clinical and laboratory studies. As increasing numbers of extremely preterm born infants are now reaching adulthood, long-term effects on both physiological and pathophysiological systems need to be considered when evaluating health outcome and well-being. Identification of underlying mechanisms and potential preventive interventions in males and females will inform clinical studies to improve long-term outcome.

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Figure Legends

Figure 1. Longitudinal data to 19 years from the EPICure cohort demonstrate persistent differences between extremely preterm (EP) and term control groups born in 1995. **A-C**: Strength and Difficulty Questionnaire Total Disability (A) and Emotional Symptoms B) and Peer Problems (C) subscale scores. **D-E**: Thresholds for heat pain (D) and cold pain (E) change with age, and group differences persist. **F**: Generalized thermal sensitivity (higher scores represent increased tolerance and reduced sensitivity) at 19 years demonstrates sex-dependent differences and the added impact of prior neonatal surgery. **G**: Cognitive test scores based on Bayley scores at 2.5 years, Kauffman Assessment Battery at 6 and 11 years, and Wechsler Abbreviated Scale of Intelligence generated Full Scale IQ score at 19 years. **H:** Full scale IQ scores for participants completing quantitative sensory testing demonstrate impact of EP birth and neonatal surgery. IQ scores do not account for sex differences in sensitivity. Data points=mean ± 95%CI. A-C reproduced from [23]; G from [25]; D-F,H redrawn from [14].

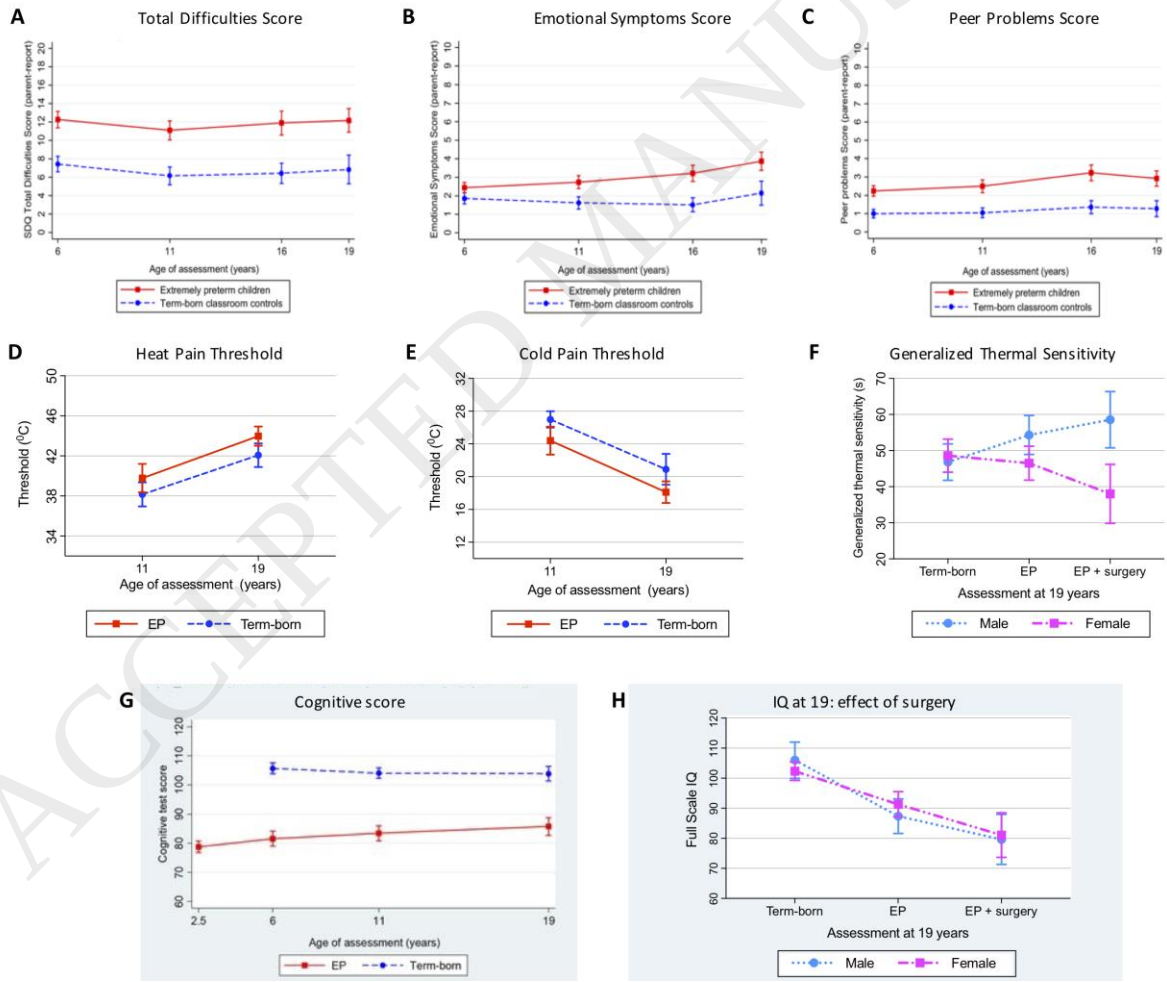

Figure 2. Schematic representing impact of neonatal tissue injury at multiple points in nociceptive pathways: initial hindpaw incision and afferent input; enhanced reflex sensitivity to adult re-incision [3,46,52,54]; alterations in spinal dorsal horn and ascending pathways [34, 49]; descending modulation [53]; and regional volumetric changes in brain following repeated needle insertion [67].

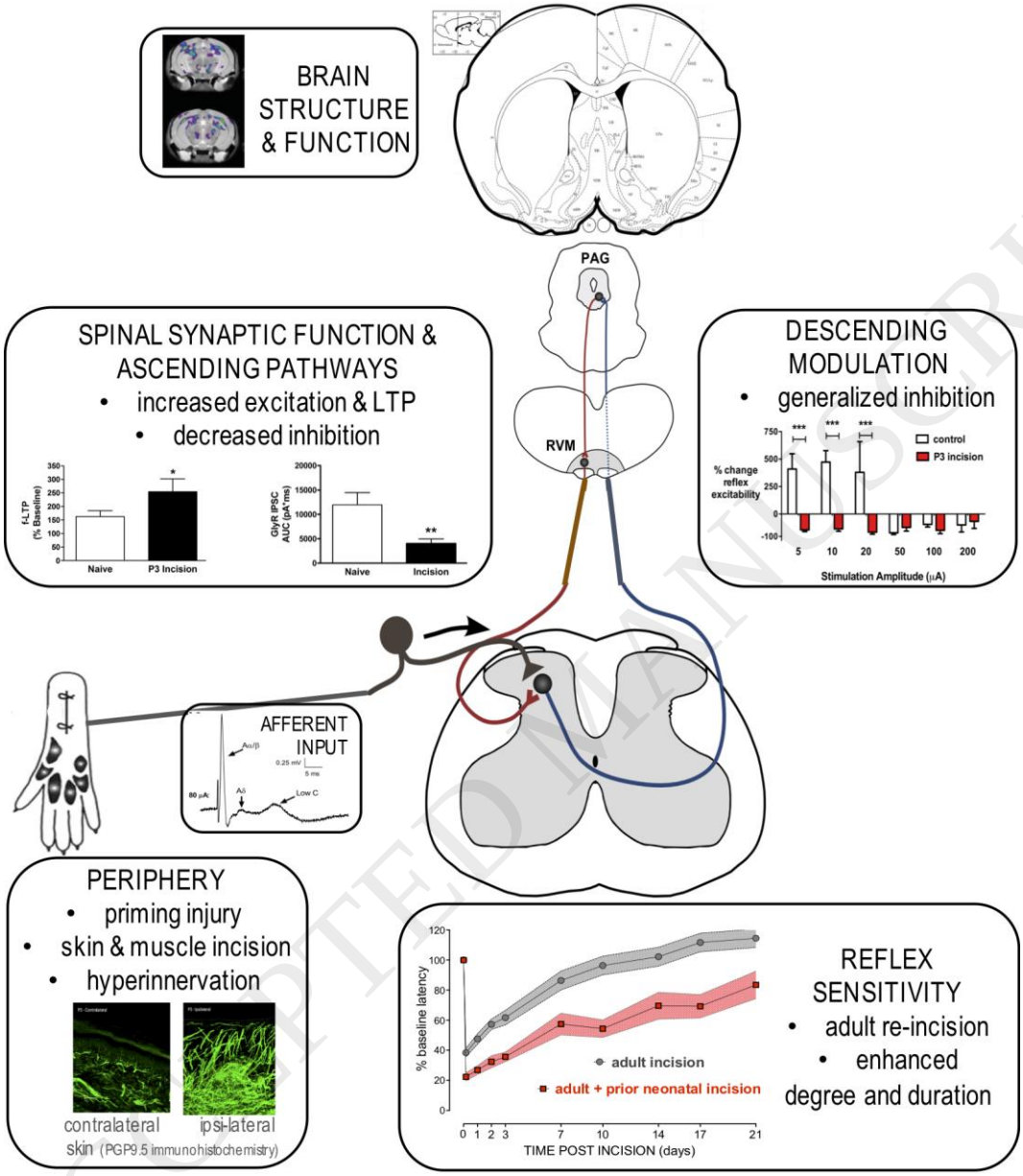

Table 1 A: Pain report in recent studies of young adults born very or extremely preterm

	Preterm	Term-born Control	Age	Assessment	Result (Preterm vs Control)
Self-reported musculoskeletal pain [21]	VP (<34 weeks) N=184 (52%F)	N=641 (54%F)	24 yrs	Any aches or pains in the last 6 months Options: no / yes / yes and health provider	• any pain 83% vs 86% • widespread pain requiring health provider 6% vs 5%
Bodily pain [20]	VP N=42	N=65	19 yrs	Bodily pain in last 4 weeks at least moderate severity	• 21% vs 15%
Bodily pain [20]	VP (29±2.7wks; 1200±257g) N=62 (48%F); NICU/ward median 75 days [23-386]	N=87 (57%F)	24 yrs	Bodily pain last 4 weeks at least moderate severity Duration > 6 months	• 29% vs 13% • 16% vs 7%
Self-reported chronic pain [28]	VP (28.8±2.6 wks; 1198±231g) N=51 (51%F) NICU/ward median 63 days [23-386]	N=86 (56%F)	28 yrs [27.3-29.9]	Pain at least moderate severity in the last 4 week Duration >6 months	• 25% vs 15%
Temporo-mandibular pain [19]	VP (28.8±2.6wks) N=145 (54%F)	N=140 (51%F)	17-19 yrs	Facial pain once a week or more Pain when open mouth wide or chew once a week or more	• 23% vs 26%
Pain History [14,18]	EP (24.9±0.8 wks; 732±127g) N=102 (60%F) NICU/ward median 126 days [73-497]	N=48 (60% F)	19 yrs [18.1-20.5]	Recurrent pain of at least moderate severity Activity interference due to pain (0-10 VRS)	• 54% vs 58% • 22% vs 8% • 3.3/10 vs 1.4/10

B: Somatosensory function in young adults born very or extremely preterm

	Preterm	Term-born Control	Age	Assessment	Result (Preterm vs Control)
QST [28]	VP (28.8±2.6 wks; 1198±231g) N=51 (51%F) NICU/ward median 63 days [23-386]	N=86 (56%F)	28 yrs [27.3-29.9]	Test site: wrist; lower leg Modalities: thermal (CDT, WDT, CPT, HPT); mechanical (PPT) Prolonged heat (change in VRS)	Thresholds: n.s. No sex differences All increase VRS with prolonged stimulus
QST [14]	EP (24.9±0.8 wks; 732±127g) N=102 (60%F) NICU/ward median 126 days [73-497] Surgery 30/102 (43%F)	N=48 (60% F)	19 yrs [18.1-20.5]	Test site: thenar; chest wall (± neonatal scar) Modalities: thermal (CDT, WDT, CPT, HPT); mechanical (MDT, MPT, PPT);	Generalized thresholds: decreased sensitivity; predominantly males with neonatal surgery Neonatal scars: decreased static

				dynamic (brush allodynia, punctate wind-up ratio)	sensitivity, increased dynamic allodynia; males and females
Cold pressor test [18]	EP (24.9±0.8 wks; 732±127g) N=102 (60%F) NICU/ward median 126 days [73-497]			Immersion: 5^0C, ≤30 secs	Tolerance to 30s: 53% vs 71% Male EP+surgery vs EP vs TC (67% vs 70% vs 68%) Female EP+surgery vs EP vs TC (23% vs 49% vs 72%)
Cold pressor test [30]	VP (31.1±2.5 wks; 1299±301 g N=412 (55%F) NICU/ward median 63 days [49-79] NEC 30/412 (7%)	Compare within subgroups	19 yrs	Immersion: 4-6^0C, ≤180 secs	Tolerance to 180s: SGA vs AGA (18% vs 27%) NEC vs no NEC (7% vs 25%) Female vs male (19% vs 29%)
Cold pressor test [29]	VP (26.8±1.8 wks; 942±209 g N=31 (58%F)	N=29 (69%F)	17-18 yrs	Immersion: 0-2^0C, ≤180 secs)	Tolerance to 180s: 32% vs 61%

Legend: VP, very preterm (<32 weeks gestational age at birth); EP, extremely preterm (<28 weeks gestational age at birth); TC, term control; F, female; g, birth weight in grams; AGA, appropriate for gestational age; SGA, small for gestational age; NEC, necrotizing enterocolitis; NICU, neonatal intensive care unit; CDT, cold detection threshold; WDT, warm detection threshold; CPT, cold pain threshold; HPT, heat pain threshold; MDT, mechanical detection threshold; MPT, mechanical pain threshold; PPT, pressure pain threshold. Numerical data are (mean±SD) or [range]