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Neonatal invasive procedures predict pain intensity at school age in children born very preterm

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Abstract

Children born very preterm display altered pain thresholds. Little is known about neonatal clinical and psychosocial factors associated with their later pain perception.

OBJECTIVE—We aimed to examine whether the number of neonatal invasive procedures, adjusted for other clinical and psychosocial factors, was associated with self-ratings of pain during a blood collection procedure in children born very preterm at school age.

METHODS—56 children born very preterm (24–32 weeks gestational age) at age 7.5 years, followed longitudinally from birth, and free of major neurodevelopmental impairments, underwent a blood collection by venipuncture. The children's pain was self-reported using the Coloured Analog Scale and Facial Affective Scale. Parents completed the Child Behavior Checklist and State-Trait Anxiety Inventory. Pain exposure (number of invasive procedures) and clinical factors from birth to term-equivalent age were obtained prospectively. Multiple linear regression was used to predict children's pain self-ratings from neonatal pain exposure after adjusting for neonatal clinical and concurrent psychosocial factors.

RESULTS—Greater number of neonatal invasive procedures and higher parent trait-anxiety were associated with higher pain intensity ratings during venipuncture at age 7.5 years. Fewer surgeries and lower concurrent child externalizing behaviors were associated with higher pain intensity.

CONCLUSION—In very preterm children, exposure to neonatal pain was related to altered pain self-ratings at school age, independent of other neonatal factors. Neonatal surgeries and concurrent psychosocial factors were also associated with pain ratings.

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Conflict of interest statement

The authors report no conflict of interest.

Keywords

pain; preterm infant; child; parents; behavior

1. Introduction

In the neonatal intensive care unit (NICU) infants born very preterm (< 32 weeks gestational age [GA]) are exposed to multiple painful and stressful procedures. There is experimental evidence in rodent studies for persistent changes in nociceptive processing and response to future pain following early life pain and injury [1]. Moreover, children born very preterm display altered pain thresholds [2,3,4], as well as exaggerated cortical activation in response to noxious stimulation [5]. Exposure to a greater number of neonatal painful interventions is associated with less endogenous pain inhibition during experimental pain in children born preterm, showing long lasting altered pain processing [6].

Rodent studies of long-term effects of early pain on pain thresholds have shown hyper- and hypo- sensitivity, depending on the nature of the pain stimulus [7,8]. Consistent with these animal findings, human studies comparing preterm and full-term children show that the direction of pain responses depends on duration and type of stimulus. For example, using experimental quantitative sensory testing in a lab setting at school age, children born preterm showed hypersensitivity to prolonged (i.e. tonic) painful stimulation and hyposensitivity to brief heat pain stimuli, compared to full term healthy controls [2]. Furthermore, adolescents born preterm were reported to have less tolerance to experimental pain tasks compared to full-term controls [4,9]. However, Walker and colleagues [3] found that in children born extremely preterm (< 25 weeks GA), neonatal surgery accounted for most of the differences in thermal pain sensitivity at age 9–11 years. Specifically, extremely preterm children exposed to surgery as neonates, were less sensitive to thermal pain compared to preterms that had not had surgery and to full-term controls. Neonatal surgery appears to be an important factor that has rarely been addressed.

According to the social communication model of pain [10], as preterm children develop, additional behavioral and psychosocial factors such as parental anxiety [11, 12, 13], caregiver pain perception [10], and child behavior [14,15] may interact with early exposure to neonatal pain and contribute to child pain experience later in life. Moreover, early adversity can contribute to a greater anxiety phenotype later, in both animals [16,17,18,19,20] and humans [21,22,23,24,25]. In children born very preterm, we have previously reported that higher neonatal procedural pain was associated with greater internalizing behaviors (i.e. anxious and/or depressive behaviors) at age 7.5 years [15]. Pain perception in children born preterm may also be associated with contextual factors, such as parental stress and anxiety levels [26,27]. Mothers of preterm children reported greater levels of solicitous behavior when their child was in pain, although maternal presence during prolonged/tonic heat pain stimuli was associated with higher pain thresholds in preterm children [26]. Parents of children born prematurely may show higher levels of stress and anxiety when compared to parents of full-term children [28], suggesting that emotional factors may also moderate the relationship between child pain responses and parental

psychosocial aspects. In summary, painful experiences have biological, psychological and social parameters [10], which are relevant when examining pain expression.

Previous studies comparing pain responses in very preterm compared to full-term infants have utilized clinical procedures such as blood collection [29,30] and vaccination [31]. However, beyond infancy, to our knowledge, all studies of pain sensitivity in childhood and adolescence have been conducted in experimental settings [2,3,4,5,6,9]. The present study, as far as we know, is the first to address in children who were born very preterm whether extent of neonatal pain exposure is related to self-ratings of pain to a procedure in a clinical setting at school age. Our aim was to examine whether exposure to invasive procedures in the NICU (adjusted for clinical confounders related to prematurity and concurrent psychosocial factors) was associated with self-ratings of pain intensity and affect during a blood collection at age 7 years in children who were born very preterm. Since exposure to prolonged experimental pain has been shown to evoke greater pain in preterm children, we hypothesized that greater exposure to neonatal invasive procedures would be associated with higher pain intensity and affective ratings at age 7.5 years, when controlled for neonatal clinical and concurrent psychosocial factors.

2. Material and Methods

2.1 Participants

The present study comprised 56 children born between 2000 and 2004 and admitted to the level III NICU at the British Columbia's Women's Hospital. Children were seen at mean age 7.5 years ($SD = 0.33$) as part of a larger longitudinal study of neonatal pain in relation to neurodevelopmental outcomes of children born very preterm [e.g. 32,33]. Children were excluded if they had a major congenital anomaly, major neurosensory impairment (legally blind, non-ambulatory cerebral palsy, sensorineural hearing impairment) or severe brain injury on neonatal ultrasound (periventricular leukomalacia and/or intraventricular hemorrhage grade III–IV). Out of the 204 very preterm infants recruited in the NICU during the initial study, 21 children had severe brain injury and/or major sensory or motor impairment, 12 lived too far away and 16 were beyond the age window of eligibility, therefore these 49 were not contacted. An additional 12 could not be reached for follow-up. Of the 143 families approached for follow-up at age 7 years, 12 had moved too far away, leaving 131 eligible children. Of those contacted, 22 refused to participate and 4 withdrew after consenting. A total of 105 families consented to a visit at age 7 years; one child with autism spectrum disorder was excluded. Of these 104 children, blood collection was part of a brain imaging substudy, in which 56 children with complete data agreed to a blood collection (1 child refused). These 56 children did not differ significantly from the other 48 seen at age 7 years with respect to GA, illness severity on day 1 (Score for Neonatal Acute Physiology [SNAP] II [34]), number of invasive procedures, number of surgeries, days on mechanical ventilation, and cumulative morphine exposure (all $P > 0.05$). This study was approved by the University of British Columbia Research Ethics Board. Parents and children provided written informed consent and assent respectively.

2.2 Measures

2.2.1 Neonatal clinical data—Medical and nursing chart review of neonatal data from birth to term equivalent age was carried out by an experienced neonatal research nurse. Data collection included, but was not limited to, birth weight, GA, number of days on mechanical ventilation, severity of illness on day 1 (SNAP-II), number of surgeries, presence of culture proven infection, and cumulative morphine dose. Morphine exposure was calculated (intravenous dose plus converted oral dose) as the daily average dose adjusted for daily body weight, multiplied by the number of days the drug was given. Neonatal pain was quantified as the number of invasive procedures (e.g., heel lance, peripheral intravenous or central line insertion, chest-tube insertion, nasogastric tube insertion) from birth to term equivalent age or NICU discharge (whichever came first), as previously described [33,35,36]. Each attempt at a procedure was counted as one invasive procedure; all NICU nursing staff were trained to precisely record each attempt, as described previously [35].

2.2.2 Pain Self-Ratings at 7.5 years—Pain self-ratings were recorded immediately following the venipuncture procedure. Two dimensions of the pain experience were measured: the Coloured Analog Scale (CAS) to assess the pain intensity [37] and the Facial Affective Scale (FAS) [37] to assess the emotional dimension of pain [38]. These approaches have been well validated as measures of pain with this age group [39,40]. The CAS is a 14.5 cm long triangular shape scale, varying in width and hue from 1 cm wide and light pink at the bottom (indicating 0/10 pain level), to 3 cm wide and deep red at the top (indicating 10/10 pain level); the words “NO PAIN” are at the bottom and “MOST PAIN” are at the top. The child was asked to “slide the marker along the scale until the intensity (strength) of the color matches the strength of your pain”, which corresponds to a score from a ruler on the back of the scale (not visible to the child).

The FAS is comprised of 9 faces each representing various affects. The face depicting neutral affect is on the far left of the card; four faces of increasingly positive affect from left to right form an upper row, and four faces of negative affect faces on the lower row. On the back of the card are the same faces with their numerical values varying from 0 to 1. The child was asked to point to the face picture that best represented how he/she felt “How did you feel deep down inside, not the face you showed the world” as a result of the blood collection procedure.

2.2.3 Child Behavior Checklist (CBCL)—Parents rated their child’s behavior using the Child Behavior Checklist (CBCL) for children ages 6 to 18 years [41], a widely used questionnaire for identifying behavioral problems in children. Ratings are on a 3-point Likert scale [ranging from 0 (not true) to 2 (very true or often true)] on 113 items. The CBCL yields two higher-order factors of Internalizing and Externalizing problems. The Internalizing scale encompasses anxious/depressed, withdrawn/depressed, somatic problems, whereas the Externalizing scale includes aggressive and rule-breaking behaviors. Raw scores were converted to age-standardized T-scores (mean = 50, SD = 10) based on the normative sample of children for age range separately by gender [41]. T-scores < 60 are considered to be in the normal range, 60–63 borderline range and > 63 are in the clinical range.

2.2.4 Parent Anxiety and Stress—Parent anxiety was measured by the self-report questionnaire State-Trait Anxiety Inventory (STAI) [42], which detects the presence and severity of current symptoms of anxiety and a generalized propensity to be anxious [43]. There are two subscales within this measure. The State Anxiety Scale (S-Anxiety) assesses the current state of anxiety, examining how respondents feel “right now,” and the Trait Anxiety Scale (T-Anxiety) evaluates relatively stable aspects of “anxiety proneness”, including general states of calmness, confidence, and security. The STAI is comprised of 40 items, 20 items allocated to each of the S-Anxiety and T-Anxiety subscales. Since we aimed to capture the general state of anxiety of the parent, we used the more stable Trait Anxiety Scale in the current study, which assesses the anxiety level of the parent as a personal characteristic [28]. Reported T-Anxiety scores > 52 indicated clinically significant anxiety disorders and scores between 48 to 52 indicating mild or subclinical disorders; T-Anxiety < 48 the probability of any clinically significant disorder is very low [44].

Parents completed the Parenting Stress Index-III (PSI) [45], that comprises 120-items rated on a 6-point Likert scale from 1 (strongly agree) to 6 (strongly disagree). The PSI yields two domain scores: Child Domain (concern about the child), Parent Domain (concern about their own parenting ability) and a Total Score. We only included the Parent Domain in the statistical analysis since our focus was on how parental factors may be related to child behavior. The Parent Domain consists of seven subscales: competence, isolation, attachment, health, role restriction, depression and spouse. Higher PSI scores indicate greater levels of stress and scores above the 85th percentile (148) are considered to be in the clinical range. Additionally, parents filled out a demographic information questionnaire.

2.3 Procedure

A small sample of blood (5ml) was collected from the children as part of a separate study on immune function and genetic analysis. According to the standard clinical protocol, Tetracaine Hydrochloride Gel 4% (AMETOP®) was applied for all children on the site of the venous blood collection 45 minutes prior to the venipuncture to anesthetize the skin. After the blood collection, children were asked to provide their intensity and affective pain ratings (CAS and FAS). During the follow-up visit, while children were going through a series of psychometric testing, parents completed questionnaires regarding their child behaviors (CBCL) and themselves (STAI, PSI, demographics).

2.4 Data Analysis

Neonatal clinical factors were inspected for normality, log transformed and/or winsorized [46] when necessary. The following variables were transformed: neonatal invasive procedures, morphine exposure and number of days on mechanical ventilation. Pearson correlations were conducted to examine associations among the predictors (i.e. between the neonatal clinical factors and concurrent psychosocial factors). The association between neonatal invasive procedures (main predictor variable) and self-reported pain ratings CAS and FAS at school age were examined separately as outcomes using stepwise multiple regression analysis by backward elimination with a Gaussian distribution. The backward method allows for the variables that contribute the most in explaining the outcome to remain in the model while excluding the non-significant predictors. This multiple regression

selection process enables the reduction from a larger set of variables by eliminating unnecessary predictors, simplifying data, and enhancing predictive accuracy, which was important given our limited sample size of 56 children. The analysis was adjusted for neonatal clinical factors (GA, illness severity on day 1, morphine exposure, days on mechanical ventilation, postnatal infection, and number of surgeries), concurrent child behaviors (CBCL-Externalizing and Internalizing T-score), and parent trait anxiety (T-Anxiety score).

Statistical analyses were performed using the Statistical Package for Social Sciences version 20.0 (IBM, Somers, NY, USA); p -values < 0.05 were considered statistically significant.

3. Results

3.1 Sample Characteristics

Demographic, clinical data, and parent factors are presented in Table 1. Children reported a low mean pain score on the CAS of 2.67 (SD = 2.6) and on the FAS of 0.51 (SD = 0.25). Child self-rated intensity pain scores on the CAS and affective ratings on the FAS were significantly correlated ($r = 0.42$, $P < 0.01$).

The mean CBCL Internalizing and Externalizing scores were in the normal range. On Internalizing and Externalizing scales respectively, most children had scores in the normal range [78.6% ($n = 44$); 89% ($n = 50$)], 9% ($n = 5$) and 4% ($n = 2$) were in the borderline clinical range. Finally, 12.5% ($n = 7$) for Internalizing scale and 7% ($n = 4$) for Externalizing were in the clinical range with a T-score > 63 .

3.2 Correlations Among Predictors

Among the neonatal clinical predictors, lower GA at birth was correlated with higher SNAP-II on day 1, higher cumulative morphine exposure, more postnatal infection, greater number of invasive procedures, and higher number of surgeries (Table 2). Among the concurrent psychosocial factors at age 7.5 years, higher CBCL-Internalizing T-scores were associated with higher CBCL-Externalizing T-scores, higher parent T-Anxiety scores, and higher PSI scores. No correlation among the neonatal or concurrent psychosocial predictors was $r > 0.80$, thus multicollinearity among predictors was not considered to be problematic [47].

However, neonatal morphine exposure and days on mechanical ventilation were highly correlated ($r = 0.76$, $P < 0.001$), since only ventilated infants received morphine in our NICU. Keeping both variables in the model did not improve the model (R square change < 0.0001 , $P = 0.93$) and the statistical significance of all other predictors remained the same. Thus, to reduce the number of neonatal factors in our statistical model we only included neonatal morphine exposure and chose to exclude days on mechanical ventilation.

3.3 Correlations Between Predictors and Child Intensity/Affective Pain Ratings

There were no statistically significant bivariate correlations between any of the neonatal or concurrent predictors and child CAS or FAS scores. However, a correlation coefficient only measures the linear dependence between two variables, without controlling for the fact that other variables might be involved in the relationship as well. In fact, the relationship might

be masked unless confounders are controlled [48]. Given that very preterm infants who undergo more invasive procedures tend to be sicker, earlier born, more likely to be exposed to infection and surgery, and undergo longer mechanical ventilation (see Table 2), it is essential to account for these confounders to address a potential association between early pain and later outcomes. Therefore we conducted exploratory multiple regression analysis on child pain outcomes, namely the child pain intensity (CAS scores) and affective (FAS scores) ratings while accounting for these important confounders.

3.4 Prediction of Child Pain Intensity and Affective Ratings at 7.5 years from Neonatal and Concurrent Factors

In the initial regression model, neonatal predictors were added simultaneously, and as the backward method was performed, the analysis provided three subsequent regression models. Illness severity on day 1, morphine exposure, and postnatal infection were excluded one at a time. The final model included the following variables: GA, number of surgery, child externalizing behavior and parent trait anxiety.

In the final regression analysis, higher pain intensity ratings (CAS scores) by very preterm children at 7.5 years were significantly associated with greater number of neonatal invasive procedures (β 0.44 [0.47, 6.35], $P = 0.02$) and lower number of surgeries (β -0.32 [-2.55, -0.23], $P = 0.02$) (Table 3). Moreover, higher CAS scores were related to lower CBCL-Externalizing T-scores (β -0.30 [-0.15, -0.009], $P = 0.03$) and higher level of parent Trait-Anxiety (β 0.38 [0.04, 0.21], $P = 0.007$). The number of invasive procedures, adjusted for neonatal clinical confounders (e.g., GA, surgeries, infection) and concurrent psychosocial variables at age 7.5 years (CBCL-Externalizing T-scores and parent T-Anxiety scores), explained 25% of the variance in the CAS pain intensity ratings in children born very preterm at school age (Figure 1).

When CBCL-Internalizing T-scores were included as a predictor in the regression analysis, instead of CBCL-Externalizing T-scores, the model did not reach statistical significance [F (8, 47) = 1.84, $P = 0.09$]. Additionally, when we used the Facial Affective Scale (FAS) scores as the outcome, rather than CAS scores, the model was not statistically significant [F (8,47) = 0.38, $P = 0.92$].

4. Discussion

This is the first study, to the best of our knowledge, to examine self-ratings of intensity and affective pain responses in a clinical context in children born very preterm at school age. Consistent with our hypothesis, **greater neonatal pain exposure** (adjusted for both clinical factors related to prematurity and concurrent psychosocial factors) predicted **higher pain intensity** ratings at 7.5 years in children born very preterm, despite the fact that children rated their pain as mild since all children received topical anesthesia. However, early pain did not predict the children's affective response to later blood collection.

Our findings suggest that, after accounting for multiple clinical factors related to NICU care and concurrent psychosocial factors, neonatal pain exposure has long-lasting effects on the sensory pain experience, despite the use of a topical anesthetic (Ametop®) for the blood

collection at school age. Importantly, the clinical setting provides a natural context to examine pain, complementary to experimental lab studies in children born preterm [2,3,4,5,6,9,26]. Unexpectedly, only the intensity dimension of pain, but not the affective dimension, was predicted by neonatal factors or concurrent psychosocial factors in our study. This finding differed from a previous study of Grunau and colleagues, who showed that among children born at extremely low birth weight (<1000g), duration of NICU stay was related to higher pain affect ratings to pictures of pain in recreational and daily living settings at age 8 to 10 years [39]. However, in the earlier study neonatal pain was not measured in detail, and there have been major changes in NICU care and pain management since the 1980s when these infants were born. Most importantly, in the previous study the children rated pictures of pain events, whereas in the present study the children actually experienced a painful procedure for blood collection.

Beyond infancy, in childhood and adolescence, experimental studies have shown that early pain exposure in preterm infant has long-term consequences on later pain thresholds [3,4,6]. For example, adolescents born preterm exhibited lower pain tolerance to a cold pressor task compared to those born full-term [4]. In that study, among the preterms, greater neonatal pain exposure, longer mechanical ventilation and more exposure to morphine predicted higher pain tolerance (i.e. hyposensitivity) to the cold pressor pain at age 17 years. Importantly, in that study, there were no differences between the preterm and full-term groups on *self-reported* pain ratings. Our present study showed higher self-ratings of pain intensity in very preterm children exposed to more neonatal pain, however we cannot compare our finding to the cold pressor study [4], since unfortunately, self-ratings were not examined in relation to neonatal factors among the preterms in their sample. Quantitative sensory testing in school-aged children revealed sensitization (i.e. hypersensitivity) to prolonged (tonic) heat pain in the preterm born compared to healthy term born controls, but hyposensitivity to brief heat pain [2]. Our present findings suggested that greater neonatal pain exposure predicted hypersensitivity to a blood draw at school age. Given that experimental studies have found hypersensitivity to prolonged pain, this suggests that children may consider a venous blood draw a prolonged pain situation. Taken together, these conflicting findings are consistent with the hypoanalgesia and hyperanalgesia seen in adult rats under different pain stimulation conditions following early pain exposure [8]. These studies show the importance of considering the type and duration of later pain stimulation in evaluating long-term effects of neonatal pain exposure on later pain expression in children born very preterm. It is important to note that both hypo- and hypersensitivity to pain are “aberrant” and this should be considered when comparing findings between studies in this population.

Long-term effects of neonatal surgery have rarely been considered when addressing the impact of neonatal pain on later pain sensitivity. Decreased sensitivity to thermal (mediated by unmyelinated C-fibers and A-delta fibers) but not mechanical (A-beta sensory function) stimuli was reported in 11 year-old children born extremely preterm (<26 weeks GA) compared to children born full term [3]. Surprisingly, this *hyposensitivity* to thermal pain was more marked in those who had undergone surgery during neonatal period. Similarly, we found in the present study that exposure to neonatal surgeries was associated with *lower* self-ratings of pain intensity in children born very preterm. Conversely, in a combined

sample of preterm and full-term infants that underwent surgery in first three months of life, nurses rated pain higher during pain assessment to a subsequent surgery performed in the same dermatome, compared to infants with no prior surgery or infants who previously underwent surgery in another dermatome [49]. In another study, infants who underwent major surgery within the first 3 months of life did not show altered pain response to immunization at 14 or 45 months compared with infants that did not have surgery [50]. However, in the infants exposed to surgery, higher number of major surgical procedures and longer stays in the NICU, were associated with greater facial response but less heart rate response to immunization at age 14 months but not at 45 months. Thus, findings on long-term effects of neonatal surgery on children's pain perception remain inconclusive, and additional factors such as age at surgery, prematurity status, type of surgery, type of anesthesia and perioperative analgesia, comorbidities, surgical history and previous pain exposure, need to be taken into account when studying these possible relationships. Addressing neonatal surgery appears to be important since preterm infants exposed to surgery and anesthesia have a greater incidence of moderate to severe white matter injury and smaller total brain volumes, although it is unclear whether this may be due to pre-existing factors. These infants also exhibit poorer performance on cognitive and psychomotor assessments at 2 years of age, although this was not significant after correction for additional risk factors [51,52]. Therefore it is possible, that neonatal surgery exposure may be a marker of other factors that affect pain expression. Basic animal studies are needed to explicate mechanisms underlying these later changes in pain perception following early surgery.

On functional brain imaging (fMRI) at age 11–16 years, children born preterm showed greater neuronal activation compared to controls, during experimental prolonged heat pain [5]. In brain regions significantly activated in the control group, a greater number of voxels were activated in the preterm group. Significant group differences were observed in the contralateral primary somatosensory cortex, anterior cingulate cortex, and the ipsilateral anterior insula. Moreover, additional regions (thalamus, anterior cingulate cortex, cerebellum, basal ganglia, periaqueductal grey) were only activated in the children born preterm. This long-term exaggerated neuronal response to pain in children born preterm highlights the consequences of developmentally unexpected nociceptive input during a vulnerable period of brain development. Additionally, greater cerebral activation may not be necessarily translated into greater pain reactivity or self-reported pain ratings.

According to the complex social communication model of pain [10] numerous factors, including interpersonal processes, could influence pain experience and expression. It has been demonstrated that concurrent psychosocial factors such as parenting stress and parent interaction, play an important role in moderating the relationship between neonatal pain and later child behavior problems [14,33,53]. Moreover, caregiver behavior, attitudes and emotional/psychological distress may also influence pain-coping strategies of their children [54] and impact their pain expression [55,56]. The present study showed that higher parent trait anxiety predicted higher self-ratings of pain intensity in very preterm children, supporting the relationship between caregiver factors and pain processing.

Preterm birth is associated with adverse outcomes such as internalizing (anxiety/depressive) behaviors, poor executive functions and attention problems later in childhood [21]. We previously demonstrated that higher number of skin-breaking procedures in preterm infants during NICU hospitalization was associated with more internalizing behaviour at 18 months of age, and that this association was buffered by psychosocial parental factors [14]. In a subsequent study in the same cohort, this association was still present at age 7.5 years, in that cumulative neonatal procedural pain and morphine exposure, as well as concurrent parenting stress contributed to higher child internalizing behaviors at school age in children born very preterm, after controlling for neonatal clinical confounding factors [15]. While most of the child behavior scores in our study did not reach the clinical problem cutoff, our present findings indicated that less externalizing behaviors were associated with higher self-ratings of pain intensity at school age. Thus, children that tended to exhibit less aggressive and rule-breaking behaviors in their day-to-day life rated their pain intensity higher to the blood draw. Given our previous findings and the positive association between externalizing and internalizing behaviors in our sample of very preterm children, it remains unclear why only externalizing behaviors predicted self-rated pain to the venipuncture.

A limitation of the present study is that we did not take into account the children's pain history between NICU discharge and pain assessment at 7.5 years, which may influence self-ratings of pain. Future longitudinal research should consider other pain exposures across childhood after NICU discharge to extend knowledge of factors involved in later altered pain perception in children born very preterm. Although the number of invasive procedures, adjusted for neonatal clinical confounders (e.g., GA, surgeries, infection) and concurrent psychosocial factors only explained 25% of the variance in children's pain intensity ratings, it does highlight the enduring effects early exposure to stress and pain can have on later pain perception in children born very preterm. Additionally, human behaviors are typically difficult to predict and thus, in the present study, being able to predict pain intensity ratings in school age children born very preterm to the extent that we have found appears important.

Moreover, parent trait anxiety was related to later pain perception in children born very preterm. Support to promote optimal parent-child interaction may moderate the long-term effects of neonatal pain exposure on later pain experience, and as a consequence help normalize child behavior.

Though our findings should be interpreted with caution, in part due to our limited sample size, they do have important clinical implications and provide ecological validity that is complimentary to experimental lab studies. Above and beyond multiple clinical factors associated with prematurity and concurrent psychosocial factors, greater exposure to neonatal pain was associated with higher ratings of pain intensity to blood collection at 7.5 years in very preterm children. In our present study, topical analgesia was applied to all children for pain control, since ethically, pain management is required for clinical procedures. Consequently, children rated their pain to the blood draw as mild and this may have affected our findings. Although, it is still ethically acceptable to conduct experimental pain studies in children without providing pain management (e.g. cold pressure task [57]), it is not the case in the clinical setting. Therefore, it is now very challenging to find ways to clinically study pain response in children without introducing factors that may confound the

research outcome; this must be kept in mind when interpreting and generalizing the present findings.

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Figure 1.

Explained variance (25%) in pain intensity ratings in relation to the number of neonatal invasive procedures adjusted for confounding factors. On the Y-axis are the self-ratings of pain intensity (CAS scores); on the X-axis are the adjusted and log transformed number of neonatal invasive procedures.

Table 1

Neonatal, child and parent characteristics (n = 56)

<i>Neonatal</i>	Values
Gestational Age at birth, weeks – mean (SD); median (IQR)	29.56 (± 2.3); 29.71 (27.8 – 31.5)
Birth weight, grams – mean (SD); median (IQR)	1,319 (± 421); 1,285 (953 – 1,562)
Sex, male/female – number (%)	23 (41%)/33 (59%)
Illness Severity day 1, SNAP-II scores – mean (SD); median (IQR)	9.73 (± 10); 8.5 (0 – 14)
Number of Invasive Procedures – mean (SD); median (IQR)	98.7 (± 77); 74 (46.2 – 130.7)
Morphine Exposure, kg/day – mean (SD); median (IQR)	1.25 (± 3.99); 0.04 (0 – 0.65)
Mechanical Ventilation, days – mean (SD); median (IQR)	8.61 (± 15); 3 (0 – 10)
Postnatal Infection – number (%)	16 (28.6)
Surgery 1 – number (%)	10 (17.9)
<i>Child</i>	
Chronological Age, years – mean (SD); median (IQR)	7.47 (± 0.33); 7.39 (7.3 – 8.4)
Coloured Analog Scale, scores – mean (SD); median (IQR)	2.67 (± 2.6); 2 (0.2 – 4.8)
Facial Affective Scale, scores – mean (SD); median (IQR)	0.51 (± 0.25); 0.47 (0.37 – 0.75)
CBCL Externalizing T-scores – mean (SD); median (IQR)	47.75 (± 9.9); 47.5 (41 – 52.5)
CBCL Internalizing T-scores – mean (SD); median (IQR)	52.45 (± 9.8); 51 (45 – 58.75)
<i>Parent</i>	
Mother's age at child birth (years) – mean (SD); median (IQR)	33.4 (± 5); 34 (29.4 – 37.2)
Marital status (married) – number (%)	51 (91%)
Ethnicity (Caucasian) – number (%)	42 (75%)
Education, years – mean (SD); median (IQR)	15.77 (± 2.66); 16 (14 – 17)
Parenting Stress Index-III, scores – mean (SD); median (IQR) *	117.2 (± 23.4); 118 (98 – 129)
T-Anxiety, scores – mean (SD); median (IQR)	35.48 (± 8.21); 36 (29 – 42)

IQR, Inter quartile range; SNAP-II, Score for Neonatal Acute Physiology-II (severity of illness index); CBCL, Child Behavior Check List; PSI, Parenting Stress Index-III (Parent Domain); T-Anxiety, Trait anxiety domain of State-Trait Anxiety Inventory for Adults;

* One parent did not provide PSI-III (n = 55).

Table 2

Pearson correlations among the neonatal, children and parent predictors (n = 56)

	SNAP-II scores	Morphine Exposure	Mechanical Ventilation	Postnatal Infection	Number of Surgery	Invasive Procedures	CBCL- Internalizing T-scores	CBCL- Externalizing T-scores	T-Anxiety scores	PSI scores
Gestational Age	– 0.57**	– 0.42**	– 0.65**	– 0.54**	– 0.16	– 0.73**	0.10	0.14	0.03	0.10
SNAP-II scores	–	0.37**	0.46**	0.29*	0.12	0.40**	– 0.06	– 0.06	– 0.09	– 0.04
Morphine Exposure	–	–	0.76**	0.35*	0.64**	0.55**	0.24	0.03	0.07	0.19
Mechanical Ventilation	–	–	–	0.53**	0.38**	0.69**	0.03	– 0.16	– 0.17	– 0.12
Postnatal Infection	–	–	–	–	0.00	0.60**	– 0.11	– 0.19	– 0.04	– 0.05
Number of Surgery	–	–	–	–	–	0.31*	0.10	0.09	0.20	0.18
Invasive Procedures	–	–	–	–	–	–	0.06	– 0.03	0.01	0.01
CBCL-Internalizing T-scores	–	–	–	–	–	–	–	0.67**	0.28*	0.34*
CBCL-Externalizing T-scores	–	–	–	–	–	–	–	–	0.39*	0.50**
T-Anxiety scores	–	–	–	–	–	–	–	–	–	0.71**

SNAP-II, Score for Neonatal Acute Physiology II on day 1 (severity of illness index); Morphine exposure, daily morphine exposure adjusted for daily weight; CBCL, Child Behavior Check List; T-Anxiety, Trait anxiety domain of State-Trait Anxiety Inventory for Adults; PSI, Parenting Stress Index-III (Parent Domain).

* *P* < 0.05;

** *P* 0.001

Table 3

Multiple linear regression analysis for CAS sensory pain scores in children born very preterm at school age (n=56).

Predictors	Standardized β	t-value	95% Confidence Intervals	p-value
Invasive Procedures	0.44	2.33	[0.47, 6.35]	0.02
Gestational Age	0.38	2.08	[0.02, 0.87]	0.04
Number of Surgery	- 0.32	- 2.41	[-2.55, -0.23]	0.02
CBCL-Externalizing T-scores	- 0.30	- 2.25	[-0.15, -0.009]	0.03
T-Anxiety scores	0.38	2.82	[0.04, 0.21]	0.007

R square = 0.25; Adjusted R-square = 0.175 ($p=0.01$)

Child Externalizing Behaviour, CBCL for ages 6 to 18 years (T-scores); T-Anxiety, Trait anxiety domain of State-Trait Anxiety Inventory for Adults.