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Internalizing behaviours in school-age children born very preterm are predicted by neonatal pain and morphine exposure

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Abstract

Background—Greater neonatal pain is associated with higher internalizing behaviours in very preterm infants at 18 months corrected age, but it is unknown whether this relationship persists to school age. Moreover, it is unclear whether morphine ameliorates or exacerbates the potential influence of neonatal pain/stress on internalizing behaviours. We examined whether neonatal pain-related stress is associated with internalizing behaviours at age 7 years in children born very preterm, and whether morphine affects this relationship.

Methods—101 children born very preterm (≤ 32 weeks gestation) were seen at mean age 7.7 years. A parent completed the Parenting Stress Index and Child Behavior Checklist questionnaires. Neonatal pain-related stress (the number of skin-breaking procedures adjusted for clinical factors associated with prematurity) was examined in relation to internalizing behaviour, separately in subjects mechanically ventilated and exposed to both pain and morphine (n = 57) and those never mechanically ventilated, exposed to pain but not morphine (n = 44).

Results—In the non-ventilated group, higher skin-breaking procedures (p = 0.037) and parenting stress (p = 0.004) were related to greater internalizing behaviours. In the ventilated group, greater morphine exposure (p = 0.004) was associated with higher child internalizing scores.

Conclusions—In very preterm children who undergo mechanical ventilation, judicious use of morphine is important, since morphine may mitigate the negative effects of neonatal pain on nociception but adversely affect internalizing behaviours at school age. Management of procedural pain needs to be addressed in very preterm infants in the NICU, to prevent long-term effects on child behaviour.

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Introduction

Internalizing behaviours (i.e. anxious/depressed, withdrawn/depressed, and somatic problems), prevalent in children born very preterm compared to full-term, are evident by 2 years corrected age (CA) (Spittle et al., 2009; Vinall et al., 2013) and persist to school-age, late adolescence and young adulthood (Anderson and Doyle, 2003; Grunau et al., 2004; Loe et al., 2011; Schmidt et al., 2010; van Baar et al., 2009). Animal studies have established that early-life adversity, including maternal separation and pain exposure, induce long-term behavioural changes (Anand et al., 1999; Bhutta et al., 2001; Brummelte et al., 2006; de Medeiros et al., 2009; Low and Fitzgerald, 2012; Maccari and Morley-Fletcher, 2007; Walker et al., 2008). Little is known about the aetiology of internalizing behaviours in children born very preterm. Exposure to repeated pain in very preterm infants (≤32 weeks gestational age [GA]) in the neonatal intensive care unit (NICU) is associated with altered cognitive and motor development (Grunau et al., 2009). Previously, we found that greater neonatal pain (quantified as the number of neonatal skin-breaking procedures adjusted for clinical confounders) was associated with greater internalizing behaviours at 18 months CA (Vinall et al., In press). However, it is unknown whether the relationship between neonatal pain and internalizing behaviours persists to school age. Greater catastrophizing to painful events has been demonstrated in children and adolescents born very preterm (Hohmeister et al., 2009).

Mechanical ventilation in very preterm infants is highly stressful, as well as a potentially painful intervention (Barker and Rutter, 1995). Although caution has been advocated in the use of opioids in very preterm infants, morphine continues to be commonly used during mechanical ventilation (Hall et al., 2007; Simons et al., 2003). The effect of neonatal pre-emptive intravenous morphine exposure during mechanical ventilation on neurodevelopmental outcomes has been examined in clinical trials (Anand et al., 2004; Simons et al., 2003). Neonatal morphine exposure had no effect on internalizing behaviours at 5 years (de Graaf et al., 2011; Ferguson et al., 2012) or at 8 to 9 years (de Graaf et al., 2013). However, the extent of neonatal pain exposure was not considered in those studies.

Our aim was to examine whether neonatal pain and morphine exposure in non-mechanically ventilated and ventilated neonates are associated with internalizing behaviours at age 7 years in children born very preterm. We hypothesized that among children born very preterm: a) higher neonatal pain, after adjusting for confounding clinical factors, would be associated with higher internalizing behaviours at age 7 years in both the non-ventilated (pain but no morphine) and ventilated (pain and morphine) exposed groups; and b) greater neonatal morphine exposure in ventilated preterm neonates, after adjusting for confounding clinical factors, would be associated with lower internalizing behaviours at age 7 years. In our NICU morphine is only given to infants undergoing mechanical ventilation. Thus we could only study morphine and neonatal pain in relation to internalizing by running analyses on the ventilated and non-ventilated infants separately.

1. Methods

1.1. Participants

School-age children were seen as part of a larger longitudinal study of neonatal pain in the aetiology of neurodevelopmental problems of children born very preterm (24-32 weeks gestation) (Grunau et al., 2007; Grunau et al., 2009). Very preterm infants who were admitted to the level III neonatal intensive care unit (NICU) at BC Women's Hospital, Vancouver, BC between 2000 and 2004 were eligible. Children were excluded if they had a major congenital anomaly, major neurosensory impairment (legally blind, non-ambulatory cerebral palsy, sensory-neural hearing impairment), or severe brain injury (periventricular leukomalacia and/or grade 3 or 4 intraventricular haemorrhage) evident on neonatal ultrasound. Out of the 204 very preterm infants recruited in the NICU during the initial study, 49 families were not contacted (21 children had severe brain injury and/or major sensory or motor impairment, 12 lived too far away, 16 study had ended) and 12 could not be reached for follow-up at 7 years. From the 143 parents of very preterm infants approached for follow-up of their children at age 7 years, 12 had moved too far away to be followed up, leaving 131 eligible children. Of the 131 eligible children contacted for the 7-year follow-up, 22 refused to participate, 109 consented (83% out of all the eligible ones), and 102 returned for follow-up at school age. One child diagnosed with autism was excluded. The final study sample comprised 101 school-age children born very preterm. The 22 children who refused to take part in the follow-up study did not differ significantly ($p > 0.05$) from the 101 participants on key neonatal factors such as GA, SNAP-II on day 1, number of skin-breaking procedures, number of surgeries, days on mechanical ventilation, and cumulative morphine exposure. The day of the follow-up visit, while children were going through a series of psychometric testing, parents (99 mothers, 2 fathers) were instructed by a research assistant to complete questionnaires regarding their child (e.g. Child Behavior Checklist- CBCL) and themselves (e.g. Parent Stress Index- PSI III).

The study was approved by the Clinical Research Ethics Board of the University of British Columbia and the British Columbia Children's and Women's Research Ethics Board. Written informed consent from parents and child assent were obtained.

2.2 Measures

2.2.1 Neonatal clinical data collection—Medical and nursing chart review of neonatal data from birth to term equivalent age was carried out by highly trained neonatal research nurses. The data collection, included but was not limited to, birth weight, gestational age, number of days on mechanical ventilation, illness severity on day 1 (Score for Neonatal Acute Physiology [SNAP]- II (Richardson et al., 2001)), number of surgeries, presence of culture proven infection, and cumulative doses of morphine. The number of accumulated doses of morphine was calculated (intravenous dose plus converted oral dose) as the average daily dose adjusted for daily body weight, multiplied by the number of days the drug was given, as previously described (Brummelte et al., 2012; Grunau et al., 2009). Neonatal pain was quantified as the number of skin-breaking procedures (e.g., heel lance, peripheral intravenous or central line insertion, chest-tube insertion, tape removal, and nasogastric tube insertion) from birth to term equivalent age or NICU discharge (whichever came first), as

previously used (Brummelte et al., 2012; Grunau et al., 2005; Grunau et al., 2009). Each attempt at a procedure was counted as one skin-break; all nursing staff in our NICU have been trained to precisely record each attempt. Child demographic information was obtained by questionnaire.

2.2.2 Child Behavior Checklist (CBCL)- Internalizing dimension—Parents rated their child's behaviour using the Child Behavior Checklist (CBCL) for children ages 6 to 18 years (Achenbach and Rescorla, 2001), a widely used questionnaire for identifying problem behaviours 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 comprises eight syndrome scales (Anxious/Depressed, Withdrawn/Depressed, Social Problems, Somatic Complaints, Thought Problems, Attention Problems, Aggressive Behavior, and Rule-Breaking Behavior) and 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. Only the Internalizing score (includes Anxious/Depressed, Withdrawn/Depressed, and Somatic Problems syndrome scales) was used in the present study, given that Internalizing, not Externalizing problems are primarily associated with prematurity (Aarnoudse-Moens et al., 2009; Grunau et al., 2004). Reliability for the Internalizing subscale is high (stability 0.82; Cronbach's alpha 0.90). Raw scores were converted to age-standardized scores (T scores with mean = 50 and SD = 10) based on the normative samples of children for age range separately by gender (Achenbach and Rescorla, 2001). Scores are interpreted as following: for Internalizing Problems, T scores below 60 are considered in the normal range, 60-63 represent borderline scores, and scores greater than 63 are in the clinical range.

2.2.3 Parenting Stress—Parents (N=100) completed the Parenting Stress Index-III (PSI) (Abidin, 1995), 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 influence child behaviour and that the Child Domain reflects parent's concerns about their child's behaviours. The Parent Domain consists of seven subscales: Competence, Isolation, Attachment, Health, Role Restriction, Depression, and Spouse. Higher PSI scores indicate higher levels of stress and scores above the 85th percentile (≥148) are considered in the clinical range. Additionally, parents filled out questionnaires concerning their demographic information.

2.3 Data analysis

Neonatal clinical factors were inspected for normality, log transformed and/or winsorized (Tukey, 1977) when necessary (neonatal skin-breaking procedures, morphine exposure, and number of days on mechanical ventilation). Demographic and parent characteristics of the two groups of preterm children (i.e., non-ventilated exposed to pain but not morphine and mechanically ventilated/exposed to pain and morphine) were compared using *t*-tests or Mann-Whitney tests, when appropriate. Pearson correlation tests were conducted to examine associations between the neonatal clinical factors. The association between the internalizing

scores and number of neonatal skin-breaking procedures and morphine exposure was examined with multiple linear regression analysis with a Gaussian distribution, adjusting for neonatal clinical factors (GA, SNAP-II at day 1, post-natal infection, number of days on mechanical ventilation, and number of surgeries) and concurrent parenting stress (PSI-III Parent Domain score), in the non-ventilated and ventilated groups separately. All independent variables were added simultaneously in each model. Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 16.0 (IBM, Somers, NY); *p*-values < 0.05 were considered statistically significant.

2. Results

3.1 Characteristics of the sample

Of the 101 very preterm born children included in this study at 7 years, 44 had not received mechanical ventilation support or morphine during their NICU stay and 57 children were mechanically ventilated and exposed to morphine. As expected, mechanically ventilated neonates, compared to non-ventilated, had significantly lower GA ($t[98.44] = 7.73$, $p < 0.001$) and birth weight ($t[98] = 5.58$, $p < 0.001$), and higher illness severity on day one (SNAP-II) ($t[83.26] = -6.14$, $p < 0.001$), higher total number of skin-breaking procedures ($t[98] = -9.17$, $p<0.001$), and more postnatal infections (Mann-Whitney $U = 737.5$, $p < 0.001$). Mothers comprised 98% of the respondents; two fathers were the primary caregivers at the time of study testing. Child internalizing scores at 7 years, concurrent parenting stress levels (PSI- Parent domain) and parental characteristics did not differ significantly between the two groups. Participant demographic and clinical data are presented in Table 1.

Means of the CBCL-Internalizing T scores were within the normal range (i.e. < 60) for both groups of very preterm children. Most children 77/101 (77%) were in the nonclinical range (32 non-ventilated, 45 ventilated), and 7/101 (7%) in the borderline range 60-63 (4 non-ventilated, 3 non-ventilated), and 17/101 (17%) in the clinically significant range with a T score greater than 63 (8 non-ventilated, 9 ventilated). Table 2 shows the mean number of skin-breaking procedures, cumulative doses of morphine and level of parenting stress according to CBCL- Internalizing T scores (non-clinical versus clinical range) and morphine/ventilation exposure. Eleven parents (3 non-ventilated, 8 ventilated) had PSI-Child domain scores above the 85th percentile (≥148). There were no outliers on the CBCL or PSI-III.

3.2 Neonatal predictors

Correlations among the neonatal clinical characteristics of the very preterm infants in each group were examined separately (Table 3). Among the non-ventilated infants, higher number of skin-breaking procedures was associated with lower GA at birth ($r = -0.54$), and higher illness severity on day 1 (SNAP-II) was correlated with lower GA ($r = -0.34$). Among the mechanically ventilated neonates, higher number of skin-breaking procedures was correlated with lower GA ($r = -0.68$), higher illness severity on day 1 (SNAP-II; $r = 0.37$), greater morphine exposure ($r = 0.65$), higher number of surgeries ($r = 0.46$), and higher days on mechanical ventilation ($r = 0.85$). In the ventilated group, higher morphine exposure was correlated with lower GA at birth ($r = -0.47$), higher illness severity on day 1 (SNAP-II; $r =$

0.40), higher number of surgeries (r = 0.68), and more days on mechanical ventilation (r = 0.76).

3.3 Multiple Linear Regression Analysis

3.3.1 Non-ventilated preterms—In this regression model, we found that higher internalizing scores were significantly associated with greater number of neonatal skin-breaking procedures (β 0.348 [1.134, 33.120]; p = 0.037) and higher level of concurrent parenting stress (β 0.447 [0.057, 0.276]; p = 0.004) (Table 4a). None of the other neonatal predictors in our model was significantly associated with internalizing scores.

3.3.2 Mechanically ventilated preterms—In the group of children that were mechanically ventilated and exposed to morphine during their NICU stay, the regression model revealed that higher morphine exposure was associated with higher internalizing behaviours (β 0.728 [7.166, 34.652]; p = 0.004), independent of the other neonatal clinical predictors, none of which were statistically significant. Greater concurrent parenting stress scores showed a trend with child internalizing (β 0.241 [-0.007, 0.204]; p = 0.066), after adjusting for the neonatal predictors (Table 4b).

Since the number of neonatal skin-breaking procedures was highly correlated with days on mechanical ventilation in this group (r = 0.85), multicollinearity (conventional cut-off of r > 0.80 (Grimm and Yarnold, 1995)) was dealt with by repeating the regression model excluding either the skin-breaking procedures or excluding days on mechanical ventilation, and examining whether predictors of internalizing behaviours at 7 years remained the same. When skin-breaking procedures was out of the model, morphine exposure remained significantly associated with internalizing behaviours (p = 0.003). When days on mechanical ventilation was excluded from the model, morphine exposure remained predictive of internalizing behaviours (p = 0.005) and parenting stress (PSI) became a significant predictor of internalizing behaviours (p = 0.04).

In secondary multiple linear regression analyses, we excluded children exposed to neonatal midazolam (n = 8); morphine exposure remained the unique predictor of internalizing behaviours (p = 0.005). We then repeated the regression analysis excluding infants exposed to neonatal dexamethasone (n = 6), and higher morphine exposure still predicted greater internalizing scores (p = 0.009).

3. Discussion and conclusions

Cumulative neonatal procedural pain and morphine exposure, as well as concurrent parenting stress contributed to higher child internalizing behaviours at age 7 years in children born very preterm, after controlling for neonatal clinical confounding factors. To our knowledge this is the first study to demonstrate the long-term adverse effect of neonatal pain on internalizing behaviours in very preterm children at school age. First, we confirmed our hypothesis that higher number of skin-breaking procedures during the NICU stay would be associated with higher internalizing scores at 7 years. However, importantly this was only the case in the non-ventilated children, thereby not exposed to morphine. In contrast, among the children who had been exposed to morphine for support of mechanical ventilation during

their NICU stay, pain was not related to internalizing behaviours at school age. Contrary to our hypothesis, rather than being protective, higher cumulative doses of neonatal morphine was associated with higher internalizing behaviours.

Specific patterns of long-term behavioural effects from exposure to repetitive acute pain or prolonged inflammatory pain in the first week of life of neonatal rat pups have been demonstrated, such as decreased locomotor activity (Bhutta et al., 2001) and increased defensive withdrawal behaviour (Anand et al., 1999) in adult rats exposed to neonatal pain. Dohrsen and colleagues (Dohrsen et al., 2013) recently showed that during the first week of life severe inflammatory pain from formalin injections, and pain caused by repeated saline injections, may induce major neuronal apoptosis and altered expression of neurodevelopmentally important proteins in the rat pup brain. Converging findings in preterm born children exposed to pain during their stay in the NICU are emerging. In recent studies, greater stress and pain exposure (independent of confounding neonatal risk factors) have been associated adversely with neonatal brain development in infants born very preterm (Brummelte et al., 2012; Smith et al., 2011; Zwicker et al., 2013), and at school age with thinner cortex in multiple regions (Ranger et al., 2013). Thus, neonatal pain may contribute to modified cerebral microstructure in children born very preterm. Furthermore, it has been recently shown that early alterations of cerebral white matter development are associated with deficits in social-emotional behaviours at age 5 years in children born very preterm (Rogers et al., 2012).

Previous work from our group on the same cohort of very preterm children found that greater cumulative exposure to morphine was associated with poorer motor development at 8 months, but not at 18 months corrected age follow-up (Grunau et al., 2009). Those findings suggested that an adverse impact of neonatal morphine on motor development may be transitory. Recently, our group demonstrated in a different cohort of very preterm infants that neonatal pain (adjusted for neonatal clinical factors), was associated with decreased early postnatal body and head growth at 32 weeks postconceptional age (Vinall et al., 2012) and altered brain microstructure (Brummelte et al., 2012). In those studies morphine exposure was not associated positively or negatively with growth or brain microstructure development. Within the present cohort, we previously reported that greater exposure to cumulative morphine in the NICU (controlling for neonatal pain) was associated with a more “normalized” pain response in extremely low birth weight infants at 8 months CA (Grunau, 2002), but morphine was unrelated to internalizing behaviours at 18 months CA (Vinall et al., 2013). Taken together, our work suggests that neonatal morphine may have a positive relationship with infant pain expression, but a negative association with internalizing behaviours that appear later in development at age 7 years. However, in the same cohort, we did not find a relationship between neonatal morphine and internalizing at 18 months. Why this finding emerges at 7 years remains to be explained. It is possible that specific negative associations between cumulative morphine exposure and internalizing behaviours at 18 months may not have been detectable given that the exposure to morphine was not isolated in that particular sample of very preterm infants at toddlerhood.

The very preterm infants who were mechanically ventilated and exposed to morphine underwent a significantly higher number of skin-breaking procedures compared to the non-

ventilated neonates ($p \leq .001$) during their stay in the NICU, however we did not find that their pain exposure predicted greater internalizing behaviours at school-age. A possible interpretation of these results is that morphine exposure may have provided pain relief and thus, may in fact have protected these neonates from the adverse effect of pain on later internalizing behaviours at school-age. However, our findings that greater cumulative morphine exposure adversely impacted internalizing behaviours, suggests that the amount and/or duration of morphine received by mechanically ventilated preterms during their NICU stay appears to be crucial. Our present study supports recent recommendations from a Cochrane review advocating careful use of opioids based on clinical judgment, together with monitoring of pain intensity levels, in mechanically ventilated neonates (Bellu et al., 2008).

Long-term effects of routine use of morphine infusions in mechanically ventilated preterm infants on neurodevelopment into childhood from large scale randomized controlled trials [Netherland (Simons et al., 2003) and NEOPAIN (Anand et al., 2004)] and cohort studies [EPIPAGE cohort (Larroque et al., 2004)] are producing mixed results (de Graaf et al., 2011; de Graaf et al., 2013; Ferguson et al., 2012; Roze et al., 2008). At age 5 years follow-up, the morphine trial from the Netherlands reported no effect of neonatal morphine infusion exposure on intelligence, visual motor integration, or internalizing/externalizing behaviours (de Graaf et al., 2011). However, they did show that neonatal morphine exposure had a negative effect on a component of executive function (response inhibition). The same group, on follow-up at age 8 to 9 years reported similar results for IQ and behaviours, except they suggested a potential protective effect of morphine on three out of eight subscales of executive functions measured by parent questionnaire, yet there were no differences on teacher's assessment of child executive functions (de Graaf et al., 2013) or on direct assessment of executive functions. Conversely, preliminary long-term follow-up results from the NEOPAIN trial (Ferguson et al., 2012) found indications that the preterm neonates exposed to preemptive morphine infusion had smaller head circumference and showed longer choice response latency in a short term memory task at age 5. However, internalizing behaviours did not differ between the morphine treated ($n = 14$) and placebo ($n = 5$) groups.

In previous work from our group, we have shown at 18 months CA follow-up of very preterm born infants, that lower levels of parenting stress buffered effects of pain on internalizing behaviours (Vinall et al., 2013) and cognitive function (Grunau et al., 2009). Parenting stress level in families with preterm infants has been shown to remain high well beyond discharge from the neonatal intensive care unit (NICU) in children whose development is not progressing well (Brummelte et al., 2011; Garel et al., 2007; Holditch-Davis et al., 2009; Singer et al., 2003). Further, maternal stress was a significant predictor of child internalizing behaviour up to 2 years post-discharge (Zelkowitz et al., 2011). Therefore in the present study to evaluate the role of neonatal pain and morphine in the aetiology of internalizing behaviours in preterm children at school age, we controlled for concurrent level of parenting stress in the primary caregiver parent. In addition to neonatal factors, we found that greater concurrent parenting stress was also associated with higher internalizing behaviours in the non-ventilated preterm children. Although parenting stress levels did not differ between the two groups of non-ventilated and ventilated children, the association between concurrent parenting stress and internalizing scores approached significance in the

latter group ($p = 0.06$), and parenting stress became significant when number of days on mechanical ventilation was removed from our model of predictors. Thus, concurrent parenting stress may at least in part contribute to internalizing behaviours in children born very preterm, who are known to be more sensitive to their environment than full-term born children (Tu et al., 2007). However, it is essential to be cautious in interpreting the direction of this association, since it may be that when children display greater internalizing behaviours, parents feel more concerned and thereby more stressed.

Our findings have important clinical implications. Attention to the necessity and frequency of skin-breaking procedures performed in the NICU is an important goal to reduce unnecessary pain exposure. Although this study was not designed to address the effects of specific morphine protocols, we showed that rather than a protective effect, greater neonatal cumulative morphine exposure adversely affected later childhood internalizing behaviours. However, our data should not be interpreted as showing that morphine *causes* adverse effects. In the randomized controlled trials, children in the placebo group were exposed to a lot of morphine during clinical care (Anand et al., 2004; de Graaf et al., 2011; de Graaf et al., 2013; Ferguson et al., 2012; Simons et al., 2003), thus all the human studies regardless of randomized or cohort design, are limited by the need to examine morphine controlling for medical confounders that affect the clinical decision to administer morphine. There is converging evidence for cautious use of continuous morphine infusion in mechanically ventilated preterm infants. Future studies are needed to evaluate various types of management of procedural pain in very preterm infants in the NICU, to prevent long-term effects on child behaviour.

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What's already known about this topic?

• Greater neonatal pain is associated with higher internalizing behaviours in very preterm infants at 18 months corrected age.

• It is unclear whether morphine may ameliorate or exacerbate the potential influence of neonatal pain on internalizing behaviours.

What does this study add?

• In very preterm children who undergo mechanical ventilation, judicious use of morphine is important, since higher exposure to morphine appears to be adversely associated with internalizing behaviours at school age.

• Management of procedural pain needs to be addressed in very preterm infants, to prevent long-term effects on child behaviour.

Table 1
Neonatal, child and parent characteristics

Neonatal Characteristics (*n* = 101)	**Non-Ventilated *n* = 44**	**Ventilated *n* = 57**	***p*-value**
Gestational age (weeks)	31.2 (1.5) [27-33]	28.4 (2.2) [25-33]	< 0.001
Birth weight (grams)	1569 (352)[884-2350]	1140(404)[520-2265]	< 0.001
Sex (male), number (%)	17 (39)	33 (58)	0.056
Illness severity on day 1 (SNAP-II score)	4.8 (5.8) [0-22]	16 (12.1) [0-45]	< 0.001
Total skin breaks (number)	45.4 (19.4) [10-91]	135.7 (77.5)[30-350]	< 0.001
Postnatal infection, number (%)	1 (2)	23 (40)	< 0.001
Mechanical ventilation (days)	-	15.9 (18) [1-71]	-
Surgery (number)		0 (0-1)	-
Morphine (exposed), number (%)	-	49 (86)	-
Daily morphine exposure (in mg adjusted daily weight)	-	2.3 (5) [0-22.9]	-
Midazolam (exposed), number (%)	-	8 (14)	-
Dexamethasone (exposed), number (%)	-	6 (11)	-
Child Characteristics			
CBCL Internalizing (T score)	52 (11) [33-80]†	51 (11) [34-76]†	0.45
Parental Characteristics (*n* = 101)			
Marital status (married), number (%)	37 (84)	54 (95)	0.15
Ethnicity (Caucasian), number (%)	33 (75)	45 (79)	0.78
Education (years)	15.8 (2.6)	16 (2.9)	0.63
PSI Parenting stress (score) (*n* = 100)	115 (29) [68-183]	117 (3) [68-188]	0.68

† 12 children in each group had T scores ≥ 60 (clinical range).

Missing PSI from 1 parent in non-ventilated group.

Mean, (standard deviation) and [range] are provided unless otherwise specified.

SNAP-II, score for neonatal acute physiology (severity of illness index); PSI, Stress Index-III (Parent Domain); CBCL, Child Behavior Check List.

Table 2

Pain, parental stress and cumulative morphine according to Internalizing T scores and neonatal morphine/mechanical ventilation exposure

	Non-Ventilated group (n=44)	**Ventilated group (n=57)**
CBCL-Internalizing	Skin-breaks 40 (17.3) [10-81]	Skin-breaks 139 (80.1) [32-350]
T scores ≤59 non-clinical	PSI 110 (27.5) [68-176]	PSI 112 (22.1) [68-161]
		Morphine 1.9 (4.4) [0-21.3]
(n=77)	(n=32)	(n=45)
CBCL-Internalizing	Skin-breaks 61 (17.3) [36-91]	Skin-breaks 123 (69.1) [30-219]
T scores ≥60 clinical	PSI 127 (28.9) [92-183]	PSI 137 (29.5) [88-186]
		Morphine 3.7(6.8) [0-22.9]
(n=24)	(n=12)	(n=12)

Skin-breaks, number of skin-breaking procedures; Morphine exposure, daily morphine exposure adjusted for daily weight.

Means (SD), [range] are provided for pain (skin-breaks), PSI scores and cumulative morphine exposure.

CBCL, Child Behavior Check List; PSI, Stress Index-III (Parent Domain).

Table 3

Correlations among neonatal clinical predictors

3a. Pearson correlations among neonatal clinical predictors in non-ventilated preterms (*n* = 44)

	SNAP-II day 1	Skin-breaks
Gestational Age	-0.34*	-0.55**
SNAP-II day 1		0.10
Skin-breaks		

SNAP-II, score for neonatal acute physiology (severity of illness index); Skin-breaks, number of skin-breaking procedures. *$p = 0.02$; **$p < 0.001$.

3b. Pearson correlations among neonatal clinical predictors in ventilated preterms (*n* = 57)

	SNAP-II	Number skin-breaks	Morphine exposure	Ventilation days	Number of surgeries
GA	-0.54**	-0.68**	-0.47**	**-0.72****	-0.30*
SNAP-II		0.37**	0.40**	0.47**	0.26
Skin-breaks			0.65**	**0.85****	0.46**
Morphine exposure				**0.76****	0.68**
Ventilation days					0.51**
Number of surgeries					

GA, gestational age; SNAP-II, score for neonatal acute physiology (severity of illness index) at day 1; Skin-breaks, number of skin-breaking procedures; Morphine exposure, daily morphine exposure adjusted for daily weight.

*$p = 0.03$;

**$p < 0.005$.

Table 4

Multiple linear regression analysis results for child internalizing scores

4a. Regression coefficients for child internalizing scores in non-ventilated preterms (*n* = 44)

Predictors	Standardized β	95% Confidence Intervals	*p*-value
Gestational Age	-0.148	[-3.427, 1.377]	0.393
SNAP-II day 1	0.013	[-0.542, 0.590]	0.932
Postnatal infections	-0.013	[-20.373, 18.603]	0.927
Skin-breaks	**0.348**	**[1.134, 33.120]**	**0.037**
PSI Parenting stress	**0.447**	**[0.057, 0.276]**	**0.004**

Adjusted R-square = 0.283 (p = 0.004)

SNAP-II, score for neonatal acute physiology (severity of illness index); Skin-breaks, number of skin-breaking procedures; PSI, Stress Index-III (parent domain).

4b. Regression coefficients for child internalizing scores in ventilated preterms (*n* = 57)

Predictors	Standardized β	95% Confidence Intervals	*p*-value
Gestational Age	0.188	[-1.007, 2.901]	0.334
SNAP-II day 1	0.129	[-0.151, 0.379]	0.391
Postnatal infections	-0.044	[-7.702, 5.817]	0.780
Number of surgery	-0.256	[-7.702, 1.423]	0.173
Ventilation days	-0.312	[-21.921, 7.232]	0.316
Skin-breaks	0.000	[-21.936, 21, 912]	0.999
Morphine exposure	**0.728**	**[7.166, 34.652]**	**0.004**
PSI Parenting stress	0.241	[-0.007, 0.204]	0.066

Adjusted R-square = 0.202 (p = 0.014)

SNAP-II, score for neonatal acute physiology (severity of illness index); Skin-breaks, number of skin-breaking procedures; morphine exposure, daily morphine exposure adjusted daily weight, PSI, Stress Index-III (parent domain).