Neonatal Procedural Pain and Preterm Infant Cortisol Response to Novelty at 8 Months

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ABSTRACT. *Objectives.* **Stress systems may be altered in the long term in preterm infants for multiple reasons, including early exposure to procedural pain in neonatal intensive care. This question has received little attention beyond hospital discharge. Stress responses (cortisol) to visual novelty in preterm infants who were born at extremely low gestational age (ELGA; ≤28 weeks), very low gestational age (VLGA; 29–32 weeks), and term were compared at 8 months of age corrected for prematurity (corrected chronological age [CCA]). In addition, among the preterm infants, we evaluated whether cortisol levels at 8 months were related to neonatal exposure to procedural pain and morphine in the neonatal intensive care unit.**

Methods. **Seventy-six infants, 54 preterm (≤32 weeks' GA at birth) and 22 term-born infants who were seen at 8 months CCA composed the study sample, after excluding those with major sensory, motor, or cognitive impairment. Salivary cortisol was measured before (basal) and 20 minutes after introduction of novel toys (post 1) and after developmental assessment (post 2).**

Results. **Salivary cortisol was significantly higher in ELGA infants at 8 months, compared with the VLGA and term groups before and after introduction of visual novelty. Term-born and VLGA infants showed a slight decrease in cortisol when playing with novel toys, whereas the ELGA group showed higher basal and sustained levels of cortisol. After controlling for early illness severity and duration of supplemental oxygen, higher basal cortisol levels in preterm infants at 8 months' CCA were associated with higher number of neonatal skin-breaking procedures. In contrast, cortisol responses to novelty were predicted equally well by neonatal pain or GA at birth. No relationship between morphine dosing and cortisol response was demonstrated in these infants.**

Conclusions. **ELGA preterm infants show a different pattern of cortisol levels before and after positive stimulation of visual novelty than more maturely born, VLGA preterm and term-born infants. Exposure to high numbers of skin-breaking procedures may contribute to "resetting" basal arousal systems in preterm infants.** *Pediatrics* **2004;114:e77–e84. URL: http://www.pediatrics.org/cgi/content/full/114/1/e77;** ***pain, stress, premature infants, novelty, cortisol, morphine.***

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Received for publication Aug 29, 2003; accepted Feb 11, 2004.

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ABBREVIATIONS. HPA, hypothalamic-pituitary-adrenal; NICU, neonatal intensive care unit; ELGA, extremely low gestational age; VLGA, very low gestational age; SNAP-II, Score for Neonatal Acute Physiology II; CCA, corrected chronological age.

Multiple lines of evidence suggest that early repeated and prolonged pain exposure may contribute to altered development of pain systems, behavior, cognition, and learning in former preterm infants later in childhood.[1–7] There is a large body of experimental animal literature demonstrating that environmental manipulations in the neonatal period can lead to permanent shifts in the hypothalamic-pituitary-adrenal (HPA) axis reflecting stress reactivity (see reviews in [8,9]). Furthermore, novelty-induced fearfulness in rodents is altered by neonatal stress[10] and pain.[11] However, there is a dearth of direct evidence in human preterm infants that neonatal pain exposure leads to shifts in later stress responses after discharge from the neonatal intensive care unit (NICU).

Although most preterm infants, even those who are born at extremely low birth weight, display intelligence within broadly normal limits during childhood, they are at high risk of learning and behavior difficulties at school age.[12–23] Self-regulation of arousal is essential to initiating and maintaining attention and to accessing higher order "executive" functions such as planning and independent problem solving.[24,25] Landmark changes have been described in the first year of life with respect to orienting to objects and control of distress[26] and subsequently in children's abilities to plan and regulate cognitive skills.[27] Preterm infants who are born at extremely low birth weight (≤800 g) show maladaptive behaviors during the stress of novel cognitive challenge compared with term-born children at 3 years[28] and at school age,[17] even when they have normal intelligence. Although it has been proposed that preterm infants are particularly vulnerable to altered self-regulation in coping with a continuum of positive and negative stimuli,[28] the response to mildly arousing stimuli, such as exposure to novelty, has not been studied in human preterm infants after NICU discharge. In previous work, we showed that infants who were born at ≤800 g birth weight displayed higher basal heart rate,[7] suggesting a shift in arousal set point.

The aims of the present study were 1) to compare stress hormone (salivary cortisol) response before and after visual novelty in extremely low gestational

age (ELGA), very low gestational age (VLGA), and term-born infants; 2) to examine whether more exposure to neonatal skin-breaking procedures (pain) is linked to higher basal cortisol and/or increased cortisol response to novelty at 8 months of age corrected for prematurity; and 3) to evaluate whether there is an association between amount of morphine exposure and the effect of early procedural pain on cortisol responsiveness. We hypothesized that 1) basal cortisol will be higher in ELGA compared with VLGA and term-born infants; 2) ELGA infants will display increased cortisol responses after structured interaction with novel toys at 8 months, whereas VLGA and term-born infants will not show a stress response to novelty and may even find exposure to novelty positive or arousal reducing; 3) among the preterm infants, higher exposure to neonatal skin-breaking procedures will be associated with increased basal and challenge cortisol levels at 8 months of age, after controlling for neonatal illness severity; and 4) among the preterm infants, greater neonatal morphine exposure will modulate the long-term effects of exposure to neonatal procedures (ie, will be associated with lower basal and challenge cortisol levels at 8 months, after controlling for neonatal illness severity). To our knowledge, this is the first study of endocrine stress response (salivary cortisol) to novelty in human preterm infants.

METHODS

Participants

The study sample comprised 76 infants: 19 preterm infants who were born at ELGA (≤28 weeks; 10 boys, 9 girls), 34 preterm infants who were born at VLGA (29–32 weeks; 17 boys, 17 girls; Table 1), and 22 term-born control subjects (13 boys, 9 girls), of mean birth weight 3584.8 g (SD: 537.9) and GA 40.1 weeks (SD: 1.4). The preterm infants were recruited from the NICU at British Columbia's Children's Hospital, which is the major tertiary neonatal unit in British Columbia. The term-born infants were recruited before discharge from 2 major metropolitan full-term nurseries at British Columbia Women's and St. Paul's Hospitals. These hospitals are affiliated with the University of British Columbia. All infants were recruited by 1 neonatal research nurse. Infants in the present study were seen in the Biobehavioral Research Unit, Centre for Community Child Health Research at 8 months of age corrected for prematurity. Infants with a major congenital anomaly, major neurosensory impairment, or maternal drug use during pregnancy were excluded.

Measures

Visual Novelty

The visual novelty paradigm followed the methods of Ruff,[29,30] which have been used to study sustained attention and exploratory play in preterm infants. Each infant was presented with 4 different toys in a standard order, 1 at a time. When the focus of a study is individual differences, a standard order is preferred.[31] The toys were plastic and washable and varied in color and texture.

Cortisol

Saliva was obtained using a small cotton dental roll (without any stimulant) and was placed into a needleless syringe. The saliva was expressed into a vial and stored in the Biobehavioral Research Unit (frozen at −20°C) until transported to Dr Weinberg's laboratory at the University of British Columbia for cortisol assay. Cortisol was assayed from saliva samples using the Salimetrics High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit for quantitative determination of salivary cortisol (Salimetrics LLC, Philadelphia, PA). Intra-assay and interassay coefficients of variation were 2.92% and 3.41%, respectively.

Medical Chart Review

Medical and nursing chart review from birth to term (39 weeks, 6 days) was conducted for each preterm infant by a neonatal nurse, including but not limited to birth weight, gestational age, illness severity (Score for Neonatal Acute Physiology II [SNAP-II]) on days 1 and 3, days of mechanical ventilation, days of oxygen, daily dosage of opioid and other medications adjusted for daily weight, and number of skin-breaking procedures. Procedural pain exposure was operationalized as the sum of every skin-breaking procedure from birth to term (eg, heel lance, intramuscular injection, chest tube insertion, central line insertion). Each attempt at a procedure was included; thus, the total sum reflected all skin breaks. Although it is recognized that procedures differ in pain intensity, in the absence of an empirical basis for assigning weights to every procedure, counting is used as a "marker" of infant acute pain exposure in the NICU.[32–34] In our study, the total number of skin-breaking procedures also provided a marker of overall stress exposure from birth to term. Total intravenous morphine exposure was calculated from birth to term as follows. The average daily dose of intravenous morphine adjusted for daily weight was multiplied by the number of days of intravenous morphine, as we have used previously.[33] For example, if an infant received an average dose of 0.39 mg/kg body weight for 24 treatment days, then the morphine score was 9.36 (mg/kg).

Procedures

Written informed consent was obtained from mothers, following the procedures approved by the Clinical Research Ethics Board of the University of British Columbia and the research review committee at the Children's and Women's Health Centre of British Columbia. Infants were tested with their mothers in the

TABLE 1. Preterm Infant Characteristics

	Preterm 22–28 Weeks' GA at Birth (n = 19)	Preterm 29–32 Weeks' GA at Birth (n = 34)
GA at birth	26.6 (1.8)	30.9 (1.4)
Birth weight	877.5 (290.4)	1495 (455.6)
Illness severity day 1 (SNAP-II)	20.2 (11.8)	9.9 (11.1)
Illness severity day 3 (SNAP-II)	11.3 (10.6)	1.7 (3.7)
Pain exposure (no. of skin-breaking procedures)*	193.4 (107.3)	72.4 (48.3)
Mechanical ventilation, d*	32.4 (26.9)	3.5 (8.3)
Oxygen, d*	36.6 (25.4)	11.56 (14.3)
Intravenous morphine exposure (daily average mg/kg × days)*	5.1 (7.6)	1.8 (7.3)

Data are mean (SD).
* Recorded daily from birth to term (39 weeks, 6 days postconceptional age).

Biobehavioral Research Unit in the morning to control for circadian rhythm.

The infant was seated in a plastic infant seat with a tray attached. His or her mother sat behind, nearby but out of sight. The research assistant placed each toy on a tray for 90 seconds sequentially. Saliva samples for cortisol assay were collected shortly after arrival in the laboratory, when the infant was quietly settled on his or her mother's lap (basal), 20 minutes after the introduction of the first toy (post 1), and after 30 to 40 minutes, during which mother–infant interaction was evaluated and the Bayley Scales of Infant Development II and the Movement Assessment of Infants were administered (post 2). Developmental assessment was administered by a pediatric physiotherapist or an occupational therapist. The order of administration was as follows: exploratory play tasks, semistructured mother–infant interaction, Bayley Scales of Infant Development, and the Movement Assessment of Infants. For ensuring valid cortisol results, no infant was fed within 30 minutes before the basal saliva sample or during the study; all infants were checked for signs of blood in the mouth or saliva as a result of teething.

Data Analysis

Bivariate Pearson correlations were used to examine associations among the neonatal measures and relationships of the neonatal factors to the cortisol values at 8 months corrected chronological age (CCA). Comparisons of cortisol levels across the groups was conducted using repeated measures analysis of variance. Hierarchical regression analyses were used to examine relationships of neonatal factors to cortisol levels at 8 months. Sample size was calculated on the basis of the standard of 10 infants per predictor variable required for regression analysis. Thus, to allow for 5 predictors (controlling for illness severity on day 1 and day 3, and total number of days of oxygen, and examining independent effects of pain exposure and morphine exposure), a minimum of 50 preterm infants were required.

RESULTS

The cortisol data were examined for outliers, defined as any value >3 SD outside the mean in either direction at a given time point.[35,36] Three infants had 1 or 2 outlier values, and all were male, preterm ELGA (22–28 weeks' GA). One of these infants was receiving cortisol injections at 8 months CCA because of adrenal insufficiency and was therefore excluded, leaving 75 infants in the study. The outlier values for the remaining 2 boys were winsorized following the method of Tukey[37] and retained for data analyses. This method involves replacing the outlier value with the closest value within the ±3 SD range. Of the 53 preterm infants, 8 had been exposed to postnatal dexamethasone, all of whom were in the ELGA group. All of the raw (nonwinsorized) cortisol scores for the dexamethasone-exposed infants were well within the range of the ELGA nonexposed infants. The perinatal/neonatal characteristics of the infants in the study are presented in Table 1.

Preterm ELGA, VLGA, and Term Groups: Cortisol

Repeated measures analysis of variance was conducted on cortisol levels at 8 months' CCA across the phases (basal, post 1, and post 2), with infant group (ELGA, VLGA, and term) as a between-groups factor. The groups differed significantly in level of cortisol across phases ($P = .006$); the ELGA infants showed significantly higher cortisol compared with the VLGA and term-born groups combined ($P = .002$). In addition, the pattern of the ELGA group responses across the phases differed significantly compared with the VLGA and term infants (Fig 1). The VLGA group dropped significantly from basal to post 1 ($P = .02$), then increased slightly from post 1 to post 2 ($P = .12$). The term infants showed a slight decrease from basal to post 1 ($P = .105$), then increased significantly from post 1 to post 2 ($P = .04$). Post hoc comparisons for the ELGA compared with the VLGA infants showed that cortisol levels were significantly higher for the ELGA group at basal ($P = .03$) and post 1 ($P = .007$) and approached significance at Post 2 ($P = .066$). Because development of the HPA axis in preterm infants may be linked to chronological age,[38] we repeated the analysis of variance controlling statistically for chronological age; the group differences remained significant ($P = .01$).

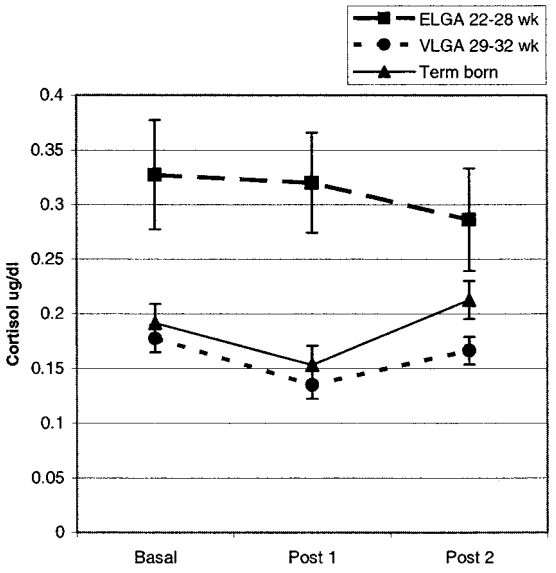

Fig 1. Salivary cortisol levels across 3 phases (basal, post 1 [after presentation of novel toys], and post 2 [after developmental assessment]) in infants born ELGA ($n = 19$) and VLGA ($n = 34$), compared with term born ($n = 22$).

Preterm Infants: Prediction of Cortisol at 8 Months' CCA

First, Pearson correlations were conducted to examine interrelationships of neonatal characteristics before regression analysis (Table 2). The number of neonatal skin-breaking procedures was significantly correlated with illness severity (SNAP-II) at day 1 ($r = .43$, $P = .001$) and day 3 ($r = .58$, $P = .0001$) and with intravenous morphine exposure since birth ($r = .58$, $P = .001$). Days of oxygen, which was only moderately related to exposure to procedures ($r = .35$, $P = .01$) and not correlated with morphine exposure ($P = -.03$, not significant) was used as a marker of long-term illness in the neonatal period. Low gestational age at birth was highly correlated with greater illness severity at day 3 ($r = -.67$, $P = .0001$); therefore, illness severity at day 3 was a marker for both variables, and gestational age was not added to the regression. Higher number of days of mechanical ventilation was very highly associated with exposure to procedures ($r = .92$, $P = .0001$) and was not entered into the regression.

Then bivariate associations between the neonatal factors and the outcome measures (cortisol at 3 time points) were evaluated (Table 3). High basal cortisol

TABLE 2. Pearson Intercorrelations of Perinatal/Neonatal Characteristics for the ELGA and VLGA Preterm Infants Combined (N = 53)

	Birth Weight	Illness Severity Day 1	Illness Severity Day 3	Mechanical Ventilation (Days)	Oxygen (Days)	Pain Exposure (No. of Skin-Breaking Procedures)	Intravenous Morphine Exposure
GA at birth	.83*	−.40†	−.67*	−.73*	−.48*	−.73*	−.34†
Birth weight		−.36†	−.59*	−.62*	−.39†	−.67*	−.33
Illness severity day 1 (SNAP-II)			.34†	.41†	.32	.43‡	.49*
Illness severity day 3 (SNAP-II)				.64*	.28	.58*	.46‡
Mechanical ventilation, d					.16	.92*	.66*
Oxygen, d						.35†	−.03
Pain exposure (no. of skin-breaking procedures)							.58*

* P = .0001.
† P = .01.
‡ P = .001.

TABLE 3. Pearson Correlations Between Neonatal Factors in the NICU and Cortisol at 8 Months of Age Corrected for Prematurity (Basal, Post 1, Post 2) for the Preterm Infants (N = 53)

	Basal Cortisol	Post 1 Cortisol	Post 2 Cortisol
GA at birth	−.31*	−.33*	−.01
Birth weight	−.20	−.22	.04
Pain exposure	.314*	.294*	−.082
Morphine exposure	−.052	.012	−.090
Mechanical ventilation, d	.194	.210	−.089
Oxygen, d	.372†	.220	−.127
Illness severity day 1	.180	.166	−.053
Illness severity day 3	.122	.050	−.173

* P = .05.
† P = .01.

was significantly associated with high number of days on supplemental oxygen (r = .37, P = .01), lower gestational age at birth (r = −.31, P = .05), and higher exposure to neonatal procedures (r = .31, P = .05). High post 1 cortisol was significantly associated with lower gestational age at birth (r = −.33, P = .05) and higher exposure to neonatal procedures (r = .29, P = .05). Post 2 cortisol was not significantly associated with any neonatal variable considered.

To examine the independent contribution of exposure to procedures and early morphine exposure to cortisol level in each phase (basal, post 1, post 2), separate regression analyses for each phase were conducted in blocks. In block 1, SNAP-II scores at day 1 and day 3 were entered to control for early illness severity, and in block 2, number of days on oxygen was entered to control for overall illness in the neonatal period. In block 3, the number of skin-breaking procedures was entered, and in block 4, morphine exposure was entered; an interaction term, to examine whether effects of procedures was associated with effects of morphine, was added in block 5. After controlling for illness severity and days on supplemental oxygen, higher number of neonatal procedures independently predicted higher basal cortisol at 8 months (standardized β = .41, t = 2.20, P = .03) and post 1 cortisol (standardized β = .44, t = 2.26, P = .029) but not post 2 cortisol (standardized β = .09, t = .44, P = .66). Morphine exposure and the interaction of morphine with procedural pain were not associated with cortisol level at 8 months in any phase. Because regression analysis examined only linear relationships between neonatal morphine and later cortisol levels, the possibility of other patterns (eg, quadratic) was explored, but no associations were found.

Exposure to skin-breaking procedures was confounded with GA. We could not include both number of skin-breaking procedures and GA into the same regression analysis, because they were highly correlated (ie, a problem with multicollinearity). Therefore, to evaluate whether GA rather than procedures was the operative factor, we repeated the same regression analyses with GA instead of number of procedures entered at step 3. After controlling for illness severity and days on supplemental oxygen, GA was not related to basal cortisol at 8 months (standardized β = −.25, t = −1.26, P = .21); therefore, we concluded that procedural pain exposure was the significant factor in relation to altered basal cortisol at 8 months. In contrast, post 1 cortisol was significantly associated with GA (standardized β = −.46, t = −2.29, P = .026) to the same extent as number of skin-breaking procedures (see above). Post 2 cortisol was not predicted by GA (standardized β = −.33 t = −1.59, P = .118).

DISCUSSION

Studies using animal models have reported that behavioral and/or hormonal responses to novelty in adulthood are altered following chronic unpredictable prenatal stress,[39,40] neonatal stress,[10] or repetitive neonatal pain.[11] In the present study, our hypotheses that at 8 months of age basal cortisol would be higher and visual novelty would elicit greater cortisol responses in preterm infants who were born at the lowest gestational ages, compared with VLGA or term-born infants, was confirmed. At 8 months, the pattern of cortisol shown by the ELGA preterm infants (22–28 weeks' GA) differed significantly from the other groups. Term-born infants and VLGA preterm infants who were born at 29 to 32 weeks' GA displayed a slight drop in cortisol after the introduction of visual novelty, consistent with sustained attention and interest. After this phase, their cortisol returned to basal levels. In contrast, the ELGA infants displayed higher basal cortisol, which was sustained in response to visual novelty, followed by a slight drop at the end of testing.

Preterm infants were seen by corrected age so that

the stimulus situation (visual novelty) used to elicit cortisol responses was developmentally appropriate for both groups. However, development of the HPA axis might be more closely linked to chronological age, and when age since birth is used, preterm infants are of course older. When the analyses were redone controlling for chronological age, the differences between the groups remained. Thus, our results were not an artifact of the HPA axis in the groups being at different developmental stages dependent on duration of postnatal life.

We confirmed the hypothesis that, after controlling for neonatal illness severity, higher neonatal procedural pain exposure was significantly linked to higher basal and cortisol levels in response to stress to visual novelty at 8 months. However, stress response to developmental assessment (the third cortisol) was not predicted by any neonatal variables. Self-regulation to stress sustained during the process of developmental assessment may be modulated by caregiver factors and the quality of the dyadic interaction with the individual infant; however, this requires additional investigation. In this study, we were unable to show that morphine administered in the neonatal period either ameliorated or exacerbated alterations in HPA axis responses, as manifested at 8 months.

There have been questions as to whether exposure to corticosteroids may have an impact on the HPA axis in preterm infants. Antenatal betamethasone was not associated with cortisol values on day 1 or day 3.[41] Whereas antenatal dexamethasone was associated with suppressed adrenal function initially in VLGA infants, these effects were no longer apparent by day 14.[42,43] However, the earlier studies examined responses to pharmacologic challenges to the adrenal axis. In this study, we used a psychological challenge, which brings into play other neural systems such as the hippocampus, which may be influenced by antenatal glucocorticoid exposure. Moreover, the timing of antenatal glucocorticoid exposure may explain differences in cortisol "set points" for ELGA and VLGA infants. That is, fetuses who are exposed to antenatal steroids at difference points in the development of the limbic-hypothalamic-pituitary-adrenal axis may exhibit different impacts on the system. In the present study, few infants were given postnatal dexamethasone in the neonatal period, and their cortisol levels at 8 months did not differ from the unexposed infants.

In a study using regression techniques, we identify contributing factors, but we cannot rule out the possibility that factors other than the target variables may be key. In our study, GA was correlated −.73 with number of skin-breaking procedures; therefore, we ran separate regression analyses to examine whether pain or GA was the key factor. After controlling for illness severity and days on supplemental oxygen, GA was not related to basal cortisol at 8 months; therefore, we concluded that procedural pain exposure (not GA) was the key factor that predicted basal cortisol at 8 months. In contrast, procedural pain and GA both were related to cortisol after novelty exposure at 8 months.

In a previous study, we reported higher basal heart rate in infants of birthweight ≤800 g at 8 months compared with term-born control subjects.[7] The number of skin-breaking procedures in the neonatal period is a marker for overall cumulative exposure to pain and stress. In ELGA infants, even routine noninvasive caregiving is more stressful than for more maturely born infants.[44] Taken together with the present findings in the ELGA infants, we concluded that previous pain and/or cumulative stress may contribute to "resetting" basal physiologic regulation in the tiniest infants. These most vulnerable infants likely are chronically stressed and may have persistently altered cortisol levels, and/or their stress responses are poorly modulated so that even entry into the test setting is stressful before the test has begun. It is possible that there are critical ontogenetic processes that require continued in utero development that are disrupted by extremely preterm delivery. We need to be cautious about concluding that pain exposure is the only or even the best explanation for our findings. The shifts that we found in cortisol levels of the tiny infants are probably determined by interactions of multiple factors.

The results of the present study are consistent with our previous work showing that children who are born at extremely low birth weight (even those with normal intelligence) display poorer self-regulatory behaviors during novel tasks during standardized cognitive assessment, compared with term-born children.[17,28] These behaviors generally reflected poorer executive planning and control. For example, at 3 and 9 years, extremely low birth weight children were more easily distracted, needed urging to attempt and/or complete unfamiliar tasks, distrusted their ability, gave up easily, sought to terminate, preferred easy tasks, and required constant praise and encouragement. These children showed problems with effortful control during problem solving, reflected in poorer attention and self-regulation, especially as tasks became more cognitively challenging. We did not record the number of skin-breaking procedures for those studies; however, behavior problems were related to longer time spent in the NICU, rather than specifically lower gestation or birth weight.

With respect to long-term effects of morphine, we have previously reported that higher morphine exposure in the neonatal period was associated with "normalized" behavioral and cardiac reactivity to pain both in the NICU at 32 weeks' postconceptional age[33] and at 8 months.[5,7] It is our impression that the overall amount of morphine exposure may be higher currently compared with our earlier cohorts, because many infants in our current sample could not be studied in the NICU phase of this research program as they were still receiving morphine at 32 weeks' postconceptional age. In our earlier pain research in the NICU, we did not encounter this, suggesting increased use of morphine in the time period between the infants who were born in 1996–1998[33] versus our present cohort of infants who were born in 2000–2002. One possibility is that the action of morphine may prevent changes to pain systems but

not changes to complex self-regulation. However, in a randomized, controlled study, morphine had no effect on composite pain scores during endotracheal suctioning.[45] Alternatively, potential benefits or risks of morphine may not be evident in infancy, perhaps only later in childhood.

Pharmacologic pain management is an intrinsic part of NICU care. In this study, we examined morphine exposure because it is commonly used in the NICU setting, frequently on an ongoing basis to provide comfort, not necessarily specifically for procedural pain management. In fact, because of the longer distribution half-life and slower onset of action seen with morphine, for routine procedures morphine would not necessarily be the agent of choice. There may be other agents better than morphine for this population. One challenge with ongoing morphine administration is that some infants require a long period of weaning, which involves a prolonged period of administration of reducing doses of morphine during the latter part of the nursery stay. This may involve additional risk, because morphine administered in the neonatal period has different long-term effects on rodents depending on whether it was given in the presence or absence of pain.[46] At the present time, the long-term effects of neonatal morphine on later development are unclear.[45,47,48] On the one hand, the results of our study are reassuring in the case of morphine, however, because higher amounts of morphine were not associated with altered levels of cortisol at 8 months. On the other hand, current attempts at pain relief in the NICU may result in prolonged or repeated sedation without preventing changes in subsequent stress responses. The use of narcotics may be counterproductive if such treatment prolongs the need for ventilator support. Moreover, very little is known about the longer or repeated exposure to any analgesic agents; for example, even repeated sucrose exposure may not be innocuous.[49] Recently it has been suggested that more attention needs to be paid to environmental adaptations to manage pain, complementary to use of pharmacologic agents.[50] Positioning and containment,[51] music,[52] sucking,[53] suckling,[54] and kangaroo care,[55] for example, may facilitate early "coping" with stressful intervention. Effective environmental interventions enhance homeostasis and stability in preterm infants in the NICU and are essential elements of neonatal nursing care.[56,57] Rodent studies have shown, for example, that maternal contact and/or nonnutritive sucking can reduce corticosterone levels in the preweaning period[58] and that consummatory behavior can reduce the corticosterone response to novelty in adulthood.[59]

Limitations

It is a major challenge to study long-term effects of pain exposure in the NICU. Although ideally one would randomize infants to receive pharmacologic pain control (eg, morphine) to evaluate long-term effects, this can be done only using an "intention-to-treat" design, whereby patients can receive open-label morphine regardless of randomized group assignment.[45] Therefore, statistical adjustment for morphine exposure (such as we used) is still needed. In a clinical setting, there are multiple confounding factors that cannot be controlled; for example, in NICU practice, morphine is given for reasons other than direct analgesia, eg, for adjustment to mechanical ventilation. It is difficult to distinguish pain from other discomfort or stress in immature preterm infants; therefore, effectiveness is uncertain, and the wrong doses may be given at the wrong frequency. The major limitation of this study is that we did not link exposure, dosing, and efficacy of morphine specifically to the timing of each skin-breaking procedure.

In addition, exposure to prenatal stress can have long-term effects on offspring,[60] and prenatal stressors may themselves cumulatively contribute to preterm labor.[61] Thus, in addition to the postnatal stress of pain in the NICU, some of the differences between ELGA and later born infants may at least partially reflect prenatal stress.

CONCLUSION

In early human development, mechanisms of attention, social-emotional regulation, temperament, and cognitive functioning are interwoven. Arousal regulation is an organizing construct for integrating relationships between sensory stimulation, stress, cortical activity, and performance. Our findings suggest that early repetitive procedural pain (and thereby stress) in ELGA preterm neonates may "reset" arousal and thereby affect interaction with the environment. Rather than considering pain reactivity and cognitive approach/avoidance as separate domains, under this view, both would be seen as different aspects of underlying difficulties in arousal regulation.[28] The specific responses likely depend on the level of the central nervous system involved, from automatic conditioned reactivity to attention and higher executive capabilities. Knowledge of the cause of altered stress response to novelty in infancy is important for understanding determinants of learning and behavior problems in preterm infants during later childhood. For addressing the broader range of arousal and self-regulation, studies at multiple time points with multiple types of stressors are needed. In conjunction with studies of human preterm neonates, more animal research is needed—with careful attention to modeling multiple aspects of neonatal intensive care.

ACKNOWLEDGMENTS

This research program was supported by the National Institute for Child Health and Human Development Grant HD39783, Canadian Institutes for Health Research Grant MOP42469, and British Columbia Ministry of Children and Family Development Human Early Learning Partnership Grant 02-2410. Dr Grunau holds a Senior Scholar Award from the Michael Smith Foundation for Health Research.

We very much appreciate the thoughtful input of the anonymous reviewers; especially one reviewer's comments, which were incorporated verbatim into the discussion. We thank the families who participated in this study and the staff of the Biobehavioral Research Unit, Centre for Community Child Health Research. In particular, we thank Colleen Fitzgerald for coordinating the research program, Colleen Jantzen and Adi Amir for data collection,

Wayne Yu for carrying out the cortisol assays, and Taryn Fay for assistance in preparation of the manuscript.

REFERENCES

1. Anand KJ. Long-term effects of pain in neonates and infants. In: Jensen TS, Turner JA, Wiesenfeld-Hallin Z, eds. *Progress in Pain Research and Management*. Seattle, WA: IASP Press; 1997;8:881–892
2. Porter FL, Grunau RE, Anand KJ. Long-term effects of pain in infants. *J Dev Behav Pediatr.* 1999;20:253–261
3. Whitfield MF, Grunau RE. Behavior, pain perception, and the extremely low-birth weight survivor. *Clin Perinatol.* 2000;27:363–379
4. Grunau RE. Long-term consequences of pain in human neonates. In: Anand KJ, Stevens BJ, McGrath PJ, eds. *Pain in Neonates*. New York, NY: Elsevier; 2000:55–76
5. Grunau R. Early pain in preterm infants. A model of long-term effects. *Clin Perinatol.* 2002;29:373–394
6. Anand KJ. Effects of perinatal pain and stress. *Prog Brain Res.* 2000;122: 117–129
7. Grunau RE, Oberlander TF, Whitfield MF, Fitzgerald C, Morison SJ, Saul JP. Pain reactivity in former extremely low birth weight infants at corrected age 8 months compared with term born controls. *Infant Behav Dev.* 2001;24:41–55
8. Caldji C, Diorio J, Meaney MJ. Variations in maternal care in infancy regulate the development of stress reactivity. *Biol Psychiatry.* 2000;48: 1164–1174
9. Walker C, Anand KJS, Plotsky PM. Development of the hypothalamic-pituitary-adrenal axis and the stress response. In: McEwen B, Goodman H, eds. *Coping With the Environment: Neural and Endocrine Mechanisms*. Oxford, UK: Oxford University Press; 2001:237–270
10. Caldji C, Francis D, Sharma S, Plotsky PM, Meaney MJ. The effects of early rearing environment on the development of $GABA_A$ and central benzodiazepine receptor levels and novelty-induced fearfulness in the rat. *Neuropsychopharmacology.* 2000;22:219–229
11. Anand KJ, Coskun V, Thrivikraman KV, Nemeroff CB, Plotsky PM. Long-term behavioral effects of repetitive pain in neonatal rat pups. *Physiol Behav.* 1999;66:627–637
12. Saigal S, Szatmari P, Rosenbaum P, Campbell D, King S. Cognitive abilities and school performance of extremely low birth weight children and matched term control children at age 8 years: a regional study. *J Pediatr.* 1991;118:751–760
13. Grunau RE, Whitfield MF, Davis C. Pattern of learning disabilities in children with extremely low birth weight and broadly average intelligence. *Arch Pediatr Adolesc Med.* 2002;156:615–620
14. Anderson P, Doyle LW. Neurobehavioral outcomes of school-age children born extremely low birth weight or very preterm in the 1990s. *JAMA.* 2003;289:3264–3272
15. Hack M, Breslau N, Aram D, Weissman B, Klein N, Borawski-Clark E. The effect of very low birth weight and social risk on neurocognitive abilities at school age. *J Dev Behav Pediatr.* 1992;13:412–420
16. Bhutta AT, Cleves MA, Casey PH, Cradock MM, Anand KJS. Cognitive and behavioral outcomes of school-aged children who were born preterm: a meta-analysis. *JAMA.* 2002;288:728–737
17. Whitfield MF, Grunau RV, Holsti L. Extremely premature (< 801 g) schoolchildren: multiple areas of hidden disability. *Arch Dis Child Fetal Neonatal Ed.* 1997;77:F85–F90
18. Hille ET, den Ouden AL, Saigal S, et al. Behavioural problems in children who weigh 1000 g or less at birth in four countries. *Lancet.* 2001;357:1641–1643
19. Hall A, McLeod A, Counsell C, Thomson L, Mutch L. School attainment, cognitive ability and motor function in a total Scottish very-low-birthweight population at eight years: a controlled study. *Dev Med Child Neurol.* 1995;37:1037–1050
20. Rickards AL, Kelly EA, Doyle LW, Callanan C. Cognition, academic progress, behavior and self-concept at 14 years of very low birth weight children. *J Dev Behav Pediatr.* 2001;22:11–18
21. Botting N, Powls A, Cooke RW, Marlow N. Cognitive and educational outcome of very-low-birthweight children in early adolescence. *Dev Med Child Neurol.* 1998;40:652–660
22. Saigal S, Hoult LA, Streiner DL, Stoskopf BL, Rosenbaum PL. School difficulties at adolescence in a regional cohort of children who were extremely low birth weight. *Pediatrics.* 2000;105:325–331
23. Taylor HG, Hack M, Klein N, Schatschneider C. Achievement in children with birth weights less than 750 grams with normal cognitive abilities: evidence for specific learning disabilities. *J Pediatr Psychol.* 1995;20:703–719
24. Rothbart MK, Posner MI. Mechanism and variation in the development of attentional networks. In: Nelson CA, Luciana M, eds. *Handbook of Developmental Cognitive Neuroscience*. Cambridge, MA: MIT Press; 2001: 353-363
25. Taylor HG, Hack M, Klein NK. Attention deficit in children with <750 gm Birthweight. *Child Neuropsychol.* 1998;4:21–34
26. Ruff HA, Rothbart MK. *Attention in Early Development: Themes and Variations*. New York, NY: Oxford University Press; 1996
27. Posner MI, Rothbart MK. Developing mechanisms of self-regulation. *Dev Psychopathol.* 2000;12:427–441
28. Grunau RE. Self-regulation and behavior in preterm children: effects of early pain. In: McGrath PJ, Finley GA, eds. *Pediatric Pain: Biological and Social Context, Progress in Pain Research and Management*. Seattle, WA: IASP Press; 2003;26:23–55
29. Ruff HA. Components of attention during infants' manipulative exploration. *Child Dev.* 1986;57:105–114
30. Lawson KR, Ruff HA. Focused attention: assessing a fundamental cognitive process in infancy. *Learn Attention.* 2001:293–311
31. Lewis M, Ramsay D. Cortisol response to embarrassment and shame. *Child Dev.* 2002;73:1034–1045
32. Porter FL, Wolf CM, Miller JP. Procedural pain in newborn infants: the influence of intensity and development. *Pediatrics.* 1999;104(1). Available at: pediatrics.aappublications.org/cgi/content/full/104/1/e13
33. Grunau RE, Oberlander TF, Whitfield MF, Fitzgerald C, Lee SK. Demographic and therapeutic determinants of pain reactivity in very low birth weight neonates at 32 weeks' postconceptional age. *Pediatrics.* 2001;107:105–112
34. Stevens B, McGrath P, Gibbins S, et al. Procedural pain in newborns at risk for neurologic impairment. *Pain.* 2003;105:27–35
35. Gunnar MR, Connors J, Isensee J. Lack of stability in neonatal adrenocortical reactivity because of rapid habituation of the adrenocortical response. *Dev Psychobiol.* 1989;22:221–233
36. Ramsay D, Lewis M. Reactivity and regulation in cortisol and behavioral responses to stress. *Child Dev.* 2003;74:456–464
37. Tukey JW. *Exploratory Data Analysis*. Don Mills, Ontario, Canada: Addison-Wesley; 1977
38. Antonini SR, Jorge SM, Moreira AC. The emergence of salivary cortisol circadian rhythm and its relationship to sleep activity in preterm infants. *Clin Endocrinol.* 2000;52:423–426
39. Walker CD, Welberg LAM, Plotsky PM. Glucocorticoids, stress, and development. *Horm Brain Behav.* 2002;4:487–534
40. Clarke AS, Schneider ML. Prenatal stress has long-term effects on behavioral responses to stress in juvenile rhesus monkeys. *Dev Psychobiol.* 1993;26:293–304
41. Terrone DA, Smith LG Jr, Wolf EJ, Uzbay LA, Sun S, Miller RC. Neonatal effects and serum cortisol levels after multiple courses of maternal corticosteroids. *Obstet Gynecol.* 1997;90:819–823
42. Ng PC, Wong GW, Lam CW, et al. Pituitary-adrenal response in preterm very low birth weight infants after treatment with antenatal corticosteroids. *J Clin Endocrinol Metab.* 1997;82:3548–3552
43. Ng PC, Lam CW, Lee CH, et al. Reference ranges and factors affecting the human corticotropin-releasing hormone test in preterm, very low birth weight infants. *J Clin Endocrinol Metab.* 2002;87:4621–4628
44. Holsti L, Grunau RE, Oberlander TF, Whitfield MF. Specific NIDCAP movements help identify acute pain in preterm infants in the NICU. *Pediatrics.* 2004; in press
45. Simons SH, van Dijk M, van Lingen RA, et al. Routine morphine infusion in preterm newborns who received ventilatory support: a randomized controlled trial. *JAMA.* 2003;290:2419–2427
46. Rahman W, Fitzgerald M, Aynsley-Green A, Dickenson AH. The effects of neonatal exposure to inflammation and/or morphine on neuronal responses and morphine analgesia in adult rats. In: Jensen TS, Turner JA, Wiesenfeld-Hallin Z, eds. *Progress in Pain Research and Management*. Seattle, WA: IASP Press; 1997:783–794
47. MacGregor R, Evans D, Sugden D, Gaussen T, Levene M. Outcome at 5–6 years of prematurely born children who received morphine as neonates. *Arch Dis Child Fetal Neonatal Ed.* 1998;79:F40–F43
48. Anand KJ, Barton BA, McIntosh N, et al. Analgesia and sedation in preterm neonates who require ventilatory support: results from the NOPAIN trial. Neonatal Outcome and Prolonged Analgesia in Neonates. *Arch Pediatr Adolesc Med.* 1999;153:331–338
49. Johnston CC, Filion F, Snider L, et al. Routine sucrose analgesia during the first week of life in neonates younger than 31 weeks' postconceptional age. *Pediatrics.* 2002;110:523–528
50. Gormally S, Barr RG, Wertheim L, Alkawaf R, Calinoiu N, Young SN. Contact and nutrient caregiving effects on newborn infant pain responses. *Dev Med Child Neurol.* 2001;43:28–38
51. Fearon I, Kisilevsky BS, Hains SM, Muir DW, Tranmer J. Swaddling

after heel lance: age-specific effects on behavioral recovery in preterm infants. *J Dev Behav Pediatr.* 1997;18:222–232
52. Butt ML, Kisilevsky BS. Music modulates behaviour of premature infants following heel lance. *Can J Nurs Res.* 2000;31:17–39
53. Gibbins S, Stevens B. Mechanisms of sucrose and non-nutritive sucking in procedural pain management in infants. *Pain Res Manag.* 2001;6:21–28
54. Blass EM, Watt LB. Suckling- and sucrose-induced analgesia in human newborns. *Pain.* 1999;83:611–623
55. Feldman R, Eidelman AI. Skin-to-skin contact (Kangaroo Care) accelerates autonomic and neurobehavioural maturation in preterm infants. *Dev Med Child Neurol.* 2003;45:274–281
56. Johnston CC, Stremler RL, Stevens BJ, Horton LJ. Effectiveness of oral sucrose and simulated rocking on pain response in preterm neonates. *Pain.* 1997;72:193–199
57. Franck LS, Lawhon G. Environmental and behavioral strategies to prevent and manage neonatal pain. *Semin Perinatol.* 1998;22:434–443
58. Rosenfeld P, Suchecki D, Levine S. Multifactorial regulation of the hypothalamic-pituitary-adrenal axis during development. *Neurosci Biobehav Rev.* 1992;16:553–568
59. Levine S, Weinberg J, Brett LP. Inhibition of pituitary-adrenal activity as a consequence of consummatory behavior. *Psychoneuroendocrinology.* 1979;4:275–286
60. Lehmann J, Stohr T, Feldon J. Long-term effects of prenatal stress experiences and postnatal maternal separation on emotionality and attentional processes. *Behav Brain Res.* 2000;107:133–144
61. Whitehead N, Hill HA, Brogan DJ, Blackmore-Prince C. Exploration of threshold analysis in the relation between stressful life events and preterm delivery. *Am J Epidemiol.* 2002;155:117–124

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