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# Relationships between adrenocorticotrophic hormone and cortisol are altered during clustered nursing care in preterm infants born at extremely low gestational age

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## Abstract

**Background:** Little is known about the effects of clustered nursing care on hypothalamic pituitary axis (HPA) responses in preterm infants in the neonatal intensive care unit.

**Aims:** To examine facial responses, adrenocorticotrophic hormone (ACTH) and cortisol levels, and the relationship between ACTH and cortisol in preterm infants in two gestational age groups (extremely low gestational age [ELGA:  $\leq 28$  weeks]; very low gestational age [VLGA: 29–31 weeks]) under basal conditions and in response to routine nursing procedures.

**Study design:** Within subjects' cross-over design in random order.

**Subjects:** Ninety preterm infants with no postnatal steroid exposure were studied at  $32 \pm 1$  weeks postconceptional age.

**Outcome measures:** Facial actions, ACTH and cortisol levels were measured after a 30 minute rest period and in response to routine clustered nursing care (CC). Changes in facial actions were analyzed using repeated measures ANOVA. MANOVA or Mann–Whitney U tests were used to

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determine differences in ACTH and cortisol between gestational age groups. Spearman rank correlations were used to examine relationships between perinatal variables and facial, ACTH and cortisol levels.

**Results:** All infants had significantly increased facial responses to CC ( $p=0.001$ ). Infants having experienced higher numbers of skin breaking procedures 24 h before basal assessment had higher basal cortisol levels ( $r=0.30$ ,  $p=0.01$ ). In response to CC, ELGA infants showed no correlation between ACTH and cortisol levels; VLGA infants showed a strong, positive correlation ( $r=0.62$ ,  $p=0.02$ ).

**Conclusion:** The pattern of relationship between ACTH and cortisol differs depending on gestational age at birth in response to clustered nursing care. Prior pain alters responsiveness and HPA dysregulation is apparent in ELGA infants.

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## 1. Introduction

Chronic exposure to stressors may alter the brain development of very preterm infants and contribute directly and indirectly to the difficulties in behavior, learning, arousal regulation and motor development observed in these children at school age [1–4]. Formerly premature children have reduced volumes of numerous brain regions including the hippocampus which is involved in central regulation of the hypothalamic pituitary adrenal (HPA) axis [5]. Some hypothesize that these changes may be due to excitotoxic damage to vulnerable immature neurons as a result of chronic early exposure to pain and other stressors [2].

In a study to elucidate the possible impact of the effects of early pain exposure as a specific stressor related to HPA activation in this population, Grunau and colleagues found that, in infants born  $\leq 28$  weeks gestational age, a higher number of skin breaking procedures since birth predicted lower cortisol responses to a clustered series of routine nursing procedures [6]. A second study found that early cumulative exposure to pain continued to influence HPA function beyond the newborn period. In preterm infants born  $\leq 28$  weeks gestation who were assessed at 8 months of age, adjusted for prematurity, after controlling for early illness severity and duration of supplemental oxygen, higher basal salivary cortisol levels were associated with higher numbers of neonatal skin breaking procedures [7]. Thus, the assessment of basal and response levels of cortisol to challenge help illuminate the development and function of the HPA axis in preterm infants. Furthermore, many factors, including the number of skin breaking and non-skin breaking procedures to which infants are exposed, can influence adrenal function in these infants. By examining the relationship between adrenocorticotrophic hormone (ACTH) and cortisol, we may gain further insight into HPA function and possibly observe more direct evidence of HPA alterations at a central level.

Except for the two studies cited above, research on HPA development and function in preterm infants in the NICU has focused on the effects of various illness states, such as chronic lung disease or hypotension, on the effects of pre- and postnatal exogenous steroids on cortisol secretion, or on the developmental patterns of cortisol secretion in preterm infants at various gestational ages [8–11]. Some studies that included measures of both ACTH and cortisol used stimulation tests to study the functional integrity of the axis, tests which are themselves stressors [12–14]. Finally, one study examin-

ing ACTH levels in this population found that a “substantial number” of premature infants had ACTH levels below limits of detection, but did not further characterize this group of infants [15]. Importantly, none of these previous studies have examined the effects of routine nursery procedures on the relationship between ACTH and cortisol in preterm infants, nor have they coupled physiological measures of stress with behavioral indicators. Therefore, the aims of our study are 1) to describe and to determine the relationship among facial activity, ACTH and cortisol in response to clustered care, 2) to describe and to compare the relationship between ACTH and cortisol in infants born at extremely early gestational age (ELGA:  $\leq 28$  weeks) with infants born at very low gestational age (VLGA: 29–32 weeks' gestational age), both at rest and in response to clustered care, 3) to describe the relationship among perinatal variables and facial, ACTH and cortisol under basal and stressor conditions and 4) to examine perinatal variables which differ between infants who have undetectable basal and stress ACTH levels and infants who have detectable ACTH levels.

## 2. Methods

### 2.1. Study participants

The study sample comprised of 90 preterm infants (48 males, 42 females) who were  $\leq 32 \pm 1$  completed weeks gestational age at birth, and who were admitted to a tertiary level NICU. Infants who had major congenital anomaly, received analgesics or sedatives within 72 h of the assessment, or whose mothers reported illicit maternal drug use during pregnancy were excluded. Infants who received postnatal steroids or who had clinical signs of acute infection were also excluded. All infants were 32 ( $\pm 7$  days) weeks postconceptional age at time of testing, and in accordance with current standards of perinatal care, 72 (80%) of the infants had been exposed to prenatal steroids. Infant characteristics are presented by gestational age group at birth in Table 1.

### 2.2. Background data

A NICU-trained research nurse who completed the prospective clinical chart review obtained information from birth to day of testing including, but not limited to the following:

**Table 1** Preterm infant characteristics by gestational age group at birth

	Preterm 24–28 wk GA at birth		Preterm 29–32 wk GA at birth	
	<i>n</i> =30		<i>n</i> =60	
	Mean; sd	<i>N</i> (%)	Mean; sd	<i>N</i> (%)
Gestational age at birth	27 (1)		31 (1)	
Birth weight	958 (247)		1515 (456)	
Illness severity day 1 (SNAP-II)*	17 (10)		9 (8)	
Illness severity day 3 (SNAP-II)	6 (6)		0.7 (2)	
Incidence of grade IV intraventricular hemorrhage and/or periventricular leukomalacia		0 (0)		2 (0.03)
Total pain exposure (number of skin-breaking procedures)†	110 (61)		40 (26)	
Mechanical ventilation (days)†	17 (15)		1 (3)	
Oxygen (days)†	11 (9)		5 (6)	
IV morphine exposure (daily average mg/kg days)†	2 (2)		0.6 (0.2)	
Mechanical ventilation on first study day		9 (30%)		2 (3%)
Any prenatal steroid exposure		28 (93%)		44 (73%)
Post-conceptual age at first test day (days)	32 (11)		11 (7)	
Time since last feed (min)*	85 (212)		73 (40)	
Time since last invasive procedure (min)	1558 (1791)		1105 (1025)	
Number of painful procedures in 24 h before the basal study day	1 (2)		2 (2)	
Number of painful procedures in 24 h before the stress response study day	2 (2)		2 (3)	

\*Score for Neonatal Acute Physiology.

†Recorded daily from birth to basal sampling day.

birth weight, gestational age at birth, Apgar score at 1 min, illness severity using the Score for Neonatal Acute Physiology (SNAP-II) [16], head ultrasound scan results, daily opioid and other analgesic and sedative exposure, numbers and types of invasive skin breaking procedures, respiratory support, type and time of last handling just prior to blood collection. Procedural pain exposure was operationalized as the sum of every skin breaking procedure from birth to the first assessment day (e.g. heel lance, intramuscular injection, chest tube insertion, central line insertion). Each attempt at a procedure was included; thus, the sum reflected all skin breaks. While 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 [17,18]. Total intravenous (iv) morphine exposure was calculated from birth to the first test day by multiplying the average daily dose of iv morphine, adjusted for daily weight, by the number of days of iv morphine, as we have done previously [18]. For example, if an infant received an average dose of 0.39 mg/kg body weight for 24 treatment days, the morphine score was 9.36 [mg/kg].

## 2.3. Measures

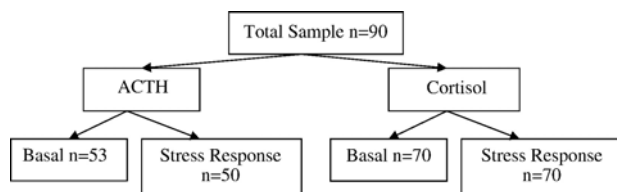
### 2.3.1. Facial actions

The Neonatal Facial Coding System (NFCS) is a reliable, well validated behavioral pain measure used widely in studies of preterm infants [19–21]. Videotapes were coded in random order of events, and coders were blind to all clinical information about the infants, to events and to the study purpose. All nine facial actions (brow lowering, eyes

squeezed shut, deepening of the nasolabial furrow, open mouth, vertical mouth stretch, horizontal mouth stretch, taut tongue, chin quiver, and tongue protrusion) were coded as occurred/did not occur for each 2 second segment during 20 s for each Phase of clustered care (Baseline=20 s period before the first contact by the nurse; Clustered Care=20 s starting when the nurse raised the hips to change the diaper; Recovery=20 s period following last contact by the nurse). In order to establish reliability, the primary NFCS coder and the reliability coder were trained to achieve a reliability coefficient above 0.85 [22]. In addition, reliability coding was carried out on 10% of the infants, with a reliability coefficient of 0.88 on this sample.

### 2.3.2. ACTH and cortisol

Blood samples were collected in plastic tubes containing 3.75 g of EDTA [Fisher Cat#: BP120-1 (FW=372.24) and 5.285 mg of Aprotinin [Sigma Cat #: A-1153 (7 TIU/mg)] were centrifuged at 4 °C and the plasma was separated immediately and frozen at –80 °C. Plasma cortisol was measured by radioimmunoassay in plasma extracted in 95% ethanol. Dextran-coated charcoal was used to absorb and precipitate free steroids after incubation. Samples were counted using ScintiSafe Econo 2 from Fisher Cat # SX21-5. The minimal detectable level for cortisol was 0.1 µg/dl, with mid-range intra- and inter-assay coefficients of variation of 1.55% and 4.26%. Plasma ACTH was assayed using a modification of the Incstar kit (INCSTAR Corp, Stillwater, MN) with all reagent volumes halved and 50 µl plasma per tube. The minimal detectable dose for ACTH was 15 pg/ml; the mid-range intra- and inter-assay coefficients of variation were 3.9% and 6.5%, respectively.



**Figure 1** Total numbers of basal and stress response ACTH and cortisol samples.

## 2.4. Procedures

The infants were recruited by a NICU-trained research nurse; written informed consent was obtained from the mother or other legal guardian according to a protocol approved by the Clinical Research Ethics Board of the University of British Columbia. The infants were studied on two separate days because blood collection was completed when required for clinical purposes only; none of our infants had indwelling lines. To control for the effects of intervening procedures which occurred between the first and second sampling, infants were randomized to have either the basal or stress response sampling done first. A video camera was positioned for a close-up view of the face and was attached to a custom made recording set-up on a moveable cart. The video signals were fed directly to a VCR and a time code was imprinted automatically. Each study phase was marked with an inaudible event cue signal recorded on the videotape. During the recording, the incubator was partially covered with a blanket, and the infant's position was supported (nested) using a continuous roll around both sides and feet.

On one test day facial recordings and basal blood samples for determination of plasma ACTH and cortisol were taken after the infant was undisturbed for a period of at least 30 min. On the other test day, facial recordings and plasma ACTH and cortisol were obtained 20 min from the start of a clustered series of tactile nursing procedures. A single research nurse carried out the clustered routine nursing procedures in the same set order: changing the diaper, measuring abdominal girth, taking the axillary temperature, cleaning the mouth with gauze and sterile water. In addition, all plasma samples were obtained between 10:00 a.m. and 2:00 p.m.

## 2.5. Data analysis

Facial activity was examined using repeated measures analysis of variance (ANOVA). Repeated measures data were examined for sphericity; when present, Greenhouse–Geisser Epsilon values were used to determine significance. Statistically significant ANOVA was followed by planned Student's *t* tests for paired comparisons to identify differences between specific phases within each observation. The significance level for each test was set at  $p \leq 0.05$ . For the physiological data, ACTH and cortisol levels were log<sub>10</sub> transformed. Separate analyses examining perinatal variables (see Table 1) used MANOVA or Mann–Whitney U tests (for non-parametric variables) with the following two grouping variables as between subjects'

factors: infants who were born  $\leq 28$  weeks gestational age at birth (ELGA) versus infants who were born 29–31 weeks gestational age (VLGA); infants whose basal or stress ACTH was  $< 15$  pg/ml versus infants whose ACTH was  $\geq 15$  pg/ml. Spearman rank correlations were then used to examine the relationship between perinatal variables and facial responses, ACTH and cortisol under basal conditions and during clustered care. Since exposure to prenatal steroids may alter HPA hormone levels taken within 7 days of delivery, we compared the cortisol levels in the infants who had been prenatally exposed to steroids and tested in the first week of life with those who were tested after 7 days of birth [23]. As a further conservative measure, we analyzed our data twice including all infants in each gestational age group first and then re-analyzing the data separating the infants who had prenatal steroid exposure from those who had no exposure.

## 2.6. Results

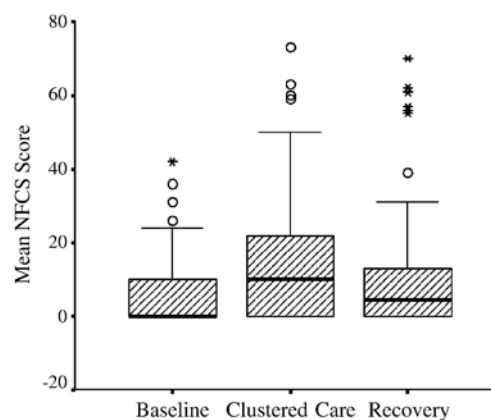
Eighty of the 90 infants (89%) had complete facial data during clustered care. The number of basal and stress ACTH and cortisol samples is shown in Fig. 1. Seventy-two (80%) infants had been exposed to prenatal steroids. Of twenty infants born within 7 days of the first test day, 6 had no prenatal steroid exposure. Cortisol levels in the infants tested within 7 days of birth were not statistically significantly different from those infants who were tested beyond 7 days of birth (2.6  $\mu\text{g}/\text{dl}$  versus 2.7  $\mu\text{g}/\text{dl}$ ).

### 2.6.1. Facial actions (NFCS)

Facial actions increased significantly from Baseline to the CC ( $p=0.001$ ), and infants continued to show increased facial activity during the Recovery Phase (see Fig. 2). No differences in facial responses between ELGA and VLGA infants were found, nor were facial actions related to ACTH or cortisol.

### 2.6.2. ACTH and cortisol

Overall, basal ACTH levels ranged from 2.0 to 53.2 pg/ml (mean: 21.5 pg/ml, SEM: 1.6), and basal cortisol levels ranged from 0.5 to 7.0  $\mu\text{g}/\text{dl}$  (mean: 2.7  $\mu\text{g}/\text{dl}$ , SEM: 0.2). In response to clustered care, ACTH levels ranged from 2.0 to



**Figure 2** Mean neonatal facial coding scores during clustered care (Baseline, Clustered Care, Recovery).

58.9 pg/ml (mean: 22.1 pg/ml, SEM: 1.7), and cortisol levels ranged from 0.8 to 7.4  $\mu\text{g}/\text{dl}$  (mean: 2.6  $\mu\text{g}/\text{dl}$ , SEM: 0.2).

### 2.6.3. Effects of gestational age and prenatal steroid exposure on ACTH and cortisol

There were no significant differences in either basal or stress ACTH or cortisol levels between ELGA and VLGA infants. However, the correlation between ACTH and cortisol differed significantly between the two groups. Under basal conditions, we found no correlation between ACTH and cortisol in either group. In contrast, in response to clustered care, ELGA infants continued to show no relationship between ACTH and cortisol, whereas VLGA infants showed a significant correlation between ACTH and cortisol levels. ( $r=0.62$ ,  $p=0.0001$ ; see Fig. 3).

Two infants in the ELGA group had not been exposed to prenatal steroids, but we were unable to obtain ACTH samples for these infants. Eight of the 16 VLGA infants who had not been exposed to prenatal steroids had plasma samples for both ACTH and cortisol levels. Exposure to prenatal steroids in the VLGA group did not alter the lack of correlation between ACTH and cortisol under basal conditions. However, in the VLGA infants free from prenatal steroid exposure, the magnitude of the correlation between ACTH in response to clustered care increased ( $r=0.83$ ,  $p<0.01$ ); whereas in the infants exposed to prenatal steroids, the magnitude of the correlation in response to

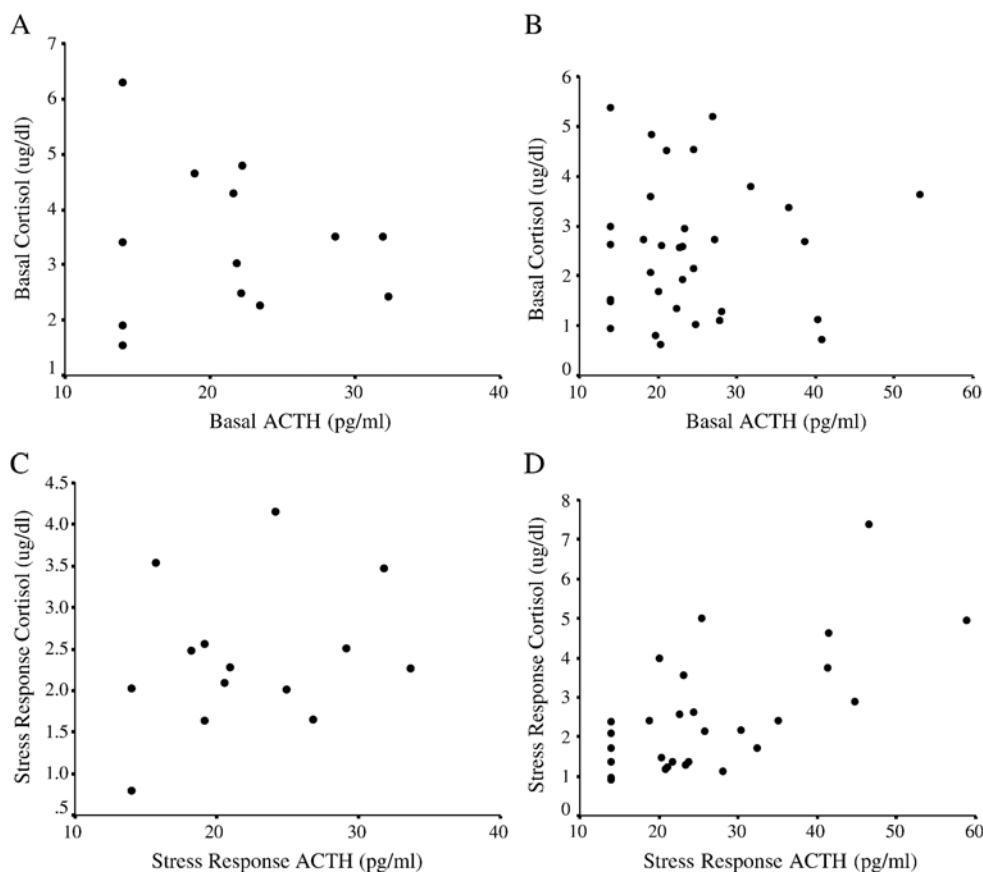
clustered care decreased, but remained significant ( $r=0.55$ ,  $p<0.01$ ).

### 2.6.4. Relationships between perinatal variables and facial, ACTH and cortisol

In the infants as a whole, higher birth weight was associated with more intense facial responses during clustered care ( $r=0.26$ ,  $p=0.02$ ) and greater numbers of skin breaking procedures in the 24 h preceding the basal assessment were associated with higher basal cortisol levels ( $r=0.30$ ,  $p=0.01$ ).

### 2.6.5. Relationships between perinatal variables in infants with ACTH < 15 pg/ml

Of the 53 infants from whom basal ACTH samples were obtained, 10 infants (19%) had basal ACTH levels that were undetectable (<15 pg/ml). No perinatal variables distinguished this group of infants from those who had detectable basal ACTH levels. Ten infants (20%) of the 50 who had stress ACTH samples collected also had undetectable ACTH values (3 of these infants also had had undetectable basal ACTH). Importantly, these ten infants had been exposed to greater numbers of skin breaking procedures in the preceding 24 hours ( $p=0.007$ ) than had infants with ACTH levels >15 pg/ml. There were no differences in cortisol levels between infants who had undetectable basal ACTH levels and infants who had normal basal ACTH levels. However,



**Figure 3** Correlation between basal and stress ACTH and cortisol (raw values). Panel A: infants born  $\leq 28$  weeks gestational age ( $n=13$ ). Panel B: infants born 29–32 weeks gestational age ( $n=33$ ). Panel C: infants born  $\leq 28$  weeks gestational age ( $n=14$ ). Panel D: infants born 29–32 weeks gestational age ( $n=28$ ).

infants with undetectable ACTH levels in response to the stressor had significantly lower cortisol levels (mean = 1.5 µg/dl versus 2.7 µg/dl; 95% CI: 2.1–2.6,  $p=0.02$ ) than infants whose ACTH levels were in the detectable range.

### 3. Discussion

This study is the first to describe facial, ACTH and cortisol levels before and in response to routine clustered nursing procedures. Importantly, none of the infants in our study were exposed to postnatal steroids. All infants exhibited significantly increased facial responses in response to the clustered care, responses which continued into the Recovery Period. The facial responses in this study and the body movement, heart rate and oxygen saturation responses to identical care-giving tasks found in our previous work indicate that preterm infants experience significant ongoing stress responses to what are usually considered relatively “innocuous” routine procedures [24]. We also found a modest but significant association between higher birth weight and more intense facial responses in response to clustered care, paralleling previous findings which show that preterm infants born at earlier gestational ages (and accordingly, with lower birth weights and greater exposure to cumulative pain) display less intense facial responses to acute pain procedures [18]. We did not find a relationship between facial actions and ACTH or cortisol in response to clustered care; however, dissociation between behavioral and physiological responses is common in infants [25–27].

The basal ACTH and cortisol levels in our sample were at the lower end of the range of values reported in other studies of preterm infants tested at 32 weeks post conceptional age in the NICU [12]. Although prenatal steroid exposure could have influenced basal hormone levels, it is noteworthy that basal samples did not differ between infants who had been exposed to prenatal steroids who were tested within 7 days of delivery and infants who were tested later and that the correlation between ACTH and cortisol in response to clustered care in the VLGA group remained irrespective of prenatal steroid exposure. Nevertheless, exposure to prenatal steroids did reduce the magnitude of this correlation in the VLGA infants. Consistent with our findings, one study of 9 preterm infants has shown alterations in cortisol responses to handling in infants exposed to prenatal steroids when they were tested within the first week of life [28]. Further research is needed to determine whether preterm infant HPA responses to NICU procedures are altered by prenatal steroid exposure beyond the first week of life, particularly in ELGA infants [29].

In this study, basal hormone levels did not differ between infants born at earlier gestational ages, and concomitantly those with greater cumulative pain exposure, and those infants born later. We speculate that basal hormone levels remain relatively well-conserved in the first few weeks or months of life since they play such an important role in physiological and behavioral system regulation, as has been shown in studies examining the developmental effects of stressors using animal models (for example [30,31]). In addition, we found no correlation between basal levels of ACTH and cortisol in either gestational age group. The only

other study to examine correlations between ACTH and cortisol in preterm infants reported a modest correlation (0.42) between basal ACTH and cortisol in a younger sample [32]. However, the lack of correlation may have occurred because we were able to take one plasma sample only and, as a result, may have missed the peak hormone levels of either ACTH or cortisol. In preterm infants free from postnatal steroid exposure, ACTH levels peak and very gradually decline after 15 min, whereas peak cortisol levels can occur between 20 and 30 min [14,23,32]. By taking our samples 20 min after the start of the handling, we tried to maximize the likelihood that our single samples would capture as close to the peak levels of both hormones as possible. Furthermore, in adults, lack of correlation between ACTH and cortisol has been observed during periods of prolonged stress, such as following major surgery [33]. The numerous invasive and non-invasive procedures experienced by preterm infants, especially ELGA infants, can be considered analogous to the prolonged stress experienced following surgery. Additional research is needed to make clear the reason for the lack of correlation between basal ACTH and cortisol in the present study.

Because blood collection could be completed only when required for clinical purposes, basal and stress blood samples were obtained on two separate days and we were unable to measure directly pre- and post-test HPA responses to clustered care. Nevertheless, we found that while ELGA infants continued to show no correlation between ACTH and cortisol in response to clustered care, we found a strong, positive correlation between ACTH production and cortisol secretion in response to the challenge of clustered care in the VLGA infants. Although Poggi Davis and colleagues found that healthy preterm infants did not mount a cortisol response to a tactile (physical exam) procedure [28], in the present study, the increased facial activity in both the ELGA and VLGA infants and the change in the relationship between ACTH and cortisol in the VLGA infants suggest that clustered care was a strong enough stimulus to activate the HPA axis. It is possible that clustered care represents a more stressful set of procedures than those in a typical physical exam and/or may reflect methodological differences in that the preterm infants in the present study, particularly the ELGA infants, were smaller and sicker and tested at a younger age than the infants studied by Poggi Davis.

In addition, the lack of change in the relationship between ACTH and cortisol in the ELGA infants may be the beginnings of the altered “set point” of the HPA activity we have observed at 8 and 18 months of age in ELGA infants who have had early repetitive exposure to intrusive and invasive procedures [6,34]. Conversely, it is possible that suppressed responsiveness in the first months of life may be neuroprotective for those infants exposed to greater numbers of stressors early in life, such as what occurs for the ELGA infants. Indeed, researchers have reported that a developing brain which is exposed to high levels of glucocorticoid will exhibit catabolic and neuroanatomical changes such as reduced myelination, inhibition of cell proliferation, reduced axonal growth, and inappropriate formation of dendritic spines and synaptogenesis [35].

Greater numbers of skin breaking procedures in the 24 h before the test day influenced the HPA axis in different ways. In the group as a whole, higher basal cortisol levels

were associated with greater pain exposure in the preceding 24 h. As in adults, this high cortisol may be providing the appropriate negative feedback to shut down the stress response. However, an alternative explanation is that we are observing "sensitization" of the HPA axis, a response consistent with other studies which found exposure to pain in the preceding 24 h induced heightened behavioral responses to intrusive procedures [36]. Furthermore, during the stressor, one fifth of the infants had below detectable limits of ACTH; these same infants also produced the lowest levels of cortisol during the clustered care. Thus, in some infants, greater immediate pain exposure may induce a "down-regulation" of the HPA axis, leaving them unable to mount appropriate HPA responses to subsequent handling [6].

Several limitations of this study should be mentioned. First, as noted, we could not measure directly pre- and post-ACTH and cortisol levels to the clustered care because blood samples could not be taken for experimental purposes. Therefore, the lack of difference between the basal and stress cortisol levels in our study must not be interpreted as "non-response" to the clustered care. Second, we were unable to determine the effects of prenatal steroid exposure on the relationship between ACTH and cortisol in the ELGA infants. We did note, however, that in the VLGA infants free from prenatal steroid exposure, the magnitude of the correlation between ACTH and cortisol in response to the clustered care increased, whereas in the infants exposed to prenatal steroids, the magnitude of the correlation decreased, suggesting that prenatal steroid exposure did exert at least some influence on the HPA response to clustered care. Third, we could not control for exposure to prenatal stress which in itself can have long-term effects on offspring [37]. In fact, prenatal stressors may themselves cumulatively contribute to preterm labor [38]. Therefore, in addition to the postnatal stress of pain in the NICU, some of the differences in infants with low basal and stress ACTH may at least partially reflect prenatal stress. Fourth, no infants had abdominal ultrasounds to examine the influence that adrenal hemorrhage may have had on our results, nor did we obtain blood pressure measures. Finally, determining the relative contributions of immaturity, prenatal steroid exposure, cumulative pain exposure and illness severity on HPA responses in preterm infants in the NICU remains difficult because these variables are highly inter-correlated. In this study, we chose to examine our results in two gestational age groups (ELGA and VLGA), and through this we were able to define more clearly how preterm birth may affect HPA development.

In conclusion, dysregulation of the HPA axis may occur in preterm infants born at extremely low gestational ages. The finding that ACTH and cortisol levels are not correlated in ELGA infants as they are in VLGA infants in response to clustered care may reflect changes at a more central level. Moreover, exposure to pain may diminish the ability of preterm infants to mount the expected cortisol response to clustered routine nursing procedures. These data highlight the difficulties faced by clinicians trying to interpret ACTH and cortisol levels in sick infants and formulating an appropriate treatment plan. Even though clustered care provides preterm infants with longer rest periods throughout

the day than would occur if procedures were spaced over time, grouping routine procedures induces responses in these infants which may contribute to the difficulties in development which we observe in the later childhood of formerly preterm children.

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