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Prior pain induces heightened motor responses during clustered care in preterm infants in the NICU

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Abstract

Background: Acute pain is a significant stressor for preterm infants in neonatal intensive care units (NICU); however, little is known about the effects of acute pain on subsequent motor responses during clusters of tactile handling.
Aims: (1) To compare facial, body and heart rate reactivity in preterm infants at 32 weeks gestational age (GA) during routine care-giving tasks following a rest period (RCC: diapering, measuring abdominal girth and axillary temperature, mouth care) with their responses to Clustered Care following blood collection (PCC). (2) To examine how GA at birth affects patterns of stress and self-regulatory behaviors during RCC and PCC.
Study Design: Within-group crossover design (random order).
Subjects: Preterm infants, N=54 (mean GA at birth 29.3 ± 2.2 weeks; mean birth weight 1257 ± 423 g) were assessed at 32 weeks GA in the NICU.
Outcome measures: The Newborn Developmental Care and Assessment Program (NIDCAP®) and Neonatal Facial Coding System (NFCS) were coded from continuous bedside video recordings. Changes in mean heart rate (HR) were computed using custom physiologic software.
Results: All infants had heightened facial, body and HR responses when CC followed a painful procedure compared to when they had not been handled prior to CC.

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Infants born at earlier GA (<30 weeks) had equal numbers of stress cues during RCC and PCC, but dampened self-regulatory behaviors during PCC.
Conclusion: Prior pain induces heightened biobehavioral reactivity in preterm infants during subsequent tactile procedures. In addition, clustering care is particularly stressful for infants born at earlier GA.

1. Introduction

Practitioners caring for preterm infants have long been concerned with the cumulative effects of pain and stress on this vulnerable population because both animal and human research demonstrate the deleterious effects of repetitive pain and stress on developing neonates [1—3]. Moreover, early pain and/or chronic stress experienced by these infants induces behavioral and physiologic changes which then may directly and indirectly contribute to persistent neurodevelopmental and behavioral alterations in the earliest born in this population [4—6]. In addition, researchers hypothesize that the reduced volumes [7—9] and diffuse abnormalities [10] in cortical and subcortical regions in children born prematurely may be linked to cellular mechanisms triggered by repeated exposure to pain and stress in the NICU [11].

In addition to studying the effects of pain in preterm infants in neonatal intensive care units (NICU), examining the effects of patterns of handling is important particularly because patterns of handling have changed substantially since the introduction of developmental care as modeled in the Newborn Individualized Developmental Care and Assessment Program (NIDCAP®) [12—14]. Now, using developmental care principles, many NICU staff base care-giving on each individual infant's needs whenever possible [15,16]. Accordingly, routine care-giving tasks, such as diaper changing and feeding, are often clustered together to allow infants longer rest periods. Indeed, stable preterm infants sleep more, [17,18] weigh more and have more rapid decline in the incidence of apnea [19] when rest periods are scheduled throughout the day. Although preterm infants appear to benefit from rest periods, these studies were not designed to examine the reactivity of infants *during* clustered care. Further examination of the specific effects of clustered care is vital because some suggest that the increased sleep following clustered interventions may be due to increased energy expenditure, [18] and less stable preterm infants may find the clustering of procedures over a short period of time very stressful [20].

Few studies have reported the effects of prior handling on reactivity during subsequent care-giving tasks. Porter et al. [21] found that preterm infants who had been handled prior to blood collection showed heightened facial reactivity and changes in heart rate (HR) during heel lance compared to infants who were not handled prior to the heel lance. However, body movement reactivity was not described. Inclusion of body movements is important because they provide important additional information regarding preterm infants' tolerance to handling [22,23].

Heightened reactivity following prior pain exposure has also been described by Taddio et al. [24] who reported heightened anticipatory behaviors and reactivity during heel lance in term infants who had received multiple heel lances in the first 24—36 h of life. Although Goubet et al. [25] found anticipatory increases in heart rate prior to heel lance in preterm infants born between 28 and 32 weeks gestational age (GA) that had experienced multiple heel lances in the previous 2 weeks, only infants born at later gestational ages showed heightened reactivity during the lance phase. Neither of these studies included full body movement reactivity. Finally, using the NIDCAP® observation system, Grunau et al. [26] found that extremely low birth weight preterm infants (≤1000 g) displayed greater facial grimacing during endotracheal suctioning if they had experienced more painful procedures in the previous 24 h. Although this study did utilize body movements in evaluating reactivity to handling of varying intensities, the timing between prior pain and suctioning was not controlled. Thus, while these four studies have examined the effects of prior pain on later pain reactivity, no studies have evaluated the effects of prior pain on the biobehavioral reactivity of preterm infants in the NICU during subsequent *tactile* procedures. Therefore, the aims of this study were the following:

1. To compare facial, body and heart rate reactivity in preterm infants at 32 weeks gestational age (GA) during routine care-giving tasks in infants undisturbed prior to handling with their responses to clustered care following an acute skin breaking procedure.

2. To compare patterns of stress and self-regulatory movements during Clustered Care following Rest (RCC) and Clustered Care following Pain (PCC) in infants born at earlier gestational ages (<30 weeks) to those of later-born infants (≥30 weeks).

2. Methods

2.1. Participants

The study sample comprised 54 preterm neonates (24 females, 30 males; 72% Caucasian ethnicity) born ≤32 completed weeks gestational age, in a major regional level III NICU at the Children's and Women's Health Centre of British Columbia, Vancouver, Canada. Infants with a major congenital anomaly, significant intraventricular hemorrhage (IVH grade III) and/or parenchymal brain injury [IVH grade IV and/or periventricular leukomalacia (PVL)], as well as infants who had received analgesics or sedatives within 72 h of the targeted study session, were excluded. All infants were 32 weeks postconceptional age (±7 days) at time of the study. GPOWER [27] was used to calculate the sample size estimate, and effect sizes entered into the program were based on differences in the frequencies of finger splays in preterm infants, a reliably occurring NIDCAP® stress cue [22,26]. Using this method, 14 infants were needed to detect differences between each phase for a power of 0.83 with the statistical significance set at 0.05.

2.2. Procedures

The infants were recruited by a NICU research nurse, and written informed consent was obtained from the parent or legal guardian according to a protocol approved by the Clinical Research Ethics Board of the University of British Columbia. Videotaping and physiologic recording were carried out continuously. Heart rate data were collected by attaching the leads from the bedside monitor to a custom-designed computer data acquisition system. Two cameras (one positioned for close-up on the face, the other on the full body) were attached to a custom-made recording set-up on a moveable cart, including two 9″ video monitors. The signals were fed directly to two VCRs, and a time code was imprinted automatically. Each study phase was marked with an inaudible event cue signal recorded simultaneously on the videotape and physiologic acquisition systems. A research technician set up the video cameras, VCRs, and operated the computerized cardiac data acquisition system and marked each event. Each infant was undisturbed for a period of at least 30 min prior to recording. Then, a single research nurse carried out clustered nursing procedures (Clustered Care) in a set order: changing the diaper, measuring abdominal girth, taking the axillary temperature, cleaning the mouth with gauze and sterile water. Blood collection following heel warming was carried out by a lab technician who cleansed the heel, applied a lancet and squeezed the heel to collect blood. Following blood collection, each infant was left nested and undisturbed for a rest period prior to the initiation of the diaper change and other tactile procedures. Each infant was tested on two separate days. Assignment to Clustered Care following Rest (RCC) vs. Clustered Care following Pain (PCC) was randomized when babies were entered into the study. Three phases were analyzed [Baseline, Handling Phases (Clustered Care following Rest or Clustered Care following Pain), Recovery].

2.3. Measures

2.3.1. Infant state

Infant sleep/wake state was coded every 2 min according to the NIDCAP® protocol [28]: 1=deep sleep; 2=light sleep; 3=drowsy; 4=quiet awake; 5=active awake; 6=highly aroused/crying. The predominant state over each 4-min period was coded for each phase.

2.3.2. Facial activity (Neonatal Facial Coding System, or NFCS)

The Neonatal Facial Coding System (NFCS) is a reliable, well-validated behavioral pain measure widely used in studies of preterm infants [29—31]. Traditionally, the full NFCS has been applied to brief periods (e.g., 20 s per phase) to capture the acute pain response. However, for this study, the frequency of NFCS brow bulge was coded continuously for 12 min using the Noldus Observer system [32] (throughout 4 min of Baseline, the first 4 min of the Handling Phase (RCC and PCC) and the first 4 min after the last contact by the technician [Recovery]) to match the NIDCAP® coding. Brow bulge was selected as a proxy for upper facial actions since it has been shown to correlate highly with the other upper facial actions of the NFCS and has been shown to reflect pain reactivity in preterm infants [22]. Lower facial actions were not used because they are sometimes obscured by tape used to secure tubes to the face of preterm infants. Videotapes were edited for coding in random order of events, and coders were blind to all clinical information about the infants and to events. In order to establish reliability, both the primary NFCS coder (LH) and the reliability coder were trained on the entire tool with a reliability coefficient of 0.87 [33]. In addition, reliability coding was carried out on 20% of the sample with a reliability coefficient of 0.88. For data analysis, the frequency of NFCS brow bulge was summed across all infants for each 4-min phase for both RCC and PCC procedures.

2.3.3. NIDCAP®

The NIDCAP® behaviors were coded continuously from video recordings of each infant and coding was carried out blind to all clinical information. Following published NIDCAP® procedures, the frequency of each infant's movements was recorded systematically in two-min time blocks [28]. The primary coder (LH) was an occupational therapist, and the reliability coder was a physiotherapist, both of whom were NIDCAP® certified. Reliability for the NIDCAP® was initially established during the certification process [34]. In addition, a randomly selected sample of 5% of NIDCAP® video segments from the study was coded to evaluate reliability. NIDCAP® reliability was calculated by determining % agreement of occurrence (both coders indicating the presence or absence of a behavior) within every 2-min time segment during each 4-min phase for each infant. Interrater agreement was 87%. Physiologic measures were recorded by custom computer software, and so were not scored using the NIDCAP® observation record.

The frequencies of each NIDCAP® behavior were reviewed, and the 31 movements which occurred in less than 25% of the infants were excluded from statistical analysis. The remaining NIDCAP® behaviors were summed across the four min of the 3 Phases [Baseline, Handling Phase (Clustered Care following Rest or Clustered Care following Pain), Recovery] for each procedure and divided into four grouping variables for each Phase (e.g., Clustered Care following Rest Stress and Stability Cues; Clustered Care following Pain Stress and Stability Cues). The NIDCAP® behaviors which were included in the NIDCAP® Stress and Stability categories are presented in Fig. 1.

2.3.4. Heart rate

Continuous electrocardiographic (ECG) activity was recorded from a single lead of surface ECG and was digitally sampled at 360 Hz off-line using a specially adapted computer acquisition system. Custom physiologic signal processing software was used to acquire, process and analyze heart rate [35]. R waves were detected from the sampled ECG and were used to form a smoothed instantaneous 4-Hz time series [36]. Mean heart rate (HR) was calculated for each 2-min segment of each study period to correspond to the 2-min NIDCAP® time blocks and averaged over 4 min of each of the 3 phases of each procedure [Baseline, Handling Phase (Clustered Care following Rest or Clustered Care following Pain) Recovery]. Prior to statistical analysis, 4% of the 2-min HR segments were dropped due to poor signal for that phase.

2.4. Background data

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

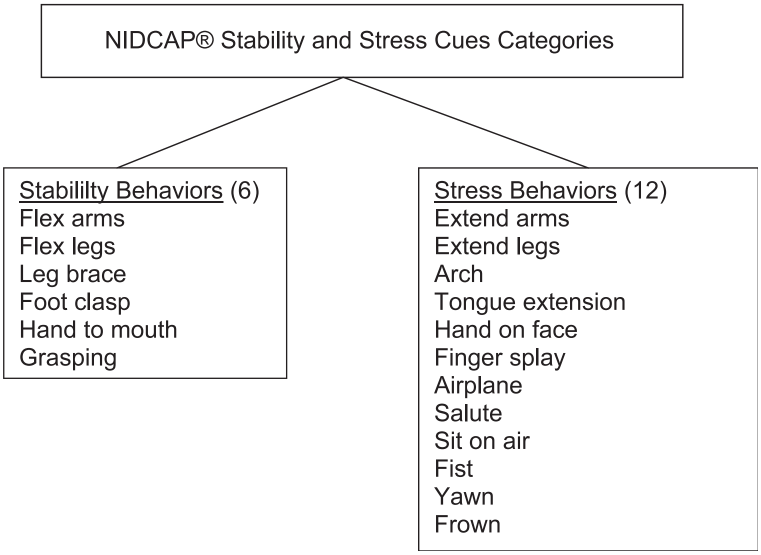

Figure 1 NIDCAP® Stability and Stress cues grouping variables.

Table 1 Demographic and study day characteristics to the first study day (n=54)

	Mean (S.D.)	Range
Birth weight (g)	1257 (423)	500–2345
Gestational age at birth (weeks)	29.3 (2.2)	24–32
SNAP-II day 1	12 (9)	0–38
Mechanical ventilation (days)	8.3 (12)	0–46
Other respiratory support (days)	7.2 (7)	0–28
Dexamethazone (days)	0.32 (1.4)	0–8
Prior pain exposure[a]	70.89 (51)	7–246
Morphine exposure[b]	0.77 (1.9)	0–9.6
Maternal age (years)	31.4 (5.7)	19–47

[a] Number of invasive (skin breaking) procedures from birth to the first study day.

[b] Morphine exposure=(daily average/kg per os dose/3 + daily average iv mg/kg)×days.

weight, gestational age at birth, Apgar score at 1 min, illness severity using the Score for Neonatal Acute Physiology[1] (SNAP II) [37], amount of opioid and other analgesic and sedative exposure, total number and types of invasive skin breaking procedures, respiratory support, type and time of last handling just prior to blood collection. Invasive procedures were defined as those involving skin breaking such as heel lance, venipuncture, insertion of arterial and venous lines, lumbar puncture and chest-tube insertion. In addition, the number of endotracheal intubations was collected. Demographic and study day characteristics of the infants are presented in Tables 1 and 2.

2.5. Data analysis

Since position of an infant may influence the occurrence of body movements [38], infant position was examined using paired Student's t-tests to determine whether there were differences in positioning during the Baseline and Recovery phases of both RCC and PCC. Then, continuous measures (NFCS, NIDCAP® and HR) were analyzed with repeated-measures ANOVA to compare biobehavioral responses across the three phases [Baseline, Handling (Clustered Care or Clustered Care following Pain) Recovery)] of each procedure with sex and gestational age at birth as between-subjects factors, with time of blood collection as a covariate and Bonferroni corrections to control for overall error. Statistically significant ANOVA was followed by planned Student's t-tests for paired comparisons to identify differences between specific phases within each observation. In addition, one-way ANOVA was used to determine differences in frequencies of NIDCAP® Stress and Stability Cues in earlier-born (<30 weeks GA) vs. later-born (≥30 weeks GA) infants. Categorical variables (sleep/wake states) were analyzed using nonparametric tests for related samples (Wilcoxon signed ranks and Friedman).

3. Results

3.1. Infant sleep/wake states

The infants differed in their behavioral state during the Baseline phases of the two procedures. Greater numbers of infants were in deep sleep during the Baseline phase of the RCC procedure than during the Baseline phase of the PCC procedure ($z=-2.2$, $p<0.03$) where more infants were in active sleep. Sleep/wake state changed significantly across phases during both the RCC ($x^2=69.2$, $p<0.0001$) and PCC procedures ($x^2=59.3$, $p<0.0001$). There were no differences in sleep/wake states during Handling or the Recovery phases between RCC and PCC.

3.2. Facial activity (Neonatal Facial Coding System, or NFCS)

The frequency of brow bulge changed significantly across the 3 phases of both the RCC [$F(1,52)=19.2$; $p<0.0001$] and PCC [$F(1,52)=10.3$; $p<0.0001$] pro-

Table 2 Infant characteristics on the clustered care following pain study day (n=54)

	Mean (S.D.)	Range	N (%)
Postconceptional age (weeks)	32 (0.7)	31–33	
Postnatal age (days)	19	3–49	
Mechanical ventilation			9 (2)
Blood collection time (min)[a]	5.4 (2.7)	1.8–16.7	
Rest period between blood collection and clustered care[b]	14.3 (2.9)	3.9–18.7	

[a] Time from first contact of lab technician to last contact following heel squeeze.

[b] Undisturbed time from last contact of lab technician to first contact of research nurse for Clustered Care following Pain Procedure.

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[1] The Score for Neonatal Acute Physiology (SNAP II) is an illness severity measure that incorporates respiratory, neurological and other physiological parameters, such as temperature, as markers of early illness in preterm neonates. The SNAP II provides a cumulative score whereby the higher the score, the sicker the infant.

Table 3 NIDCAP® stress and stability cues across three phases of Clustered Care following Rest (RCC) and Clustered Care following Pain (PCC) (n=54)

Procedure	Baseline [mean (S.D.)]	Handling [mean (S.D.)]	Recovery [mean (S.D.)]	*F*	P
RCC stress cues	2.3 (4)	14.3 (7)	6 (8)	17.8	<0.0001
RCC stability cues	1.8 (3)	5.3 (4)	4.4 (7)	4.6	<0.02
PCC stress cues	3.8 (6)	16.7 (8)	4.2 (6)	23.4	<0.0001
PCC stability cues	3.5 (5)	7.4 (6)	3.6 (6)	4.4	<0.02

cedures. During RCC, the frequency of NFCS brow bulge rose from 0.20 during Baseline to 2.30 during the Handling phase and returned to 0.90 during Recovery. During PCC, the frequency of NFCS brow bulge increased from 0.30 during Baseline to 3.80 during the Handling phase, and returned to 1.00 during Recovery. Thus, infants showed greater frequencies of brow bulge during PCC ($t=-2.5$, $p<0.01$) compared to RCC. The differences between the Baseline and Recovery phase NFCS frequencies were not significant for the two procedures, nor were there sex effects.

3.3. NIDCAP®

3.3.1. Total group analyses

There were no differences in the infants' position during the Baseline and Recovery phases of the RCC and PCC procedures. There was a main effect of phase for both NIDCAP® Stress and Stability cues for both RCC and PCC (see Table 3). During the Baseline phase, all infants exhibited more NIDCAP® Stability behaviors during the RCC procedure than to the PCC procedure ($t=2.2$, $p<0.03$). During the Handling phases, the infants showed greater numbers of both NIDCAP® Stress ($t=-2.5$, $p<0.02$) and Stability cues ($t=-2.8$, $p<0.008$) during the PCC procedure than to the RCC procedure. For the PCC procedure, three infants had had less than 10 min of rest time between the end of the blood collection and the beginning of clustered care. Reanalysis of the data with these infants removed did not alter the results. In addition, there were no significant effects of blood collection time on the frequencies of either NIDCAP® Stress or Stability cues during PCC. There were no sex effects on frequency of NIDCAP® Stress or Stability cues during the Baseline or Handling phases of either procedure. Although as whole group, the infants showed no statistically significant differences in the numbers of NIDCAP® Stress or Stability cues during the Recovery phases of the two procedures, male infants showed greater numbers of NIDCAP® Stress cues during the Recovery Phase of the PCC procedure than did females [$F(1,52)=4.7$, $p<0.03$].

3.3.2. Gestational age effects

There were no statistically significant differences between infants born <30 weeks gestational age (GA) and those born at later GA (≥30 weeks) in the frequencies of NIDCAP® Stress or Stability cues during the Baseline phases of either the RCC or the PCC procedure. In addition, gestational age at birth did not influence the total numbers of NIDCAP® Stress cues occurring during the Handling phases for either the RCC or the PCC procedures. However, infants born >30 weeks gestational age at birth had twice as many Stability cues during the Handling phase of the PCC procedure as did infants born at earlier gestational ages (see Table 4 and Fig. 2). More specifically, infants born at later gestational ages (≥30 weeks) were able to flex their legs [$F(1,52)=10.4$, $p<0.002$] and bring hands to mouth [$F(1,52)=4.5$, $p<0.04$] during the Handling phase of the PCC procedure.

During the Recovery phase, the infants born at later gestational ages (≥30 weeks) continued to exhibit increased Stability cues during both the RCC [$F(1,52)=9.1$, $p<0.004$] and PCC procedures [$F(1,52)=4.6$, $p<0.04$]. The later-born infants also

Table 4 Differences in NIDCAP® stress and stability behaviors of earlier (<30 weeks) and later-born preterm infants (≥30 weeks) during the handling phases of Clustered Care vs. Clustered Care following Pain procedures

Procedure	Gestational age at birth <30 weeks (n=34)	Gestational age at birth ≥30 weeks (n=20)	*F*	P
Clustered Care following Rest				
Total stress cues [mean (S.D.)]	14.7 (7)	13.7 (6)	0.2	ns
Total stability cues [mean (S.D.)]	4.6 (3)	6.4 (4)	3.1	ns
Clustered Care following Pain				
Total stress cues [mean (S.D.)]	16.2 (8)	17.7 (8)	0.4	ns
Total stability cues [mean (S.D.)]	5.5 (4)	10.5 (8)	10.2	<0.002

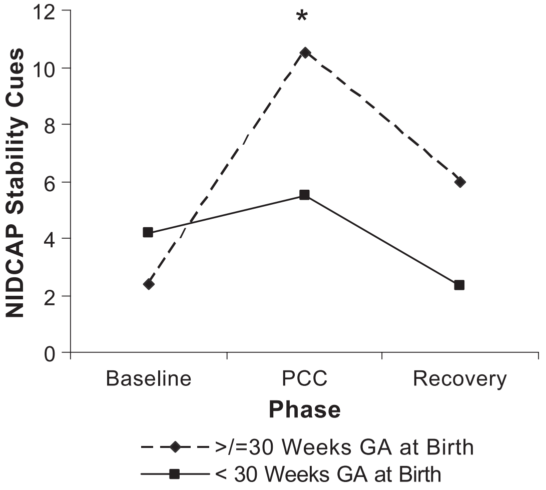

Figure 2 Mean number of NIDCAP® Stability cues during Clustered Care following Pain (PCC) in infants born <30 weeks gestational age compared to infants born ≥30 weeks gestational age. *$p<0.002$.

showed ongoing Stress cues during the Recovery phase following the RCC procedure [$F(1,52)=4.3$, $p<0.04$].

3.4. Heart rate

Mean heart rate (HR) changed significantly across the three phases of RCC [$F(1,53)=41.7$; $p<0.0001$] and across PCC [$F(1,49)=50.0$; $p<0.0001$]. There were no statistically significant differences between the Baseline phases of the two procedures, nor in the Recovery phases. During RCC, heart rate increased from 155.7±13 during Baseline to 167.6±15 during the Handling phase and returned to 156.5±14 during Recovery. During PCC, heart rate increased from 157.0±11 during Baseline to 171.6±12 during the Handling phase and returned to 156.8±13 during Recovery. The increase in mean HR during the Handling phase was significantly greater during PCC than during RCC (t=2.2, $p<0.03$).

4. Discussion

This study is the first to examine the specific effects of a prior pain procedure (routine blood collection) on the biobehavioral reactivity of preterm infants in the NICU during a routine cluster of tactile procedures. While the infants showed increased reactivity during both the handling phases of the Clustered Care following Rest and the Clustered Care following Pain procedures compared to Baseline and Recovery phases, their facial, body and heart rate reactivity was *heightened* when the tactile procedures were preceded by blood collection required for clinical management. Our findings are supported by others who have reported heightened reactivity to painful procedures following either prior handling or an acutely painful procedure in the preceding 24 h [21,26]. These heightened facial, body and heart rate responses occurred even when time of blood collection was statistically controlled and despite there being a rest period between the last contact of the lab technician completing the blood work and the initiation of the Clustered Care by the nurse. Thus, the infants' heightened reactivity to the second procedure in this study likely reflects sensitization [39,40].

More importantly, while the frequency of NIDCAP® Stress and Stability movements increased more during the Handling phase of the PCC procedure in the infants as a whole group, we found that infants born at earlier GA (<30 weeks) lose their ability to use flexor behaviors, such as hands to mouth, to self-regulate when they experience a painful procedure prior to a cluster of tactile handling. Since infants born at earlier gestational ages exhibited the same number of Stability cues during the Baseline phases of both procedures, their lack of flexor regulation was not developmental in nature (e.g., Ref. [41]). While the result of different mechanisms is likely, it is interesting that infants born at earlier gestational ages in this study displayed dampened self-regulatory body movements following a pain procedure, a pattern which may be similar to the dampened facial reactivity seen in preterm infants who have experienced an acutely painful event just prior to the one being assessed [42]. Some researchers speculate that dampened reactivity may reflect dysregulation or, alternatively, that decreased reactivity to routine handling may be the result of habituation to the handling [43]. However, determining the short- and long-term neurodevelopmental implications of this pattern of responses in preterm infants will require much further study.

In addition to the effects of gestational age on the reactivity of preterm infants to Clustered Care following Pain, infants who were born later continued to show self-regulatory behaviors during the Recovery phases, whereas the earlier-born infants returned to Baseline. These findings likely reflect the more robust behavioral characteristics of infants born at later gestational ages. We also found that male but not female infants continued to demonstrate NIDCAP® Stress cues into the Recovery Phase of the PCC procedure. While Sell et al. [44] found that preterm infants continued to show disorganized motor skills following care-giving, they did not specify what kind of tasks were observed, nor did they examine differences in NIDCAP® behavior between male and female infants. In addition, our finding was not the result of male infants being in a different position during Recovery than female infants [38]. Our findings may be in contrast to other studies which have examined sex differences in pain processing which show that term female infants have greater facial displays of pain [45]; however, Guinsberg et al. did not include body reactivity. Reconciling sex differences in neonatal body movement reactivity will require further study with a larger sample.

Our findings highlight the effects that acute skin breaking procedures have on preterm infants' responses to subsequent tactile handling. Providing developmentally sensitive strategies, such as facilitated tucking, kangaroo care and limited use of sucrose, may buffer the effects of acute pain in these high-risk infants [46—48]. Our findings also have implications for choice of care-giving patterns for preterm infants in the NICU. Rest periods between procedures, now standard in many units practicing developmentally sensitive care, clearly have benefits for preterm infants [17—19]. However, providing rest periods often results in a number of procedures being clustered together in between the rest times. Some researchers suggest that speed is the critical factor which determines how well very ill preterm infants tolerate clustered care [49]. But even healthier preterm infants who have their care clustered to provide longer rest periods show increased sleep following the clustered care, a sign which may indicate significant energy expenditure [18]. Similarly, our study shows that even preterm infants whose care is paced demonstrate increased reactivity during the subsequent handling. Moreover, there are selective effects on those infants who are most vulnerable to the effects of repeated stress on the developing nervous system, those born at earlier gestational ages.

In conclusion, acutely painful procedures prime preterm infants' biobehavioral response systems to subsequent handling producing heightened reactivity. In addition, clustered care produces significant behavioral and autonomic reactions in preterm infants, particularly those born at earlier gestational ages. While the intention of using clustered care is to provide vitally needed rest periods for these vulnerable infants, this practice needs careful review so that relative risks and benefits of differing care-giving patterns can be evaluated. More specifically, the amount of time that preterm infants should remain undisturbed requires further investigation. Although the temporal patterns of sleep/waking states of preterm infants have been described (e.g., [50,51]), little is known about the optimal quantity of sleep that these infants need to ensure their robust growth and development. Thus, evaluating the tolerance to handling of each individual infant remains a vital component of developmentally sensitive care. While "random" patterns of care-giving are not likely optimal either, it may be a balance of shorter rest periods interspersed with one or two care-giving tasks that would produce less intense biobehavioral responses and increased opportunities for infants born at earlier gestational ages to self-regulate.

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