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Developmental Changes in the Responses of Preterm Infants to a Painful Stressor

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Abstract

The purpose of this investigation was to examine longitudinally gestational age and developmental differences in preterm infants' self-regulatory abilities in response to a painful stressor, as well as associations between behavioral and cardiovascular responses. Participants included 49 healthy premature infants. Behavioral and cardiovascular responses to a heel stick blood draw were compared between infants of 28–31 and 32–34 weeks gestational age at birth. Both gestational age groups displayed behavioral and cardiovascular indications of stress in response to the blood draw. However, both shortly after birth and several weeks later, infants born at younger gestational ages (28–31 weeks) were more physiologically reactive. Evidence that the behavioral stress responses of 28–31 weeks gestational age group preterm infants do not reflect their physiological responses suggests that evaluation of preterm infants' experiences and risk require assessments of both physiology and

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behavior. The greater stress vulnerability of the 28–31 relative to the 32–34 week gestation infants and the implications of this for subsequent development are discussed.

Keywords

Stress; Prematurity; Self-regulation; Physiological reactivity

Medical and technological breakthroughs over the last few decades have increased the survival rates of infants born at the youngest viable gestational ages. Yet many of the procedures that are a necessary part of their post-natal care can be, by nature, painful and stressful. In a Canadian survey, Johnston, Collinger, Henderson, and Anand (1997) found that infants residing in a NICU had an average of two invasive procedures a day, with some infants having as many as eight. One common procedure is the heel stick blood draw (Barker & Rutter, 1995). Although at one point the prevailing belief was that preterm infants could not feel the pain of invasive medical procedures, more recent research has indicated that premature infants have the anatomic and brain structures necessary for nociception (Stevens, Johnston, & Grunau, 1995). The recognition that preterm infants experience pain has led to a proliferation of studies aimed at understanding the development of the pain system and pain responses. It is now clear that preterm infants display appropriate hormonal stress responses (Barker & Rutter, 1996), increases in heart rate to painful procedures (Craig, Whitfield, Grunau, Linton, & Hadjistavropoulos, 1993; Grunau, Linhares, Holsti, Oberlander, & Whitfield, 2004), and hypersensitivity to tissue damage (Fitzgerald, Millard, & McIntosh, 1989). In addition, research suggests that preterm infants are actually more sensitive to pain than their term counterparts, as structures in the central nervous system that prevent the spread of pain signals may be undeveloped (Stevens et al., 1995). Indeed, younger preterm infants have lower pain thresholds than their older preterm and term counterparts (Andrews & Fitzgerald, 1994).

An important question, therefore, is how preterm infants regulate responses to the painful stressors that they regularly encounter. Preterm infants have a less organized self-regulation system than term infants (Als, Duffy, & McAnulty, 1988), which may contribute to a less healthy stress response. A healthy stress response is one that is activated quickly in the face of a stressor to enable the organism to manage the challenge and is then deactivated after the stressor has passed. Preterm infants are overwhelmed more easily and, under extreme stress, may be unable to organize a response to the pain and stress (Als, Lester, Tronick, & Brazelton, 1982; Johnston, Franck, & Stremler, 1999). Rather than mounting an effective stress response, these infants may 'shut down' (Als, 1993; Als et al., 1994). Furthermore, preterm infants remain in a higher state of arousal after a stressor has passed as compared to full-term infants (Holsti, Grunau, Oberlander, & Whitfield, 2004) and take longer to recover from painful procedures (Craig et al., 1993).

A growing body of work suggests that pain and stress exposure early in infancy can have long-lasting negative consequences (Anand, Coskun, Thrivikraman, Nemeroff, & Plotsky, 1999; Anand, Grunau, & Oberlander, 1997; Bhutta et al., 2001; Grunau, Whitfield, & Petrie, 1998; Grunau, Whitfield, Petrie, & Fryer, 1994; Hack et al., 1995; Porter, Grunau, & Anand, 1999; Shanks, Larocque, & Meaney, 1995). There has long been particular concern that the nature of life in the NICU is stressful enough for the fragile systems of preterm infants that it may also do lasting damage manifested as health, sensory, and cognitive deficits (Gorski, 1991; Gorski, Huntington, & Lewkowicz, 1990; Gottfried, Hodgman, & Brown, 1984; Gottfried et al., 1981; Long, Lucey, & Philip, 1980; Lucey, 1977). Increasing self-regulation abilities appears to be an important avenue for improving the medical and neurodevelopmental outcomes of preterm infants and perhaps ameliorating some of these long-term consequences. Developmental care aimed at supporting infants' self-regulation leads to better health outcomes

(Als et al., 2003; Als et al., 1994). At the root of these interventions is training health-care providers to identify and distinguish between "stress" and "regulatory" behaviors, based on a scheme developed and validated by Als and colleagues (Als, 1984; Als et al., 1982). "Stress" behaviors, also called extension or avoidance behaviors, signal withdrawal, defense, and overload (Als, 1984) and have been linked with decreases in oxygen saturation levels (Peters, 2001). In contrast, "regulatory" behaviors, also called flexion or approach behaviors, signal appropriate self-soothing (Als, 1984). The identification of these behaviors is then used to formulate specific suggestions for how to increase each infant's self-regulatory competence (Als et al., 1994).

The success of these developmental programs emphasizes the importance of understanding stress and regulatory behaviors. However, much about these behaviors remains relatively unexplored. Research suggests that younger preterm infants display more stress behaviors than older preterms during non-invasive care procedures (Holsti, Grunau, Oberlander, & Whitfield, 2005b). However, it is unclear whether there is a link between gestational age at birth (GAB) and the display of stress and regulatory behaviors in response to a *painful stressor* such as a heel stick blood draw. In addition, although stress and regulatory behaviors are theoretically linked with physiological responses (Als, 1984), a link with physiological responses other than oxygen saturation has not yet been empirically established.

A third aspect of adaptive and maladaptive behavioral responses to stress that warrants exploration is how the differential experiences of developing *in utero* versus in the NICU are associated with the ability to manage responses to a stressor. Evidence from cross-sectional studies suggests that preterm infants not only have a greater difficulty regulating their responses to stressors as compared to term infants, but also that they may not ‘catch up’ with increasing post-conceptional age. Even at 42 weeks post-conception, infants born at the youngest GAB have the lowest stress thresholds and highest hypersensitivity (Als et al., 1988). Furthermore, medically healthy preterm and term infants of the same postconceptional age (PCA) differ significantly on measures of self-regulation (Duffy, Als, & McAnulty, 1990; Ferrari, Grosoli, Fontana, & Cavazzuti, 1983; Holsti, Grunau, Oberlander, & Whitfield, 2005a; Mouradian, Als, & Coster, 2000). However, evidence does suggest improvement with development. Over the eight-week period after birth, preterm infants become less likely to demonstrate facial flaccidity (Johnston, Stevens, Yang, & Horton, 1996), a behavioral pattern that is often argued to represent an inability to mount an appropriate pain response due to overload (Als, 1993; Als et al., 1994; Johnston et al., 1999). If facial flaccidity is in fact representative of overload, this suggests that preterm infants may become more capable of appropriate self-soothing as they age.

These findings suggest that early experiences may impact the development of the stress regulation system in ways that have lasting consequences for the preterm infant. However, a longitudinal examination of the specific behaviors that represent overload and self-soothing is necessary to determine the pattern of associations between PCA and self-regulatory competence. This is of particular importance in terms of the consequences of self-regulatory competence for physiological regulation and the ability of preterm infants to manage a challenge.

Based on this review, a need exists for a greater understanding of the vulnerability of preterm infants by longitudinally examining developmental differences in preterm infants’ abilities to self-regulate in response to stressors. In addition, there is a need for integrating assessments of behavior and physiology to gain a clearer picture of whether behavioral responses to stress reflect physiological responses, or whether both must be considered to fully understand when preterm infants are at risk. The goal of the present study was to advance understanding of preterm infants' self-regulation by addressing three questions: 1) How does GAB predict infants' physiological, overload, and self-soothing responses to a painful stressor? 2) Several weeks after birth, do infants of the same postconceptional age (PCA) but of differing GAB show similar or different behavioral and physiological responses to a painful stressor? Finally, 3) Are behavioral indications of stress related to physiological indicators of stress, and are they associated to the same degree for infants of different GAB?

Methods

Participants

Participants included 49 healthy premature infants; 21 were of a younger GAB (YGAB), born between 28 and 31 weeks' gestation (*M* = 29.81; *SD* = .98; 9 girls, 12 boys) and 28 were of an older GAB (OGAB), born between 32 and 34 weeks' gestation (*M* = 33.11; *SD* = .69; 15 girls, 13 boys). Participants were recruited from two large metropolitan hospitals with NICUs: University of Minnesota Children’s Hospital-Fairview University Medical Center and Hennepin County Medical Center, with 670 and 250 admissions per year to the NICU, respectively. Both verbal and written consent were obtained prior to each infant's enrollment in the study; consent was obtained from 90% of parents solicited for participation.

Only medically stable, healthy infants born at the appropriate weight for their GAB were included. Exclusion criteria were chromosomal or other genetic anomalies (e.g., trisomy 21), congenital infections, chronic lung disease, mechanical ventilation over 24 hours, intraventricular hemorrhage, neonatal illness (e.g., sepsis), maternal history of adrenal illness or endocrine problems (e.g., diabetes mellitus), major maternal illness, and maternal substance use during pregnancy (e.g., alcohol). These exclusion criteria were designed to ensure that the resulting sample was comprised of relatively healthy infants born to mothers free of major health complications.

Procedures

The first assessment occurred between postnatal days three and five. At this assessment (t1), the YGAB infants were between 28 and 31 weeks’ PCA and the older preterm infants were between 32 and 34 weeks’ PCA. Fourteen of the YGAB (7 girls, 7 boys) were assessed a second time three to five weeks after birth (t2) when they were between 32 and 34 weeks PCA (*M* = 33.54; *SD* = .81), allowing for a comparison of the two groups matched for PCA. The second assessment occurred between postnatal days 21 and 35. Seven of the YGAB infants could not be assessed at time two because they were transferred to a different hospital or were no longer receiving heel stick blood draws as part of their postnatal care. The older preterm infants were discharged from the hospital before the second assessment and thus were only assessed at time one.

While in the NICU, infants were kept on a 3-hour feeding schedule. Each assessment period began one hour after the feeding that occurred between 0400 and 0700 hours. To ensure that they were in a baseline state prior to the manipulation, infants were observed continuously during the hour prior to the blood draw. This was done to ensure that infants were not handled and were in either quiet or active sleep prior to the manipulation. There were no differences in behavioral state during this one-hour period based upon GAB [$\chi 2(3) = 2.18$, $p = .54$]. Baseline behavioral and physiologic measures were taken at the end of this hour; thus, baseline began two hours after the infant's last feeding and after the infant was observed to be sleeping for an hour. During the five-minute baseline period, the heel stick blood draw, and the five-minute recovery period, heart rate was collected and the infant was videotaped for behavioral observations. Following hospital protocol, infants were not handled after the blood draw. Six of the older preterm infants were missing behavioral data due to technical difficulties such as videotape failure or a shortage of video cameras; there were no significant heart rate differences between those missing and not missing behavioral data, therefore, they were included in the analyses.

Measures

Physiological Responses—Throughout infants' stay in the NICU heart rate was continuously monitored using either a Space Labs (Space Labs Medical Inc., Redmond, WA) or an Air Shields (Draeger Medical Systems, Telford, PA) monitor. However, it was not possible to retain continuous heart rate data from these monitors. Therefore, for the purposes of this study, heart rate was recorded at 30-second intervals during a five-minute resting baseline just prior to the heel stick blood draw, during the blood draw, and during a five-minute recovery period. Mean heart rate was calculated for each of the 3-periods (see Table 1).

Behavioral Responses—Two types of behavioral responses were coded based on the work of Als (1984): stress/extension and regulatory/flexion. Extension behaviors signal withdrawal, avoidance defense, and overload in response to a stressor and include spitting up, gagging, hiccoughing, grunting or straining, grimacing, truncal arching, finger splaying, airplaning (infants' arms are extended out to the side at shoulder level), saluting (infants' arms are fully extended into midair), sitting on air (infants' legs are extended into midair), sneezing, yawning, sighing, coughing, averting eyes, and frowning. Flexion behaviors signal appropriate self-soothing reactions to a stressor and include extending the tongue, placing hands on face, making sounds (often undifferentiated or whimper-like), clasping hands, clasping feet, folding fingers, tucking, adjusting the body into a more flexed position, placing hands over the mouth, grasping, bracing legs or feet, mouthing, suck searching, sucking hands or fingers, hand holding, making an 'ooh' face, locking visually and/or auditorially, and cooing. During the baseline, event (heel stick blood draw), and recovery periods, the number of 10-second epochs in which each of the specific behaviors occurred was recorded. The frequencies of the behaviors in each category were then summed and divided by the number of epochs in that period (see Table 1 for the display of these behaviors during each period).

Two independent raters who were blind to study group coded these behaviors from videotape. Raters were trained using videotapes of infants who were not part of the current sample; coders achieved at least 85% agreement prior to coding of study tapes. Raters were not involved in data collection, and were blind to the infants' medical histories. Fifteen-percent of the tapes were selected at random for reliability coding. Reliability was calculated based on agreement of occurrence of each specific behavior during each epoch. Percent agreement over all epochs was calculated for each behavior and then averaged across extension and flexion behaviors. Percent agreement between the two coders was over 90% for extension behaviors for all reliability tapes and over 85% for flexion behaviors.

Clinical characteristics and potential confounding variables

Medical history was obtained through chart review. None of the infants had experienced any significant medical events (e.g., apnea spell) in the last 24 hours. There were no significant differences at the first assessment between the YGABt1 and OGAB infants in the number of previous heel stick blood draws [$t(46) = -1.26$, $p = .21$]. However, as expected based on their longer history of NICU experience, by the time two assessment, YGA infants had undergone significantly more blood draws than at time 1 [$t(11) = 5.34$, $p < .001$]. In addition, at the time to assessment YGAB infants had significantly more heel stick blood draws than the OGAB infants [$t(15.03) = 3.64$, $p = .002$]. See Table 2 for additional clinical characteristics of the sample. At each time point, the duration of each blood draw was also recorded. The blood draws ranged from two minutes to 14 minutes. There were no significant differences at time one in the length of each blood draw based on GAB [$t(47) = .67$, $p = .51$; YGABt1, $M = 3.99$ min., $SD = 3.36$; OGAB, $M = 3.48$, $SD = 1.21$]. However, the heel stick blood draws of the

YGABt2 infants (*Median* = 5.75, *SD* = 3.24) were longer than those of their older counterparts [*t*(40) = 4.27, *p*< .001] and their time one blood draws [*t*(13) = −2.30) *p* = .04]. All analyses, therefore, control for the number of prior and duration of the heel stick blood draws.

Data Analysis

Generalized Estimating Equations (GEE) models were used to analyze the data. GEE models are a regression-based, non-parametric and appropriate approach to examine repeated measures (Ballinger, 2004; Liang & Zeger, 1986; Zeger, Liang, & Albert, 1988; Zorn, 2001). GEE models produce more efficient and unbiased estimates when data are correlated than ANOVA-based models (Liang & Zeger, 1986; Zeger & Liang, 1986).

Heart rate, extension, and flexion behaviors were the dependent variables in the first set of analyses. GAB and period (event, recovery) were the primary independent variables. Interactions between GAB and period were then examined. As mentioned previously, the number of prior heel stick blood draws and the duration of the blood draws were included as control variables. In addition, sex was also included as a control variable because of the theoretical and empirical indications that sex is associated with behavioral and physiological responses to pain (Grunau & Craig, 1987; Guinsburg et al., 1999, Holsti et al., 2005a; Morison et al., 2003). Finally, baseline levels of heart rate, extension, or flexion behaviors were included as control variables for the heart rate, extension, and flexion analyses, respectively.

A second set of analyses, also using GEE models, was conducted to examine the associations between behavioral and physiological responses. Heart rate was the dependent variable; extension and flexion behaviors were the independent variables, and the previously mentioned controls were also included. Interactions between GAB and extension behaviors, as well as GAB and flexion behaviors, were then sequentially tested.

Results

How do GAB and PCA predict infants' physiological and behavioral responses to a painful stressor?

Heart Rate

Baseline levels: As expected, the baseline heart rate of the YGABt1 infants was significantly higher than the heart rate of the OGAB preterms [*t*(47) = 2.11, *p* = .04; see Table 1]. In addition, the baseline heart rate levels of the YGABt2 infants remained significantly higher than the heart rate levels of the OGAB infants [*F*(1,40) = 12.74, *p* = .001] and were not significantly different from their own baseline heart rate levels at time one [*F*(1,33) = 2.91, *p* = .10].

Responses to the heel stick blood draw: Controlling for baseline heart rate levels (as well as the additional control variables discussed previously), heart rate levels were significantly higher during the blood draw than during the recovery (at both t1 and t2, for both GAB groups). In addition, YGABt1 infants had significantly higher heart rate levels throughout the blood draw and recovery periods as compared to the OGAB infants, suggesting that they were more physiologically reactive to this stressor (see Table 3). This same pattern was evident at trend-levels at time two, when the YGAB infants were the same PCA as the OGAB preterms at time one. This suggests that YGABt2 infants still had greater heart rate responses to the painful stressor than OGAB infants. Furthermore, there were no significant differences in heart rate responses between YGABt1 and YGABt2 infants, suggesting that, regardless of PCA, YGAB infants display similar physiological responses to the heel stick blood draw.

Examination of the interactions revealed one trend-level interaction between GAB grouping and period of the blood draw, for the comparison of YGABt1 and OGAB infants. Heart rate levels decreased from the event to the recovery for both YGABt1 and OGAB infants; however, there was a greater decrease in heart rate for the OGAB infants. Planned follow-up tests revealed that although the heart rate levels of the YGABt1 infants decreased significantly from the event to the recovery [*t*s < −2.75, *p*s < .018], their heart rate levels remained significantly higher than during baseline [*t*s > 2.42, *p*s < .031]. In contrast, the heart rate levels of the OGAB infants decreased significantly from the event to the recovery [*t*(27) = −5.12, *p* < .001] to a level that was not significantly different from heart rate during baseline [*t*(27) = .25, *p* = .81].

Behavioral Responses

Baseline levels: There were no significant baseline differences between YGABt1, OGAB, or YGABt2 preterm infants in the mean number of extension or flexion behaviors displayed (all *t*s < .1.57, all *p*s > .20; see Table 1).

Responses to the heel stick blood draw: Across comparisons, and adjusting for control variables, infants displayed more extension/stress behaviors during the blood draw than during the recovery period. There were no such differences in flexion/self-soothing behaviors. In addition, GAB grouping was not significantly associated with displays of extension or flexion behaviors (see Table 4).

Examination of the interactions revealed that GAB grouping and period (event, recovery) of the blood draw interacted to predict flexion behaviors; the interaction was significant for the comparison of YGABt2 and OGAB infants and reached trend-levels for the YGABt1 and YGABt2 comparison. YGABt1, YGABt2 and OGAB infants all displayed similar levels of flexion behaviors during the event. In addition, YGABt1 and OGAB infants displayed similar levels of flexion behaviors in both the event and the recovery; planned follow-up tests revealed that, for both YGABt1 and OGAB infants, displays of flexion during the event and recovery were not significantly different (*t*s < .78, *p*s >.44). However, displays of these self-soothing behaviors decreased significantly during the recovery for YGABt2 infants [t(11) = 3.04, *p* = .011].

Are behavioral indications of stress related to physiological indicators of stress, and are they associated to the same degree to infants of different GAB?

To examine the association between the physiological and behavioral responses to the heel stick blood draw, extension and flexion behaviors were included as independent variables in a GEE predicting heart rate responses. For all comparisons there was a main effect of extension behaviors, such that greater displays of these stress behaviors were associated with higher heart rate levels. Flexion behaviors and heart rate were never significantly associated (see Table 3). In addition, for comparisons of both of the YGAB groups to the OGAB infants, there was a significant interaction between GAB grouping and extension behaviors. Heart rate levels of the YGAB infants were similar regardless of their display of extension behaviors; however, for OGAB infants, heart rate levels were highest when they were demonstrating many behavioral indications of stress and lowest when they also were demonstrating few stress behaviors, suggesting a stronger association between physiological and behavioral responses for the OGAB infants than for the YGAB infants at both time points.

Discussion

The first goal of the present investigation was to longitudinally examine developmental differences in preterm infants' ability to self-regulate in response to stressors to gain a better understanding of the vulnerability of preterm infants to stress exposure. The second goal was to integrate assessments of both physiology and behavior to examine whether behavioral responses reflect physiological responses, and whether the association between behavioral and physiological responses differs by GAB. The results of this study indicate both GAB and developmental differences in the self-regulatory and physiological responses of preterm infants to a painful stressor. All of the preterm infants displayed both physiological and behavioral indications of stress in response to the heel stick blood draw. However, preterm infants of a younger GAB (28–31 weeks, YGAB) were more physiologically reactive to the blood draw than infants of an older GAB (32–34 weeks; OGAB), suggesting that younger GAB preterm infants may be more vulnerable to stress than older GAB preterm infants. Furthermore, associations between behavioral and physiological stress responses differed depending on GAB. Although OGAB infants displayed a positive association between extension behaviors and heart rate levels, there was no association between the physiological and behavioral responses of the YGAB infants. This suggests that integrating measurements of both behavior and physiology will allow for a better understanding of when younger preterm infants are at risk.

Compared to OGAB preterm infants, YGABt1 and YGABt2 preterm infants had greater heart rate increases in response to the heel stick blood draw; this elevation continued into the recovery period. This pattern was evident at both assessment points, during postnatal days three to five and again during postnatal weeks three to five. This suggests that YGAB infants remain more physiologically reactive to a painful stressor several weeks after birth, even when assessed at the same PCA as OGAB infants. These results are consistent with previous indications that physiological responses to pain remain similar over the eight weeks after birth (Johnston et al., 1996), and suggest that the physiological stress responses of young GAB infants do not mature over this period of time. Furthermore, although the heart rate levels of the OGAB infants returned to baseline during the five-minute recovery period, the heart rate levels of the YGAB infants remained significantly elevated. Taken together, these results suggest that, shortly after birth, younger preterm infants are less capable than older preterm infants of regulating their physiological responses to a stressor. Thus, this research provides evidence that the less developed self-regulatory abilities of infants born at younger GAs may render them more vulnerable to early postnatal stressors. These infants may lack the control to organize a competent response to pain and stress (Als et al., 1982; Johnston et al., 1999), leading to physiological patterns that indicate high-levels of arousal and overload well after the end of the stressor. Prolonged physiological arousal beyond the end of a stressor may contribute to the behavioral and physiologic sensitization to repeated pain and stress that is often demonstrated by younger preterms (Andrews & Fitzgerald, 1994; Fitzgerald et al., 1989; Holsti et al., 2005a; Morison et al, 2003; Grunau, Oberlander, Whitfield, & Fitzgerald, 2001; Storm, 2000).

Previous research suggests that infants of older and younger GAB differ in their behavioral responses to pain, as measured by facial pain responses (e.g., Goubet, Clifton, & Shah, 2001; Johnston, Stevens, Craig, & Grunau, 1993; Johnston et al., 1999), and that, with increasing PCA, preterm infants respond with increasing robustness to a painful stressor (Johnston et al., 1996). However, the results of the current study suggest that GAB is not associated with either behavioral stress or self-regulatory responses to the heel stick blood draw. In response to the blood draw, preterm infants across GAB and PCA groups increased displays of stress behaviors; in contrast, no such increase in self-soothing behaviors was demonstrated. However, there were indications that there were changes in the behavioral responses of the YGAB infants when observed three to five weeks after birth, suggesting a possible effect of their NICU experience on their behavioral stress responses. Although all infants displayed similar levels of self-soothing behaviors during the blood draw, only YGABt2 infants decreased their self-soothing behaviors during the recovery period. This suggests that preterm infants who remain for longer periods in the NICU are less capable of behavioral self-soothing.

Taken together, the results indicate that the experience of developing in a NICU (instead of *in utero*) may have lasting consequences for stress regulation. Infants born at an earlier GA display less well-regulated stress responses, even after controlling for PCA. Specifically, these infants are not able to deactivate their physiological response after a stressor has passed; again, this continued activation and overload may lead to negative outcomes for preterm infants' in the long-term. As a whole, these results indicate that YGAB and OGAB infants display similar behavioral responses to pain, yet YGAB infants display more immature regulation of their physiological responses. This suggests the possibility that younger infants are less *effective* self-regulators than older preterms in that these behaviors do not lead to a reduction in physiological arousal.

In support of this idea, the results of the current study suggest that behavioral and physiological responses to pain are not associated in the same way for OGAB and YGAB infants. This study is the first to directly examine the link between cardiovascular responses and the behaviors identified by Als and colleagues as representing overload and self-soothing. The expected positive association between cardiovascular and behavioral responses was demonstrated by the OGAB infants, such that the infants who had the highest physiological arousal were also displaying the most behavioral indications of stress; this indicates that the behavioral responses of older preterm infants reflect their physiologic responses. However, the physiological stress responses of the YGAB infants were not reflected in their behavioral responses, as these infants showed similar levels of stress behaviors regardless of their heart rate levels. These results underscore that a reliance on measuring behavioral responses is insufficient, and needs to be balanced by a consideration of physiological responses to more fully understand when preterm infants are at risk because they have difficulty regulating stress (e.g., Morison, Grunau, Oberlander, & Whitfield, 2001). Furthermore, these findings have particularly meaningful implications for developmental care programs aimed at improving self-regulatory competence by tailoring support based on infants' displays of stress/extension and regulatory/flexion behaviors (e.g., Als, 1984, Als et al., 1982). Although younger infants may be less likely to be identified as being overloaded by stressors, they may still be vulnerable to the long-term consequences of chronic arousal and reactivity (e.g., Epel et al., 2006; Silberman, Wald, & Genaro, 2003). Furthermore, these results suggest that the utility of using extension and flexion behaviors as indicators of physiological reactivity and vulnerability differs based on GAB.

An additional implication of these findings is that they do not support an association between flexion behaviors and a more rapid physiological recovery. Peters (2001) found that flexion behaviors were associated physiological recovery, as measured by oxygen saturation levels. This raises the possibility that flexion behaviors are distinctly related to different aspects of physiological arousal and recovery. It is important to determine the behavioral responses to pain that reflect more adaptive cardiovascular responses. Clearly, based on the results of this study, it remains equivocal whether improving preterm infants' abilities to self-regulate and soothe in response to a stressor may ameliorate the long-term consequences of the pain and stress that they are exposed to as part of NICU care (Bhutta et al., 2001; Grunau et al., 1994; Grunau et al., 1998; Hack et al., 1995; Porter et al., 1999; Shanks et al., 1995).

Although evidence of this less adaptive behavioral pattern has important implications for researchers and care providers working with preterm infants, the cause of this pattern remains unclear. Experiences in the NICU, including painful stressors like heel stick blood draws and overstimulating visual and auditory stimuli (e.g., Gorski, 1991; Gottfried et al., 1981; Long et al., 1980), may lead to this less adaptive behavioral pattern. It is also possible, however, that infants who are less capable of self-soothing are more likely to remain in the NICU for extended periods of time. In addition, it is possible that the less mature behavioral responses displayed by the YGAB infants resulted from prenatal experiences that affect stress and behavioral regulation such as maternal stress (Davis, Glynn, Dunkel Schetter, Hobel, Chicz-Demet, &

Sandman, 2007; De Weert, Van Hees, & Buitelaar, 2003) or treatment with synthetic glucocorticoids (Davis, Townsend, & Gunnar et al., 2006).

Limitations and Future Directions

Although illuminative, the present study is not without limitations. As with much work in this area, our findings are based on a naturalistic design. A more controlled administration of the painful stressor may have been theoretically preferable, but ethical concerns preclude the use of a more methodologically rigorous design. Similarly, it would have been preferable to study the infants over a longer time-span. However, we were only able to study the infants while they were being treated in the NICU. Although following preterm infants for a longer period of time would allow us a better understanding of how their stress responses continue to develop, it is ethically undesirable to expose these already vulnerable infants to additional discomfort that is not medically necessary after their release from the NICU. Of particular importance for future research, therefore, is further investigation of how self-regulatory behaviors are associated with physiological responses to stress, and whether they play a role in buffering the risks of early, repetitive stress exposure. In addition, it would be desirable to have a continuous measure of heart rate, along with measures of oxygen saturation and respiratory rate to get a broader picture of physiological responses to stressors. It is meaningful that differences based on GAB were found using a less nuanced measure; however, future studies should utilize more continuous and varied measures of physiological responses.

Conclusions

The aim of this research was to investigate longitudinally the previously unexplored links between GAB and stress, regulatory, and physiological responses to a painful stressor in order to more fully understand the vulnerability of preterm infants to stress, as well as the associations between behavioral and physiological stress responses and GAB. The findings of this research indicate that younger preterm infants are less able to physiologically regulate their responses to a painful stressor than older preterm infants, a pattern that remains consistent over the first several weeks after birth. This study also provides evidence that associations between behavioral and physiological responses to pain differ based on GAB, suggesting that younger preterms may be less effective at regulating their physiological responses to stressors even though they display behavioral cues of self-soothing. Therefore, they may continue to be vulnerable to the consequences of pain and chronic reactivity even though they are not displaying behaviors that signal overload. Taken together, these results suggest that younger preterm infants are more vulnerable to stress than older preterm infants and that future developmental care programs and evaluations of preterm infants' experiences and risk would benefit from a simultaneous consideration of behavioral and physiological responses.

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Table 1

Descriptive Statistics: Heart Rate and Display of Extension and Flexion Behaviors During Baseline, Blood Draw, and Recovery Periods

	YGABt1		**OGAB**		**YGABt2**	
	M	***SD***	***M***	***SD***	***M***	***SD***
Heart Rate:						
Baseline	151.78	12.07	144.04	13.16	158.62	10.91
Event	169.27	10.89	158.18	15.19	175.94	12.66
Recovery	161.21	14.35	144.69	13.94	166.30	11.88
Extension/stress Behaviors						
Baseline	.67	1.39	1.74	3.99	2.50	5.98
Event	8.43	10.33	8.39	6.32	15.21	16.24
Recovery	5.29	7.04	2.74	4.03	5.14	8.41
Flexion/regulatory Behaviors						
Baseline	3.81	5.94	2.78	4.43	2.79	3.33
Event	4.48	9.31	4.74	3.02	6.29	6.91
Recovery	3.76	4.52	5.78	6.13	2.79	2.67

Table 2

The Clinical Characteristics of the Study Sample[a]

	YGAB Preterm Infants		OGAB Preterm Infants		*p*-Value
	M	***SD***	***M***	***SD***	
Gestational Age (wks)	30	.98	33	.69	<.001*
Method of Delivery	31% Vaginal 69% C-Section		48% Vaginal 52% C-Section		.41
1 min Apgar	7	1.32	7	1.81	.52
5 min Apgar	8	.60	8	.79	.97
Birth weight (grams)	1456	252	1928	300	<.001*
Birth length (cm)	38.53	4.30	43.96	2.35	<.001*
Head circum. (cm)	29.30	4.95	30.69	1.35	.17
Mother's Age	29.05	5.87	28.64	5.28	.80

[a] Comparisons were made using independent sample *t*-tests, except for method of delivery. Comparisons for method of delivery were made using Pearson chi-square.

Table 3

Heart Rate During the Blood Draw and Recovery Period

	YGABt1 vs. OGAB		YGABt2 vs. OGAB		YGABt1 vs. YGABt2	
	Estimate(SE)	**95% CI**	**Estimate(SE)**	**95% CI**	**Estimate(SE)**	**95% CI**
Heart Rate						
Step 1: Main Effects						
Prior blood draws	−.44(.40)	1.22, .35	.05(.29)	−.52, .61	.25(.22)	−.18, .68
Baseline heart rate	.74(.10)***	.54, .93	.77(.11)***	.56, .97	.67(.12)***	.44, .89
Length of blood draw	−.06(.09)	−.24, .12	.10(.10)	−.09, .28	.02(.07)	−.12, .15
Infant sex (0 = Male)	−2.49(2.51)	−7.40, 2.43	−7.31(2.59)**	−12.37, −2.24	−3.33(2.65)	−8.53, 1.86
Period (0=Event)	−11.04(1.64)***	−14.25, −7.83	−11.72(1.97)***	−15.57, −7.87	−8.51(1.75)***	−11.94, −5.07
GA group (0=YGAB)^{a}	−8.22(2.53)***	−13.18, −3.26	−7.35(4.14)$^{+}$	−15.47, .78	−1.71(3.53)	−8.64, 5.21
Step 2: Interactions						
GA × Period	−5.83(3.16)$^{+}$	−12.01, .36	−3.70(3.91)	−11.36, 3.96	−2.89(3.60)	−9.94, 4.17
Step 3: Associations with Behavioral Data^{b}						
Extension	.43(.15)**	.14, .72	.36(.13)**	.11, .62.	.27(.11)*	.06, .48
Flexion	.03(.21)	−.38, .44	−.09(.24)	−56, .38.	.08(.21)	−.33, .50
Step 4: Interactions with Behavioral Data						
GA × Extension	.60(.27)*	.07, 1.13	.57(.27)*	.05, 1.09	.05(.19)	−.32, .41
GA×Flexion	.19(.39)	−.57, .94	.46(.51)	−.54, 1.45	.10(.38)	−.65, .84

$^{+}$ $p < .10$

* $p < .05$

** $p < .01$

*** $p < .001$

^{a}For the YGABt1 vs. YGABt2 comparisons, 0 = Time 1 and 1 = Time 2.

[b] Controlling for all variables included in Step 1.

Table 4

Displays of Behavioral Responses During the Blood Draw and Recovery Period

	YGABt1 vs. OGAB		YGABt2 vs. OGAB		YGABt1 vs. YGABt2	
	Estimate(SE)	95% CI	Estimate(SE)	95% CI	Estimate(SE)	95% CI
Extension/Stress Behaviors						
Step 1: Main Effects						
Prior blood draws	−.05(.25)	−.53, .44	−.37(.21)$^{+}$	−.78, .03	.02(.23)	−.42, .47
Baseline extension	.51(.25)*	.02, 1.00	.15(.18)	−.21, .51	.26(.28)	−.29, .80
Length of blood draw	.18(.06)***	.07, .30	.23(.07)***	.10, .37	.23(.06)***	.12, .34
Infant sex (0 = Male)	−1.93(1.54)	−4.95, 1.09	2.02(1.87)	−1.65, 5.68	−2.59(2.26)	−7.02, 1.84
Period (0 = Event)	−4.37(1.31)***	−6.94, −1.80	−7.53(2.34)***	12.11, −2.95	−5.91(2.68)*	−11.15, −.66
GA group (0 = YGAB)^{a}	−1.34(1.56)	−4.39, 1.71	−3.12(2.82)	−8.66, 2.41	−.62(3.23)	−6.95, 5.72
Step 2: Interactions						
GA × Period	−2.40(2.60)	−7.50, 2.69	4.52(4.64)	−4.58, 13.61	−7.68(5.29)	−18.06, 2.69
Flexion/Self-soothing Behaviors						
Step 1: Main Effects						
Prior blood draws	.31(.19)	−.06, .68	−.24(.13)$^{+}$	−.49, .01	−.03(.13)	−.28, .23
Baseline flexion	−.03(.11)	−.25, .19	.02(.14)	−.25, .29	.05(.14)	−.22, .33
Length of blood draw	.27(.04)***	.18, .35	.13(.04)**	.04, .21	.20(.04)***	.13, .27
Infant sex (0 = Male)	1.53(1.15)	−.72, 3.78	−.09(1.17)	−2.37, 2.19	.64(1.44)	−2.17, 3.46
Period (0 = Event)	−.14(.90)	−1.91, 1.63	−.31(1.24)	−2.76, 2.14	−1.73(1.25)	−4.18, .73
GA group (0 = YGAB)^{a}	1.41(1.15)	−.85, 3.66	1.15(1.76)	−2.29, 4.59	−1.94(1.93)	−5.73, 1.85
Step 2: Interactions						
GA × Period	−1.12(1.80)	−2.40, 4.65	4.54(2.18)*	.28, 8.81	−4.07(2.47)$^{+}$	−8.92, .78

$^{+}p < .10$

$^{*}p < .05$

$^{**}p < .01$

[***] $p < .001$

[a] For the YGABt1 vs. YGABt2 c006Fmparisons, 0 = Time 1 and 1 = Time 2.