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# Sensitization of Cardiac Responses to Pain in Preterm Infants

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## Key Words

Heelstick · Preterm heart rate · Pain

## Abstract

**Background:** Preterm infants are repeatedly exposed to painful experiences as part of their care in the neonatal intensive care unit. There is evidence from both animal and human studies that exposure to pain during the neonatal period may have persisting consequences for development. **Objective:** To perform serial assessments of three heelstick blood draws to examine early changes both in physiological and behavioral responses to repeated exposure to painful stimuli in preterm infants. **Methods:** Heart rate and behavioral responses to three serially administered heelstick blood draws were evaluated in 22 medically stable preterm infants with less than 48 h of mechanical ventilation who were admitted to the neonatal intensive care unit. **Results:** Heart rate and behavioral agitation significantly increased during each heelstick as compared to baseline. The heart rate response was larger to the third heelstick as compared to the first two procedures. Behavioral responses did not change across the three assessments. **Conclusions:** Healthy preterm infants sensitize to heelstick-induced pain, as measured by their heart rate responses. These data suggest that greater attention to the effects of repeated pain for the neonate is needed.

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Increasing evidence indicates that experiences early in life have consequences for health that persist through adulthood [1]. Because of the rapid developmental changes that occur during the gestational period, the fetus is vulnerable to both organizing and disorganizing influences. The developmental trajectory of the preterm infant is uniquely challenged during this vulnerable period because of exposure to stimulation outside of the protected environment of the uterus. Many preterm infants are exposed to numerous painful procedures as part of their medical care. Exposure to these procedures during this vulnerable period of rapid central nervous system development may have lasting implications for the development of the preterm infant [2]. Chronic exposure to early painful experiences may contribute to the high rate of adverse developmental outcomes among individuals born prematurely, including impairments in learning, behavior regulation and motor development [3, 4].

Results from animal studies provide significant evidence that the consequences of exposure to early pain both are widespread and persist to adulthood. In rodents, exposure to neonatal pain results in long-term hyperinnervation of injured areas [5, 6], and alterations to sensory pathways [7], pain thresholds [8, 9], and behavior [10]. Furthermore, it has been shown that the timing of exposure, the extent of the injury, and the presence of re-injury have an impact on the consequences of exposure to neonatal pain [11].

In human preterm infants, exposure to painful procedures alters behavioral and cardiac responses to subsequent painful procedures. Fitzgerald et al. [12] demonstrated that repeated exposure to heelstick procedures resulted in hypersensitivity to the von Frey hair stimulation test. Several researchers have shown that a history of previous pain exposure predicted dampened behavioral responses, but had inconsistent effects on physiological responses to a target painful procedure [13–15]. These patterns, however, appear to be influenced by gestational age, maturation, health status, and frequency and type of painful procedure [14–17].

Most of the studies that have examined the consequences of repeated exposure to painful procedures in preterm infants utilized a cross-sectional design. Only a few studies of early pain exposure systematically evaluated serial exposures to skin-breaking procedures in preterm infants [12, 15]. No published study of preterm infants has performed serial assessments to examine early changes both in physiological and behavioral responses to repeated exposure to painful stimuli in the early neonatal period. In this study, we report the behavioral and physiological effects of neonatal exposure to three serial heelsticks in medically stable preterm infants.

## Methods

### Subjects

The study sample comprised 22 infants (11 male, 11 female) admitted to a major regional tertiary neonatal intensive care unit (NICU) for prematurity. Written consent was obtained from a parent according to a protocol approved by the Institutional Review Board at the University of California, Irvine, Calif., USA. Infants were excluded who had congenital anomalies, major neonatal illness (e.g. sepsis), fever, intraventricular hemorrhage greater than grade I, 5-min Apgar score less than 7, mechanical ventilation for more than 48 h, surgery, or evidence of maternal substance use during pregnancy (e.g. alcohol). Three twin pairs were included. The pattern of results described below did not change when analyzed after randomly removing one twin from each pair. The subjects were born by vaginal delivery ( $n = 10$ ) or cesarean section ( $n = 12$ ). Mothers were  $28.4 \pm 9.2$  years of age, had a median parity of one, and 36% of them were Hispanic while 32% were non-Hispanic White. Infant characteristics are presented in table 1.

### Procedure

Infants were evaluated at three heelstick blood draws, chronologically labeled T1, T2, and T3. Postnatal age at each assessment and the number of previous skin-breaking procedures and the number of heelstick blood draws are summarized in table 2. Each assessment consisted of two phases. During the first 10-min phase, resting baseline heart rate and behavioral data were collected. Monitoring of the infant continued during the second

**Table 1.** Subject characteristics

	Mean (SD)	Range
Birth weight, g	1,948 (593)	1,210–3,194
Gestational age, weeks	32.8 (2.2)	28.7–36.1
Apgar score at 5 min	8.5 (0.8)	7–9
SNAP-II score	6.9 (7.7)	0–25

**Table 2.** Postnatal age and number of prior skin-breaking events at each assessment

	T1	T2	T3
Postnatal age, days	1.3 (1.5)	2.6 (3.1)	4.7 (4.5)
Prior skin-breaking events	5.1 (4.1)	8.1 (6.1)	12.0 (7.3)
Prior heelsticks	2.6 (2.5)	4.9 (3.6)	7.5 (4.6)

Data are presented as mean (SD).

phase, the heelstick procedure, which was performed by a neonatal nurse. The nurse cleaned the heel with an alcohol swab, applied an automated lancet, and then repeatedly squeezed the heel until a sufficient blood sample was obtained. Because the duration of the heelstick varied across infants, only the first 120 s of the heelstick event was used in all analyses. Thus, for the purposes of analysis, the heelstick event was standardized across all infants as to include the heel lance and the first 120 s of heel squeezing. Infants were not fed during the study visit and were not handled beyond the blood draw protocol unless medically necessary.

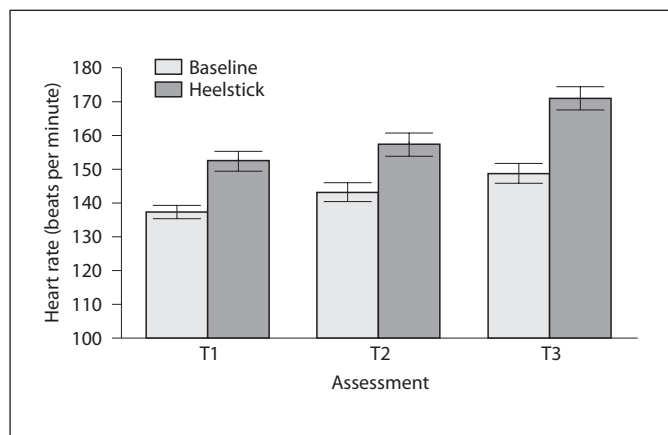
### Measures

#### Heart Rate

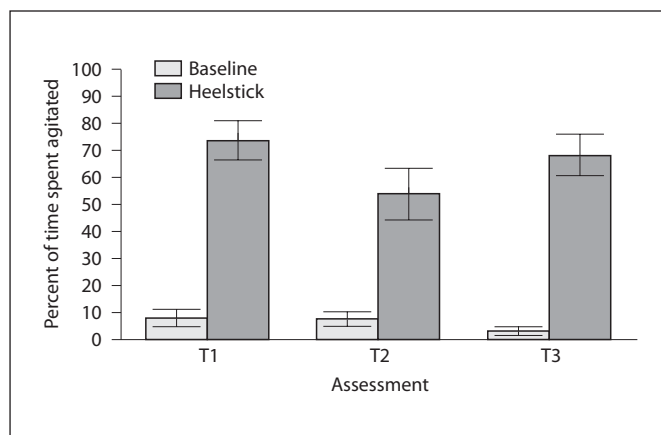
An Agilent/Philips (2001 or V26C – Neonatal, Böblingen, Germany) monitor was used to record infant heart rate. Heart rate was recorded continuously and retained for the purposes of this study at 20-second intervals during the resting baseline phase and the heelstick phases. Mean heart rate was calculated for each study phase. Additionally, baseline-corrected area under the curve (AUC) was calculated for the heelstick phase.

#### Behavioral State

Behavioral state was assessed using a reliable and validated coding scheme designed for preterm infants [18] and used previously by our group [19] and others [16]. This scheme was used to categorize infants' state into the following six categories: quiet sleep, active sleep, quiet awakeness/drowsy, awake and alert, fussy, and crying. Codes recorded represented the highest level of state or activity noted during each 20-second epoch. Coders were trained to 85% reliability prior to the start of the study. Percent agreement for behavioral state codes, obtained on 15% of the assessments, was 85.1%. For each infant, the percent of time spent in an agitated state



**Fig. 1.** Mean heart rate during the baseline and heelstick phases of each assessment. Data are presented as mean  $\pm$  SD.



**Fig. 2.** Percent of time spent in an agitated behavioral state during the baseline and heelstick phases of each assessment. Data are presented as mean  $\pm$  SD.

(fussing or crying) during each study phase was calculated and used in all subsequent analyses of behavioral state.

#### Background Characteristics

Medical history was obtained through review of the patients' medical records. Data collected include: birth characteristics (gestational age at delivery, birth weight, Apgar scores, delivery method), demographic data (gender, maternal race, maternal age), illness severity using the Score for Neonatal Acute Physiology II (SNAP-II) [20], medication, and postnatal experiences including number of heelsticks and total number of skin-breaking events. Skin-breaking events were defined as the sum of every skin-breaking procedure from birth. In this cohort, skin-breaking procedures included venipunctures, heelsticks, and the insertion of central and peripheral vascular lines.

#### Data Analysis

Heart rate and behavioral changes from baseline in response to the heelstick event were analyzed using paired-sample *t* tests. Next, repeated-measures ANOVAs were implemented with post hoc tests as needed to determine whether the heart rate (baseline-corrected AUC) or behavioral state (percent of time spent in agitated behavior) differed at baseline or in response to the heelstick manipulation across the three assessments. Finally, background variables were examined to determine whether they were correlated with either of the dependent measures.

## Results

### *Does the Heelstick Blood Draw Elicit an Increase in Heart Rate and Behavioral Agitation?*

We first examined whether the heelstick manipulation elicited physiological and behavioral changes at each of the three assessments implemented during the first post-

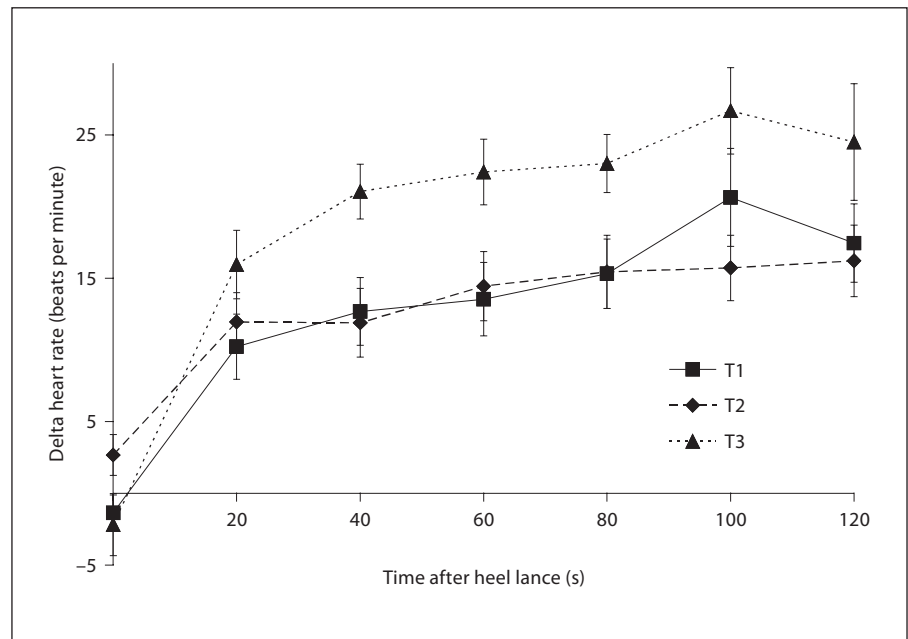
natal week. As shown in figure 1, the mean heart rate increased from baseline in response to the heelstick procedure at all three assessments ( $t = 7.0, 7.3, 10.3$  at T1, T2, and T3, respectively; all  $p$  values  $< 0.001$ ). Additionally, as illustrated in figure 2, infants displayed significantly more agitated behavior during the heelstick phase relative to baseline at all assessments ( $t = 9.0, 5.0, 8.6$  at T1, T2, and T3, respectively; all  $p$  values  $< 0.001$ ). A significant proportion of infants displayed agitated behavior during the entire 2-min heelstick phase (41%, 36% and 36% of subjects at T1, T2, and T3, respectively).

### *Does the Heart Rate Response to the Heelstick Procedure Change across Assessments?*

AUC was calculated for each of the three assessments to evaluate the heart rate response to heelstick. Because baseline heart rate differed among the three assessments ( $F_{2,20} = 5.5, p < 0.05$ ), baseline-corrected AUC was calculated. Repeated-measures ANOVA indicated that the heart rate response differed significantly across the three assessments ( $F_{2,20} = 4.7, p < 0.05$ ). As shown in figure 3, post hoc tests revealed that the heart rate response was greater at T3 than at T1 or T2 (all  $p$  values  $< 0.05$ ).

### *Does the Behavioral Response to the Heelstick Procedure Change across Assessments?*

By repeated-measures ANOVA, baseline behavior did not differ among assessments ( $F_{2,20} = 2.7, p = 0.1$ ; fig. 2). Furthermore, the amount of agitation in response to heelstick also did not change significantly across assessments ( $F_{2,20} = 1.1, p = 0.4$ ).



**Fig. 3.** Heart rate response to the heelstick blood draw at T1, T2, and T3. Data are presented as change scores from baseline.

### Background Variables

We next examined background characteristics of the infants that could have affected their heart rate or behavioral responses to the heelstick. Neither the number of skin-breaking procedures nor the number of heelsticks prior to assessment were associated with behavioral agitation or heart rate response to the heelstick (all  $p$  values  $> 0.1$ ). Previous total number of skin-breaking procedures, but not the number of heelsticks, was significantly associated with baseline heart rate at T1 and T3 (all  $p$  values  $< 0.05$ ) with a nonsignificant trend at T2 ( $p = 0.09$ ). Potential confounding variables such as postnatal age at assessment, gestational age at birth, birth weight, Apgar scores at 1 and 5 min, use of methylxanthine therapy ( $n = 4$ ), delivery method, gender, maternal race, maternal age, or SNAP-II score were not related to behavioral or heart rate responses at T1, T2, or T3 (all  $p$  values  $> 0.3$ ).

### Discussion

This study examined prospectively preterm infants' physiological and behavioral responses to serial painful events within a brief postnatal period. As expected, infants displayed increases in heart rate and behavioral agitation in response to the heelstick blood draw at all three assessments indicating that, as in previous studies, pre-

term infants continued to experience distress during this event [15, 19]. The primary aim of this project, however, was to examine the influence of repeated skin-breaking procedures on subsequent responses. We discovered that there was evidence for sensitization to repeated exposures to the heelstick procedure. Sensitization, enhancement of a response resulting from repeated exposure to a noxious procedure, was observed in measures of infant heart rate, but not in behavioral responses. Furthermore, this sensitization developed rapidly, as early as 25 h after birth. Importantly, gestational age at birth, birth weight, infant health, and postnatal age did not account for this effect. Increases in baseline heart rate across the measurement periods were observed, possibly reflecting a state of chronic arousal due to the NICU experience [13]. In support of this explanation, the number of previous painful experiences was correlated with baseline heart rate. Importantly, sensitization in the heart rate response was seen after controlling for baseline differences.

These findings of sensitization are conceptually consistent with prospective studies that examined changes in response patterns with subsequent exposures to painful events. Fitzgerald and colleagues [21, 22] have shown that preterm infants' withdrawal response becomes sensitized with repeated exposure to stimulation. A study utilizing the von Frey hair stimulation test determined that the infants' experience of pain was necessary for sensitization to the heelstick procedure. They found that repeated

exposure to the heelstick procedure lowered the pain threshold in preterm infants. Infants receiving analgesics during the heelstick procedure, however, displayed no significant change in pain threshold in response to subsequent stimulation [12]. These findings support the role of pain in sensitization to the heelstick procedure. Gunnar et al. [23] found physiological evidence for sensitization (a greater cortisol response) to the second of two heelsticks in term infants, providing further evidence for the consequences of repeated exposures to painful procedures. In preterm infants, Johnston et al. [15] reported sensitization for behavior, but not physiological responses. Several procedural factors may account for the differences between the findings by Johnston et al. and the current study. Johnston et al. [15] assessed preterm infants four times over an 8-week period and employed a precise behavioral measure, but a less precise measure of heart rate responses. Despite the differences among studies, converging evidence indicates that the preterm infant displays a decreased pain threshold with repeated pain exposure. The unique finding of the current study is that this sensitization develops rapidly.

The present results, in conjunction with other findings [12, 21], indicate that preterm infants experience in-

creased pain in response to repeated exposures to skin-breaking procedures. There are at least two central implications of these observations. First, these data suggest that infants react to the painful events they are exposed to as part of their NICU care, and that their pain responses increase with repeated events. Second, these data suggest that the organization of the infant nervous system is responding and possibly changing with repeated exposure to these painful procedures. There is substantial evidence that early experiences have persisting or programming effects on development [24]. Results from animal studies show both long-term changes in behavior and in the structure of the nervous system [7, 9]. It is probable that painful experiences in the NICU have persisting effects on the nervous system. This may be a contributing factor to the long-term developmental difficulties noted in this population [25].

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