

ORIGINAL ARTICLE

Mode of delivery modulates physiological and behavioral responses to neonatal pain

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Objective: To study whether the mode of delivery alters pain expression.

Study Design: Full-term infants born by vaginal delivery or elective caesarean section were observed following high- and low-intensity pain stimuli, with recording of electrocardiogram, facial expression and vocalization.

Result: Graded physiological and behavioral responses occurred, with greater responses to higher than lower intensity pain stimuli. Elevation in heart rate following both stimuli increased with time after vaginal delivery. Infants delivered by elective caesarean section showed stronger facial expressions and briefer time in vocalizations response to both interventions.

Conclusion: Diminished responses following vaginal delivery suggest that physiologic events associated with a normal delivery reduce the physiologic and sympathoadrenal activation by nociceptive mechanisms. Pain and stress reactivity appear to be inhibited during fetal life and sensory inputs during vaginal delivery may reverse this inhibition. To minimize neonatal pain, we recommend that postnatal invasive procedures to be performed shortly after vaginal birth.

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Introduction

Normal vaginal delivery (VD) in humans involves intense squeezing of the fetal head, with intermittent pressure as high as 2 kg cm^{-2} for several hours.¹ Although application of similar pressure to an adult would probably cause severe distress and pain, this situation appears

to be well tolerated by the fetus and may even facilitate the transition from intrauterine to extrauterine environments.² We propose that the fetus exhibits a high pain threshold during delivery (fetal inhibition) and that this threshold decreases significantly within hours after spontaneous vaginal birth.

If VD itself inhibits response to pain perceived by the fetus, then the response to pain in infants delivered by elective Caesarean section (CS) should be more pronounced first hours after birth. This hypothesis receives support from a number of studies demonstrating significantly higher plasma levels of catecholamine (CA)³ and β -endorphin in infants born by VD than by CS.^{4–6}

Historically as early as 1872, Charles Darwin described facial expression and cry responses followed pain as an important evidence of emotional feeling in the newborn infant,⁷ confirmed by a considerable amount of research since then. Facial expressions are more consistent indicators of pain than cry or body movements. Specific facial actions, such as brow bulge, eye squeeze, nasolabial furrow and horizontal mouth stretch (HMS) occur more frequently in response to painful stimuli among very preterm infants (24 to 30 weeks gestational age (GA)),⁸ preterm infants of greater maturity (that is 31 to 34 weeks GA), term infants⁹ and older infants. Facial expression provide the most reliable and consistent indicator of pain across all situations and populations^{10–12} and were also labeled as the gold standard for behavioral responses to neonatal pain.⁸ Acoustic analysis of the infant's vocalization response is also used frequently to assess pain and discomfort. Acoustic parameters found to be relevant are the latency, duration and fundamental frequency (F_0) of the first cry after a painful stimulus. Other parameters which may indicate a pain response are the chaotic changes in spectrograms from sound recordings.^{10,13–15}

Besides these behavioral responses, there are a number of physiological indicators reflecting activation of the sympathetic nervous system, most frequently used in the Neonatal Intensive Care Unit is the heart rate (HR).⁸

We hypothesized that the mode of delivery would have a pronounced effect on physiological and behavioral responses of neonates to noxious sensory stimuli. This trial was designed to

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investigate the newborn infant's response to pain or cold stimulation following normal VD and elective CS the first 4 h after birth.

Methods

Subjects

Women admitted to the delivery ward at Karolinska Hospital for spontaneous VD or planned elective CS were asked to allow their newborn infant to participate in this trial. Parents received both oral and written information before delivery, concerning the purpose of this study, which was approved by the local ethics committee. All parents to eligible subjects, with two exceptions, gave their consent.

Inclusion criteria were full-term birth following an uncomplicated pregnancy, GA of 37 to 42 weeks and appropriate weight for GA, Apgar score of ≥ 7 at 5 min after birth, and normal physical appearance. We excluded infants born following premature rupture of membranes, labor contractions lasting longer than 24 h, those infants delivered by vacuum extraction or acute CS, or whose mothers required epidural anesthesia or pudendal nerve block. For subjects included, all CS were performed under spinal anesthesia using Bupivacaine with the mothers being awake during the surgical procedure, whereas analgesia during VD was achieved with 50% nitrous oxide (N₂O). The VD ($N = 53$) and elective CS infants ($N = 23$) were similar with respect to gender, birth weight and postnatal age (in minutes) with a difference in GA at the time of testing (Table 1).

Procedure and methods

Infants' response to three standardized stimuli, one painful and two cold stimuli were assessed. These stimuli were used, as pain and cold both activates the same nerves (c- and A δ -fiber) but with different intensities. Our high-intensity pain stimulus was caused by the routine intramuscular injection of vitamin K (Konakion Novum, that is, 1 mg phytymenadione injected in a volume of 0.1 ml). After 2 min, the low-intense stimuli with a cooled metal spoon applied on the abdominal skin for 10 s. After further 2 min, 10 drops of cold water were dropped on the infant's foot. To secure the same temperature of the spoon, it was stored in the fridge at 10 °C whereas water was stored in a cool cupboard at 15 °C. Both spoon and water were taken out immediately before stimulation.

The sequence of stimulation was the same for all infants, and no stimulus was repeated. Infants were blindly, by closed envelopes, randomized to receive these stimuli either within >30 to ≤ 90 or at >90 to <260 min after birth. The time span was supposed to cover an interval of wakefulness between the first arousal hours followed the period when the infant fell asleep for the first time after birth.

Recordings were performed when the infant were calm and held in their parent's arms.

Physiological response

A standard three-lead ECG was recorded using a multichannel system (BIOPAC system, inc. Goleta, USA), with a sampling frequency of 1000 Hz. The signal was digitized employing a 16-bit A/D converter and displayed in real-time using the AcqKnowledge software. The baseline HR in beats per minute (bpm) was evaluated during a 1 min reference period immediately before the stimulus. Directly following stimulation, the HRs were measured at 1-s intervals for a period of 10 s; these readings were averaged and then expressed as a percentage of the baseline HR (HR %).

Behavioral response

Facial expression. The Neonatal Facial Coding System (NFCS) is a fine grained, anatomically based measure of behavioral pain response, which has shown consistently high reliability, construct validity in term and preterm infants.¹¹ The origin NFCS scale was used to describe responses to acute pain occurring across different sleep/wake states (Supplementary Figure S1). Later versions also differentiate acute pain from nonpain events, tissue damage (painful) from nontissue damage (stressful) phases of a procedure, therapeutic effects of pharmacologic analgesia or sucrose during invasive procedures, and nonpharmacologic interventions to manage pain. The first original NFCS scale includes nine facial expressions (Supplementary Table S1).¹¹ However, due to technical problems in differentiating details and recommendations from educated staff, we assessed infants' responses to seven of the nine facial expressions (see local Form; Supplementary Table S2).

All infants were videotaped at a distance of 2 m, with a Sony Handycam Video at high resolution. NFCS score were evaluated during the first 15 s immediately following each of the three stimuli. Collected data were coded and trained staffs estimated

Table 1 Descriptive data

Characteristics	All	Vaginal delivery	Caesarean section	P-value
Number (<i>n</i>) total	76	53	23	
Girls/boys	42/34	32/21	10/13	0.10
Weight (g) mean (s.d.)	3636 (418)	3649 (408)	3605 (449)	0.68
GA week/days (min–max)	39/4 (37/0–42/5)	40/0 (37/0–42/5)	38/4 (37/3–39/5)	<0.001
Postnatal age at first test (min)	101.3 (59.0)	105.2 (53.5)	92.4 (70.6)	0.39

Abbreviation: GA, gestational age.

independently each of the seven facial expressions second by second. The analysis of the videotapes was performed with a slow motion replay with frequent stops and 10% were studied repeatedly.

Vocalization. Infant vocalization was recorded by a Digital Audio Tape recorder (TCD-D7; Sony Corp., Tokyo, Japan), by an electric condenser microphone (JEFE AV TCM110; Sony Corp.), which was attached to the parent's clothes at a distance of 15 cm from the infants' mouth. Background sound in the room was monitored using a sound level meter (Ono sokki LA-215) and maintained at or below 60 dB. All recordings were calibrated to tones of different intensity levels with constant distance of 15 cm from the microphone.

Vocalization was analyzed with respect to both acoustical and perceptual features by a computerized voice analysis program (Swell). Vocalization occurring within 30 s from each stimulus was included in this analysis, with a primary focus on the first five expiratory vocalizations and total number of inspiratory vocalizations. Subsequent vocalizations following 30 s have been characterized as infant's basic cry reflecting hunger cry.^{13,16,17}

Acoustical analysis includes the quantitative and qualitative aspects of infant vocalization. To quantify, five objective parameters were analyzed: (1) time to response, that is the latency of an infant's response from start of applied stimuli to start of vocalization, (2) time of vocalization, is the total duration of expiratory and inspiratory vocalization within the first 30 s from stimuli, excluding pause, (3) total time of vocalization, from the start to the end of vocalization, including pause, (4) sound intensity in decibel (dB) which corresponds to the strongest of the first five vocalizations that were analyzed and (5) F_0 which corresponds to the perceived pitch of the vocalization, that is the strongest of the first five vocalizations. The Swell subprogram FoX was used to analyze the segment of vocalization with the maximal sound intensity (dB) using the sound calibration to extract the sound intensity from each recording. A semiautomatic analysis with CORR software was used to extract the ground tone curve for further analysis. Weak sounds that were difficult to separate from external noise or disturbance were excluded.

Perceptual analysis was performed by six listeners (three midwives and three speech therapists) with experience in professional sound judgment. Each listener received an individual training session with six vocalizations and used the VISOR program.¹⁸ Beside the original vocalization, 22% were randomly selected for repeated analyses. The five expiratory phonations were compressed in the Swell program with the GLUE subprogram to create a listener's test. Each listener received an individual training session with six vocalizations and used the VISOR program in the evaluation.¹⁸ Six perceptual parameters were selected (Supplementary Table S3). These are used routinely in clinical voice estimation.¹⁹ Listeners were instructed to classify the infant's vocalization and assign it in a numeric value on a visual analog

scale (VAS) from 0 to 1000 as described in Supplementary Table S3.

Statistical data analyses

As the group of patients was small, nonparametric statistics were used (median, min–max). The possible differences between response to high-intensity (injection) and low-intensity (two cold stimuli) stimuli were analyzed by Kruskal–Wallis analysis of variance. As no differences were observed between the responses to the two cold stimuli (spoon and water) these results were pooled together and analyzed as responses to cold stimuli. All data are presented as median and range.

The Statgraphics Plus software was used in the analysis of data. Power was calculated to indicate a 20% difference in response to the stimuli within the two modes of delivery.

Correlation between change in HR (%) in response to stimuli and postnatal age (in minutes) of infants was analyzed by linear regression, the slope of which defined the HR changes per minutes of postnatal age.

Relationship between the predictors' independent variables (birth weight, gender, mode of delivery and postnatal age in minutes at stimulation) and dependent variable (change in HR, facial expression, and vocalization) were examined in two steps. First, all possible correlations between the variables were analyzed (Spearman's rank correlation coefficient). Variables that were significantly correlated with the predictor ($P < 0.05$), were analyzed for independent association using stepwise multiple regression analysis (backward selection). Results were expressed as correlation coefficient, standard error (s.e.), significance level (P) and explained variance (%).

The perceptual estimation of pain intensity using VAS in relation to qualities of vocalization such as pitch, intensity, instability and pressure were evaluated by linear regression analysis. Correlation between the two NFCS observers and the six perceptual listeners was analyzed to calculate the inter-rater reliability of these methods.

Results

All behavioral and physiological responses such as HR change, facial expression and vocalization were stronger during high-intensity pain (vitamin K injection) as compared to the two low-intensity pain (cold) interventions (P -value between 0.04 and 0.000).

Physiological response

The median baseline HR was similar in infants born by VD and CS (135.5 (65.0) vs 138.3 (50.8) bpm, respectively, $P = 0.12$). The HR change in response to high- and low-intensity pain stimuli were affected predominantly by the postnatal age (min) and the mode of delivery to a lower degree (Table 2).

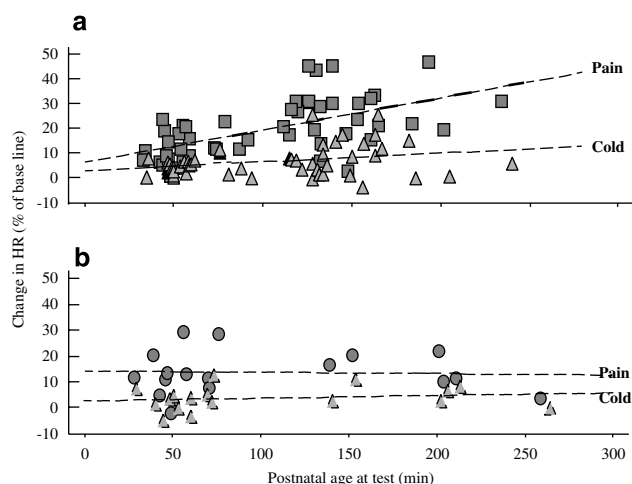


Figure 1 (a, b) Progressive increase in heart rate (HR) with age. Scatter plot diagrams illustrating the HR response to high- and low-intensity pain stimuli in relationship to time after birth in vaginal delivery (VD) infants with a progressive increase at a rate of 0.12 beat per minute of postnatal age (slope) ($r = 0.62$, $P < 0.001$) and an increase at a rate of 0.06 beat per minute of postnatal age (slope) ($r = 0.29$, $P = 0.04$), respectively (**a**), no such change was observed in caesarean section (CS) infants ($P = 0.96$) and ($P = 0.52$), respectively (**b**), linear regression analysis.

Table 2 Mode of delivery and postnatal age

Dependent variable	Independent variable (predictor)	P-level	Explained variance (%)
HR%: pain	Postnatal age (min)	0.001	14.2
	Mode of delivery	0.03	4.2
HR%: cold	Postnatal age (min)	0.000	11.5
HMS: pain	Mode of delivery	0.000	36.6
HMS: cold	Mode of delivery	0.000	12.5
Mean F_0 : pain	Mode of delivery	0.04	7.3
Mean F_0 : cold	Mode of delivery	0.004	13.9
Phonation time (%): pain ^a	Mode of delivery	0.002	21.6
Phonation time (%): cold ^a	Mode of delivery	NS	

Abbreviations: HR, heart rate; HMS, horizontal mouth stretch; F_0 , fundamental frequency.

^aOn the basis of first five vocalizations during 30 s in response to stimuli.

During the first 4 h after birth, HR response to high-intensity pain stimuli increased progressively by 0.08 bpm per minute of postnatal age ($r = 0.39$, $P = 0.001$) and to the low-intensity stimuli by 0.05 bpm per minute of postnatal age ($r = 0.35$, $P < 0.001$) disregarding the mode of delivery (all infants). Following VD, the HR response to pain increased by 0.12 bpm per minute of postnatal age ($r = 0.62$, $P < 0.001$) whereas no such change was observed in the CS group ($P = 0.96$). The HR response

to low-intensity stimuli also increased progressively but to a lower degree in the VD group (0.06 bpm per minute of postnatal age $r = 0.29$, $P = 0.04$) whereas no significant change was seen in CS group ($P = 0.52$; Figures 1a and b). Nine recordings (three VD, six CS) were excluded due to technical difficulties.

Behavioral response

Facial expression. The facial expression HMS was specifically affected by the mode of delivery whereas the other expressions were not (Table 2). As shown in Figure 2, the HMS expression was less pronounced in VD than in CS infants in response to higher pain intensity (3 (15) vs 9 (16); $P < 0.001$) and lower intensity of pain stimuli (0 (7) vs 1 (13); $P = 0.05$).

A total of 19 recordings (15 VD, 4 CS) were excluded due to technical problems.

Vocalization: acoustical and perceptual analysis. The mean F_0 response to high- and low-intensity pain stimuli was affected by the mode of delivery ($P = 0.04$ and 0.004 , respectively; Table 2). Mode of delivery also affected the response time to both high- and low-intensity stimuli with longer vocalization latency in VD infants than in CS infants ($P = 0.02$ and 0.002 , respectively; Figure 3).

Strong correlation occurred between the perceptual judgment of pain intensity and different modality of vocalization such as pitch, intensity, instability and pressure (the Pearson's product correlation coefficient were 0.92, 0.97, 0.62 and 0.68, respectively). Multiple regression analysis further revealed that perceptual assessment of pain intensity was based predominantly on pitch and pressure; both pitch and pressure explained 89.3% of the total variance in perceptual pain intensity, where the pitch contributed by 82.8% and pressure 6.4% of the variance. Similar results were obtained from vocalization following low-intensity pain stimuli, where the pitch contributed to 75.8% and pressure to 6.8% of variance. Figure 4 illustrates the linear correlations between the perceptual VAS judgments of pain intensity to pitch (a) and pressure (b).

No vocalization occurred in 10 of 53 VD infants and 1 of 23 CS infants following high-intensity pain stimuli ($P = 0.09$, χ^2 -test); these infants could not be included in the analysis of vocalization. A total of 22 recordings (17 VD, 5 CS) were excluded from analysis because the infant cried before application of the stimuli ($N = 7$), mother attempted breast feeding to calm the infant ($N = 4$) and due to technical difficulties while recording ($N = 11$).

Discussion

Our main finding was that infants born after spontaneous VD show dampened behavioral (facial expression and vocalization) response to both pain and cold stimuli as compared to infants born by CS. Physiological responses (HR%) in VD infants were dampened just

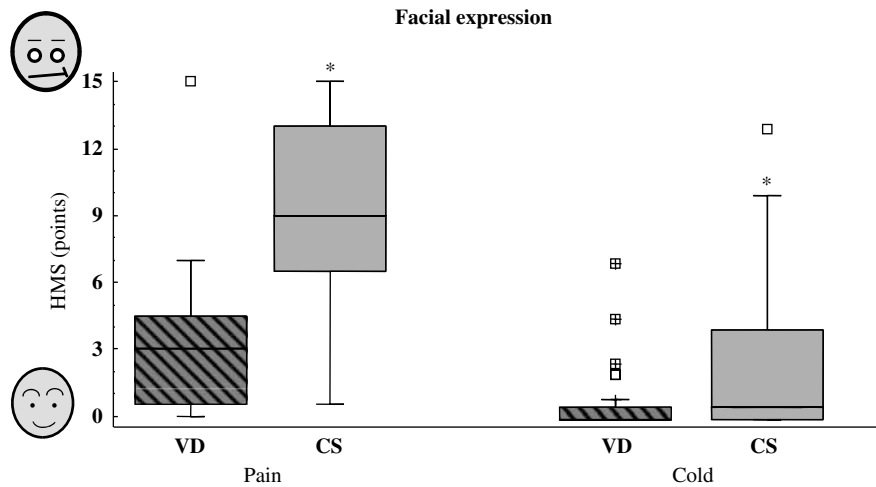


Figure 2 Horizontal mouth stretch (HMS) expression vaginal delivery (VD) vs caesarean section (CS) infants. Mode of delivery affects facial expression to higher and lower intensity pain stimuli with a weaker response in the VD infants than CS infants with HMS 3 (15) vs 9 (16), $P < 0.001$ and 0 (7) vs 1 (13), $P = 0.05$, respectively.

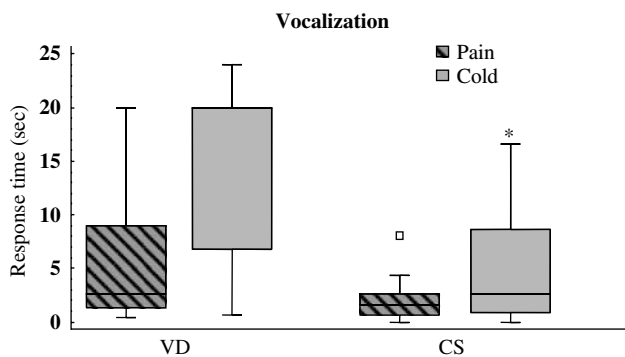


Figure 3 Vocalization response time in vaginal delivery (VD) vs caesarean section (CS) infants. Mode of delivery affects response time to high- and low-intensity pain stimuli with longer time to start of vocalization in VD infants than CS infants' 2.6 (19.6) vs. 1.6 (8.0) $P = 0.02$ and 20 (23) vs 2.6 (17) $P = 0.002$, respectively.

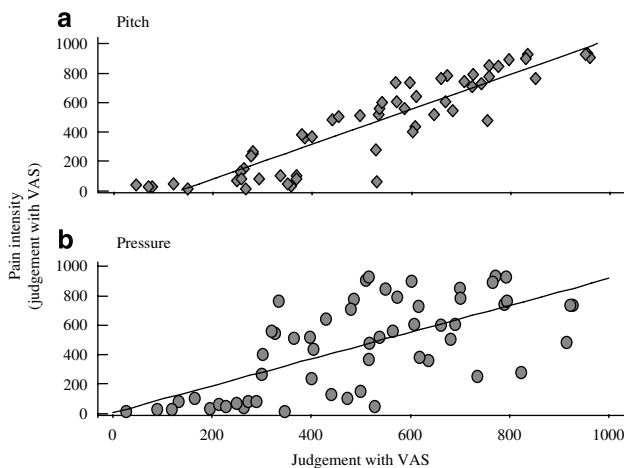


Figure 4 Perceptual pain intensity with (a) pitch and (b) pressure. The relationship between infants' perceptual vocalization, estimation with visual analog scale (VAS) of pain intensity and VAS estimation of pitch $r = 0.92$, $P = 0.000$ (a) and pressure (b) $r = 0.68$, $P = 0.000$.

after birth, but increased progressively during the first hours after birth. This pattern was not evident among CS infants.

Behavioral responses to pain, includes facial expression and vocalization. Facial expression is the most reliable and consistent indicator of all the unidimensional approaches.^{9–11,20} Vocalization is an indicator that signals distress to the caregiver and provides information about the infant state and biological integrity. In the present study it was found that the perceptual impression of pain vocalization was predominantly based on the pitch vocalization.

The objective assessment of pain perception based on physiological criteria lacks specificity for newborn infant.²¹ An increase in HR is, however, one of the most frequently reported physiological sign of acute pain or stress. We monitored such increase in response to two types of stimuli, which affect the same nerves fibers (c- and A δ -fiber) with different intensity. Strong correlation between HR increase and tissue damage followed circumcision, heel lance or immunization are well documented in infant,^{8,12,22,23} although no previous studies have examined these responses just after birth, their progression in the hours following delivery, or the mode of delivery affects the strength of the response.

The dampened behavioral and physiological responses to pain and cold stimuli in our VD infant population, especially within the initial hours after birth, suggest that newborn infants may remain in a state of fetal inhibition for a variable period of time immediately after birth. To further investigate this phenomenon, we randomized infants to receive pain and cold stimuli directly after birth (>30 to \leq 90 min after delivery) or later (>90 to <120 min) in a more sleepy state.^{2,3}

A number of functions are partially inhibited in the fetal animal during delivery, for example breathing movements, gastrointestinal activity and thermoregulation. We speculate that this inhibition, coupled with a reduced pain response may serve as protection against the stress of spontaneous birth. In uterus, human fetus,

however, respond to pain by pronounced hormonal²⁴ and hemodynamic^{25,26} changes. When fentanyl analgesia was administered to the fetus during invasive procedure at GA of 20 to 35 week, the attenuated stress response to painful stimuli was described, indicating that these response were indeed induced by pain and stress.²⁶

Our findings suggest that VD itself triggers the activation of an analgesic mechanism. Very high levels of CA are released following VD. The associated arousal is mediated by sympathoadrenal activation by noradrenergic inputs from the locus coeruleus into the reticular activating system.^{2,3,27} A possible mechanism behind the attenuated response to pain in VD infant may be due to the mobilization of noradrenaline both in the periphery and brain.^{2,3} Activation of adrenergic α_2 -receptor through the released noradrenaline may have strong analgesic effects like clonidine.²⁸ VD neonate also exhibits significantly higher plasma levels of β -endorphin than infant born by CS, which may further promote this difference.^{4–6} The similar baseline HR in our VD and CS infant with gradual increase in HR after VD in response to pain and cold stimulus during the first 4 h after birth may involve this endogen mechanism of stress-induced analgesia.²⁹ The rapid decline in circulating levels of β -endorphin during the initial 2 h following VD is in agreement with our findings.

CS mothers in the present study received spinal anesthesia with Bupivacaine, whereas VD mothers used N₂O during uterine contractions in the first and second stage of labor. Bupivacaine given spinally remains inaccessible to the paravertebral venous plexus and is unlikely to cross the placenta to produce systemic effect in the fetus or neonate. Such effect of Bupivacaine would have resulted in a lower baseline HR in the CS than in VD infants, but no such difference actually occurred. As VD mother rapidly eliminate N₂O through the lungs, it seems unlikely that N₂O could affect their newborn infant studied at ≥ 30 min after delivery.³⁰

Clinical implication

The data presented here have a significant clinical implication suggesting that invasive neonatal procedure, such as vitamin K injection, Hepatitis B immunization and metabolic screening, may be less painful for VD infant if performed soon after birth. This is particularly important as many hospitals have postponed painful or stressful procedure in order to allow mother–infant bonding in the period immediately after delivery.³¹ We therefore recommend vitamin K injection or blood sampling to be performed within 90 min after birth in VD infants in order to minimize neonatal pain and possible long-lasting changes in pain behavior.^{9,32,33}

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Conflict of Interest/Disclosure

There are no conflicts of interest within these people and the publication.

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Supplementary Information accompanies the paper on the Journal of Perinatology website (<http://www.nature.com/jp>)