The Journal of Maternal-Fetal and Neonatal Medicine, 2011, 1-6, Early Online

ISSN 1476-7058 print/ISSN 1476-4954 online
DOI: 10.3109/14767058.2011.587919

informa
healthcare

RESEARCH ARTICLE

Skin conductance indices discriminate nociceptive responses to acute stimuli from different heel prick procedures in infants

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***Aim*: To evaluate the ability of SC indexes in discriminating acute responses to different heel prick procedures. *Methods*: Observational cohort study of a systematic, convenience sample of neonates with clinical indication of capillary blood sampling by heel prick, either for glycaemia or for blood gas analysis. The Neonatal Infant Pain Scale (NIPS) was used to confirm the painful nature of the stimuli. NIPS assessment and SC measurement (Med-Storm Pain Monitor™) were simultaneously performed by two independent observers before, during and after blood sampling. *Results:* Sixty-eight heel prick procedures (46 for glycaemia and 22 for blood gas analysis) were applied to 16 infants. Both NIPS scores and SC peaks/s index were significantly higher during blood sampling than before or thereafter (Wilcoxon Signed Ranks, $p < 0.001$). There was no significant difference in NIPS score and SC peaks/s between the different heel prick procedures. Significantly higher SC area under low peaks index (Mann–Whitney, $p = 0.001$) and lower SC average rise time index (Mann–Whitney, $p = 0.037$) were registered when blood was drawn for blood gas analysis than for glycaemia, related to a sustained acute nociceptive response to a more prolonged stimulus. *Conclusion*: Using the conjunction of available SC indices, SC seems able to differentiate the nociceptive response to acute pain of different durations.**

Keywords: Pain, nociceptive response, neonates, heel prick, skin conductance

Background

The skin conductance (SC) algesimeter is a recent non-invasive portable and automated method for evaluating pain and discomfort[1]. It has been validated in term and preterm infants (from 25 weeks of gestational age onwards) for assessment of pain[2–8]. This method is based on SC variations that occur when nociceptive stimulation induces sympathetic nerve impulse bursts to the skin. When the skin sympathetic nervous system is firing, sweat is released within 1–2 s. As a result, the palmar and plantar sweat glands are filled, diminishing skin resistance and increasing the SC before sweat is reabsorbed[9]. This creates SC peaks, which are used to evaluate pain and discomfort. The number of SC peaks per second (peaks/s) index directly reflects the firing rate of sympathetic nerves, the emotional component of the sympathetic nervous system (emotional sweating[9]). The area under the SC curve shows how strongly the skin sympathetic nerves are firing[9,10]. Emotional sweating differs from temperature dependent sweating in its independence from environmental temperature[11].

During acute pain, functional MRI identifies activation in higher brain areas, simultaneous with an increase in the SC response[12]. The SC response is not influenced either by changes in blood circulation or by neuromuscular blockers acting on nicotine receptors, because SC responses are activated by acetylcholine acting on muscarine receptors[13–15].

SC measurements require high technology equipment, but provide continuous quantitative measurements with great precision (milliseconds), for as long as required to obtain the measurements. The SC peaks/s index was compared with the modified COMFORT sedation score, and SC peaks/s was considered an objective supplementary measure of discomfort in artificially ventilated neonates and children[16].

Currently, several scales are available for clinical pain assessment in neonates, but none has yet been considered a gold standard[17]. The Neonatal Infant Pain Scale (NIPS[18]) is one of the most used scales for pain assessment in clinical practice in term and preterm infants[19]. It is a relatively simple multidimensional behavioral scale based on facial expression, cry, breathing patterns, activity of arms and legs, and state of arousal[18]. Construct validity of NIPS has been widely assessed in term and preterm infants[20–22]. Furthermore, NIPS has been favorably compared with the Visual Analogue Scale[18], Premature Infant Pain Profile[23], CRIES Pain Scale[24], Neonatal Facial Coding System and Douleur Aiguë du Nouveau-né[25].

Infants under intensive care are frequently submitted to painful procedures, some of them apparently simple and of short duration as heel prick. In fact, heel prick is used for different purposes that may imply the squeezing of the heel for shorter or longer time. This difference is usually not taken into account when providing comfort during these procedures. We hypothesize that the response of the neonate to heel prick procedures for different purposes may be physiologically different, and this difference might not be detected by the usual psychometric methods to evaluate pain and discomfort in infants, but might be identified by the measurement of sympathetic nervous activity, such as SC.

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The aim of this study was to assess the ability of SC indices in differentiating the acute response of neonates to heel prick procedures for glycemia or for blood gases sampling.

Methods

A blind observational cohort study on the assessment of neonatal acute nociceptive response through a psychometric scale (NIPS) and a physiological measurement (SC) was conducted in a tertiary neonatal intensive care unit. Heel prick is assumed to be a painful procedure, and the NIPS score was used to provide additional evidence that the applied stimuli were indeed painful.

A convenience sample of neonates with clinical indication for collection of capillary blood by heel prick, either for blood gas analysis or for glycaemia, was systematically recruited. Consecutive assessments were performed from Monday to Friday, from 9 a.m. to 4 p.m. Infants in early post-surgical period, receiving muscle relaxants and those with impaired skin integrity, precluding the application of monitoring devices over the skin were not included.

None of the heel prick procedures was done specifically for the study purpose. Informed parental consent was obtained for any eligible newborn before recruitment, and the protocol was approved by the research ethics committee of the institution.

The standard protocol for capillary blood sampling involved warming the foot, picking it up, making a small incision with a metal scalpel (2.2 mm long and 1 mm wide), and gently squeezing the heel for as long as required. If needed, the incision was repeated to obtain sufficient blood for the purpose. Between 0.6 and 0.8 μL of blood was collected for capillary glycemia, using the blood glucose test strips for the glucose meter Precision Xcced™, Abbott. Seventy or 140 μL of blood were collected in the capillary tube for blood gas analysis, respectively for microsample or for regular sample options, in the Rapidlab 1265 Blood Gas Analyser™, Siemens. Following the Unit protocol for distressful procedures, cuddling and oral sucrose was routinely given, even when infants were already on systemic analgesics.

Two methods of assessment of nociceptive response to capillary blood sampling procedure were simultaneously applied by different observers. NIPS was performed by one of two observers (IM or SG), after previously achieving a good inter-observer reliability of NIPS scores as stated below. The SC measurements were always done by the same observer (PR), after an adequate training period, using the SC algesimeter Med-Storm Pain Monitor™ (Oslo, Norway). In every assessment, measurements were blindly performed and registered by each observer (concerning the measures obtained by the other observer). Observation started when the patient was awake but not agitated or crying (Prechtl's scale Behavioural state 1[26]).

The SC measurements were performed in three time periods: 120 s before blood sampling (pre-procedure), 30 s after starting the blood sampling procedure (independently of the actual duration of the procedure), and 120 s after its end (post-procedure). The SC measurements in the last 30 s of the pre- and post-procedure periods were, respectively, registered and analyzed separately. NIPS was applied in the 30 s after starting the procedure, and in the last 30 s of the pre- and post-procedure periods, respectively.

Any administered drugs, such as analgesics, sedatives, anticholinergics and amines, and respective dosages were recorded.

Previous agreement on the application of NIPS between the two observers (IM or SG) was double blindly evaluated in 21 blood sampling procedures. An inter-observer Spearman coefficient of correlation $r = 0.911$ and a coefficient of agreement of 95.99% were reached. Time required for performing NIPS was 10–20 s.

The main index used for assessing SC measurements was the number of peaks/s, which is the best validated SC index for pain score in infants[3,10]. This index, as shown in Figure 1, represents the number of peaks in a time span, and corresponds to the number of bursts per second in the skin sympathetic nerve[27]. Other three SC indices were analyzed: the area under huge peaks, the area under small peaks and the average rise time[27] (Figure 2). These indices contribute to understand the physiology of the skin sympathetic nerve response to the nociceptive stimuli by showing how forcefully the sympathetic nervous system is firing[3].

The difference in the duration of the blood sampling procedure concerning its purpose was evaluated by Mann–Whitney *U* test. The differences between the assessments (before, during and after blood sampling) were evaluated with the Wilcoxon Signed Ranks test, both for NIPS and for SC. The difference in the assessment of the nociceptive response to the blood sampling procedure (gases analysis or glycaemia), was evaluated by Mann–Whitney *U*

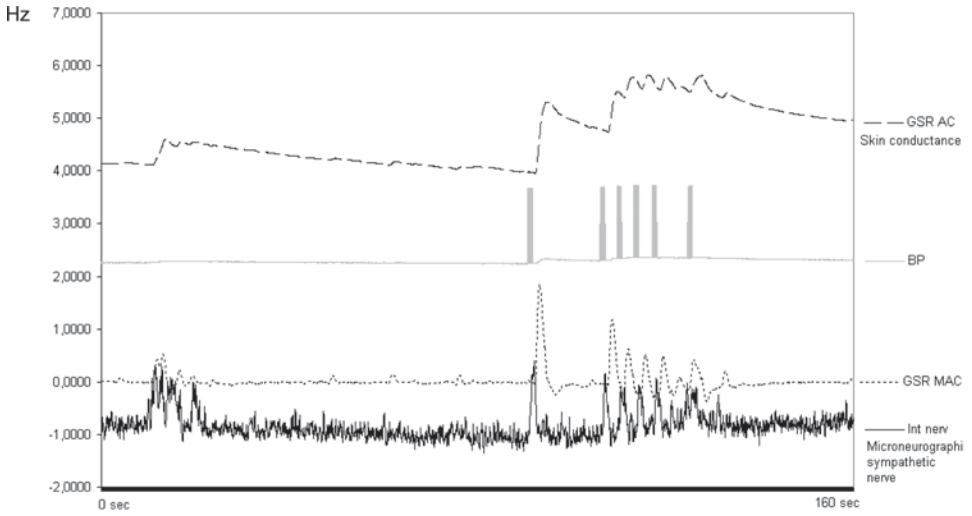

Figure 1. The four traces show changes in skin conductance (SC) (GSR AC = galvanic skin response alternating current), in blood pressure (BP), in converted skin resistance (GSR MAC = galvanic skin response mean amplitude converted), and in intra nerve activity (Int Nerve) of the nervus medianus, by microneurographi of the skin sympathetic nerve[10] (With permission). For every burst in the sympathetic nerve, there is one peak in the SC measurement. One SC peak is defined as an increase in SC of more than 0.02 microsiemens (μS), after one minimum point. Both peaks and minimum points are defined when the derivate of the SC curve is 0[3,10].

test. The potential interference of the different purposes of blood sampling, gestational age, postnatal age and administered drugs was evaluated by multivariate logistic regression analysis.

Results

The study sample included 68 heel prick events applied to 16 infants (8 females), with mean ± SD (range): birth weight (g) 1885 ± 856 (600–3605), gestational age (weeks) 34 ± 6.1 (24–41), postmenstrual age (weeks) 37 ± 5 (25–45), and median postnatal age (days) 22.4 (0–96). The main diagnoses of the infants were: prematurity/hyaline membrane disease (5), pneumonia (3), asphyxia (2), transient tachypnea of the newborn (2), prematurity *plus* necrotizing enterocolitis (2), esophageal atresia (1) and prematurity *plus* meconial ileus (1). Two infants had stable grade I intraventricular hemorrhage and two had stable grade II intraventricular hemorrhage, one infant was under invasive ventilation and two under non-invasive ventilation (nasal constant positive airway pressure).

In 9 out of the 86 events, infants were on drugs with sedative and/or analgesic effect: fentanyl *plus* midazolam (5), fentanyl (2), fentanyl *plus* phenobarbital (1), and phenobarbital (1). The administered doses were fentanyl 0.6–1.7 μg/kg/h, midazolam 0.1–0.2 mg/kg/h, and phenobarbital 3–5 mg/kg/day.

Each infant was assessed in up to 15 events (median 2, minimum 1); in 6 events, 2 heel pricks were required (4 for blood gases). SC was assessed in the 68 events (46 for glycaemia and 22 for blood gas analysis), and NIPS was assessed in 67 events (45 for glycaemia and 22 for blood gas analysis).

The duration of blood sampling was significantly higher for blood gases (median 88 s, min-max 16–214 s) than for glycemia (median 21 s, min-max 10–133 s) (Mann–Whitney *U* test, $p < 0.001$). This difference remained significant when considering only the events requiring one heel prick (Mann–Whitney *U* test, $p < 0.001$).

During blood sampling, significantly higher NIPS scores were registered, than before and after (Wilcoxon Signed Ranks Test; $p < 0.001$): 25th centile = 2, 50th centile = 4, 75th centile = 5, minimum = 0, maximum = 7. Pre- and post-procedure periods: minimum, 25th, 50th and 75th centiles score was 0, and the maximum score was 5, respectively. The NIPS scores were not significantly different concerning the purpose of blood sampling (Mann–Whitney *U* Test). There was no influence of gestational age or postnatal age on the NIPS score during the procedure (multivariate logistic regression).

Significantly higher values of peaks/s were registered during the procedure than before and after (Wilcoxon Signed Ranks Test; $p < 0.001$) (Table 1 and Figure 3). There was no influence of gestational age or postnatal age on the SC peaks/s index during the procedure (multivariate logistic regression). When analyzing the whole SC indices according to the purpose of blood sampling, no differences were found in the peaks/s index (Mann–Whitney *U* Test). However, significantly higher values of the area under small peaks (Mann–Whitney, $p = 0.001$) and significantly lower average rise time (Mann–Whitney, $p = 0.037$) were registered when blood was drawn for blood gas analysis than for glycaemia. There was no influence of gestational age or postnatal age on both these indices during blood sampling.

During blood sampling females had significantly higher NIPS scores; infants already medicated with analgesics or sedatives had significantly lower NIPS scores (multivariate logistic regression $p = 0.053$ and $p = 0.000$, respectively). SC peaks/s index was significantly higher in females but was not affected by concomitant medication with analgesics or sedatives (multivariate logistic

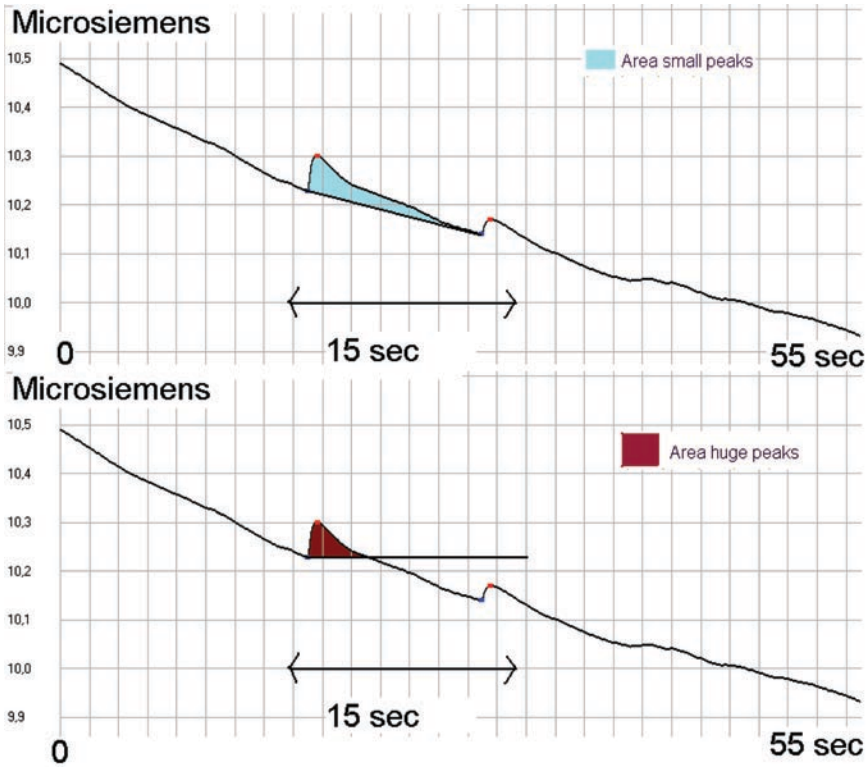

Figure 2. The area under small peaks (area small peaks) index is calculated by establishing a line between two adjacent peak minimum points, representing the accumulated difference between the line and the skin conductance registration curve values when they are larger than the baseline. The area under huge peaks (area huge peaks) index is calculated by establishing a horizontal base line from the first peak minimum in the time window, representing the accumulated difference between the conductance values at the registration curve and the established baseline when they are larger than the baseline[27]. (With permission).

regression $p = 0.004$ and $p = 0.138$, respectively). Both the area under small peaks and the average rise time indices were not significantly affected by gender or with analgesics or sedatives.

Discussion

"Pain is stressful, but not all stress is painful[28]". In fact, differentiating between pain and stress is difficult in nonverbal individuals. To provide evidence that the applied stimuli were indeed painful, NIPS was chosen in this study as a psychometric scale for pain assessment, since the staff of the neonatal intensive care unit is trained in using it, and it is one of the most used and validated pain scales in term and preterm infants[18,20–23]. Sympathetic nerve activity to the skin is activated both during pain and other stressful stimuli[29], and besides assessing response to pain, SC may reflect emotional sweating[9]. Changes in skin sympathetic nerves should therefore be interpreted according to the stimuli to which the infants are exposed. It has been debated whether tactile stimulation could be considered a stressful event, since in both preterm and term infants plasma catecholamines were found to be more elevated following nursing procedures (washing, nappy change) than following heel lance[30]. In this study, both NIPS scores and SC peaks/s index were significantly higher during capillary blood sampling than before or after, thus providing strong evidence that painful stimuli have indeed been applied.

Neither NIPS score nor SC peaks/s index were able to differentiate the response between blood gases sampling and sampling for glycemia. However, using other SC indices it was possible to identify a different pattern of response to each procedure. Sampling for blood gases by heel prick was found to induce larger area under the small peaks, and changes in SC with swifter rises (average rise time) over a more elevated baseline (area under small peaks), than blood sampling for glycemia. The SC index related to the area under the curve, as the area under small peaks, indicates how forceful the sympathetic nervous system is firing. Larger area under the curve may suggest a more intense stimulus[9,10]. Differences in capillary blood sampling technique for glycaemia or for blood gases may explain the difference, since blood gases requires collecting more blood for longer period with more heel squeezing, and therefore a more painful stimulus.

NIPS is a point in time assessment that requires several seconds to be applied, depending on the observer, while SC is continuously measured with a millisecond precision, providing an array of continuous quantitative measurements for as long as required. It seems logical that the two methods might have different ability to discriminate stimuli of different duration. The fast reaction time (1–2 s) of the SC variables[9] may contribute to explain this ability.

The SC peaks/s may benefit from being used together with other pain assessment tools, like NIPS, when the SC peaks/s increases for unknown reasons[31]. It has not been yet clarified

Table I. Skin conductance peaks/s index in the 120 s before the procedure, in the 30 s immediately before the procedure, during the procedure (30 s), in 120 s after the procedure, and in the last 30 s of this period. The number of peaks/s was significantly higher during the procedure than before or thereafter (Wilcoxon Signed Ranks Test; $p < 0.001$).

		Centiles				
Peaks/s (Hz)	N	Minimum	25th	50th	75th	Maximum
120 s pre-procedure	68	0	0.0100	0.0300	0.1275	0.38
30 s pre-procedure	68	0	0.0000	0.0300	0.1300	0.53
Procedure (30 s)	68	0	0.0550	0.1700	0.3300	1.20
30 s post-procedure	68	0	0.0000	0.0300	0.1225	0.65
120 s post-procedure	68	0	0.0200	0.0750	0.1575	0.40

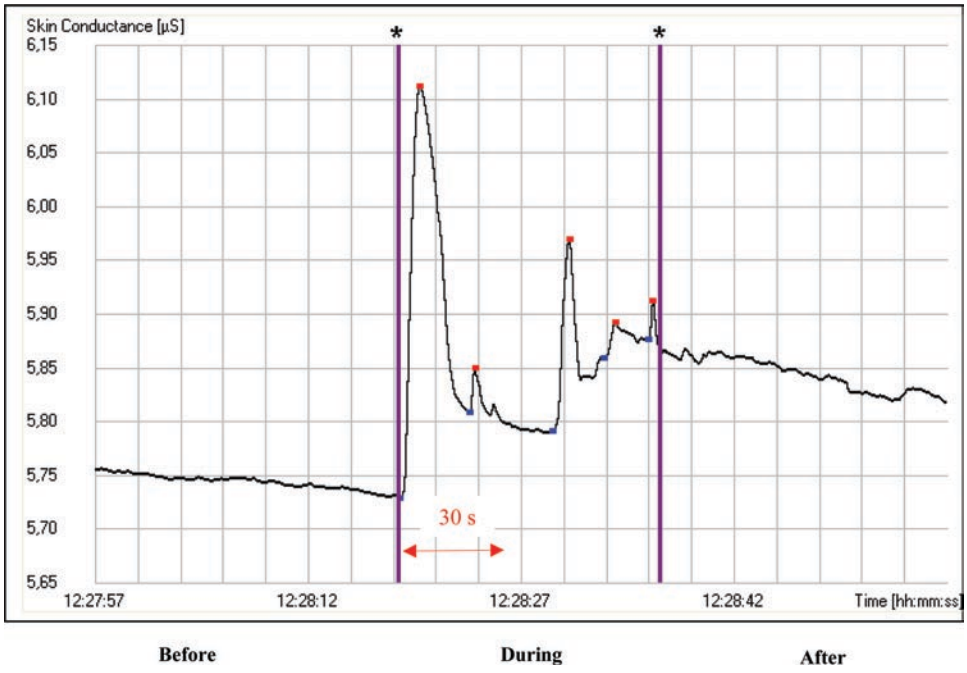

Figure 3. Variation of skin conductance in a neonate subjected to sampling for blood gases analysis. A marked increase in peaks/s (Hz) was registered during the procedure (left * - heel prick; right * - end of sampling procedure). The peaks/s values in the first 30 s of the procedure (arrow) were compared with the immediate 30 s before the procedure and with the last 30 s of the 120 s post-procedure period.

whether other SC indices used in combination with SC peaks/s index may improve the discriminative power of SC to distinguish between pain and general stress responses.

Infants under 30 weeks of gestation have a low water loss from palms and soles, measured by the water vapour pressure gradient[32]. Their palms and soles are not fully keratinised until 6–7 months after birth. Inversely to the rest of the body, the water loss from the palms and soles rises within the first month after birth, which is seen as the onset of emotional sweating[33,34]. The neurological structures necessary for nociception are developed by 24 weeks of gestation[35]. Both acute muscle and skin pain cause an increase in skin sympathetic nerve activity, sweat release and gender-dependent changes in skin blood flow[36]. The SC peaks/s reflects the pain response to heel prick in preterm infants with more than 25 weeks of gestation[8]. This indicates that the SC peaks/s index discriminates pain responses from water loss.

Whereas NIPS is dependent on the gestational age[19], and different clinical pain scores are recommended for the different age groups of preterm infants[37], this is not the case for SC peaks/s index[2,8], as confirmed in this study.

During painful stimuli, the amplitude of the SC peaks has been shown to be influenced by postnatal age, reflecting previous painful stimuli[2]. The SC peaks/s was not found to be influenced by postnatal age both in this study and in previous studies[2,8].

When infants are at the same level of behavioural state, the inter-individual variation of the SC peaks/s is lower than the intra-individual variation[38]. Since SC peaks/s increases when the pain or discomfort of the infants increases[2–8] and the inter-individual variation at the same intensity of pain or discomfort is low, SC peaks/s index is feasible to be used in any infant. The high sensitivity of SC indices may result from its objectivity, its low variation between individuals at the same level of behavioural state, its independence from gestational and chronological age, and its fast reaction time.

To summarize, the acute response of infants submitted to two different heel prick procedures is different. Both NIPS and the SC peaks/s index increased during blood sampling through heel prick, but only SC area under small peaks and average rise time were able to differentiate the nociceptive response between blood gases sampling and blood sampling for glycemia. This is due to the inability of NIPS to differentiate acute nociceptive responses to stimuli of different duration, an advantage provided by the SC algesimeter.

Declaration of interest: One of the c-authors, Hanne Storm, has a potential conflict of interest as she is part owner of Med-Storm Innovation AS, which has been developing the skin conductance equipment used in this study. All co-authors disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) this study.

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