33rd Annual International Conference of the IEEE EMBS
Boston, Massachusetts USA, August 30 - September 3, 2011

Heart rate, oxygen saturation, and skin conductance: a comparison study of acute pain in Brazilian newborns

Jose Alfredo L. de Jesus, Rosana M. Tristao, Hanne Storm, Adson F. da Rocha, *Member*, *IEEE*, and Dioclecio Campos, Jr.

Abstract— **Heart rate variability (HRV), oxygen saturation variability (OSV) and skin conductance activity (SCA) are recognized physiological markers of acute pain. In order to verify which of them has the best correlation with psychophysical parameters of pain (*intensity*, *reactivity*, *direction*, *regulation* and *slope*), an observational prospective study was performed, including 41 healthy full term newborns. The measurements studied were the HRV, the OSV, and the following SCA variables: number of waves per second (NWps) and relative area under the curve of waves (AUC). The measurements were performed in periods labeled *before*, *during*, and *after* a heel prick. The variation measured for *intensity* between periods was significant for the NWps ($p=0.001$), AUC ($p=0.03$), HRV ($p=0.001$) and OSV ($p=0.004$). Also, the *reactivity* and *direction* were significant for all variables, except AUC. The *regulation* parameter was significant for the variables NWps ($p<0.01$), AUC ($p<0.05$), HRV ($p<0.01$) and OSV ($p<0.01$). The *slope* was statistically significant only for the OSV variable ($p=0.000$). We concluded that the responses of the SCA, HRV and OSV to painful events fit the psychophysical parameters of a physiological marker and serve as valuable measures for pain diagnostic working the use in accordance with the needs of the context.**

I. INTRODUCTION

Behavioral responses to a painful event are limited in critically ill newborns as they are unable to display them, especially when sedated or receiving muscle relaxants [1]-[4]. Physiological measures such as heart rate variability (HRV) [3],[5],[6], oxygen saturation variability (OSV) [5],[6], and skin conductance activity (SCA) [7]-[13] have been studied as indicators of response to noxious stimuli in newborns in such critical contexts. Heart rate (HR) and oxygen saturation (OS) are relatively easy and of low cost to obtain, being often regarded as a useful measure of pain reactivity. However, HRV and OSV may have limited clinical use due to their low specificity and efficacy in emergency contexts [3],[5],[6],[10],[14]. Recent studies have considered SCA a physiological measure more linked to pain, as it shows faster reaction (within seconds after noxious stimuli). Moreover, it is an objective method, the variability between individuals is minimal, and it is not influenced by circulatory changes, cardiovascular drugs, environmental temperature, or changes in respiratory rhythm like apnea. Thus, it seems to be more sensitive and specific to pain than other available methods [15]-[22].

The SCA reflects changes in secretions in the palm and sole sweat glands after activating the sympathetic nervous system (SNS) from the skin surface. Each time the SNS is activated, the sweat glands of those areas are filled up and a spontaneous wave of SCA occurs. SCA is an electrical phenomenon translated by a conductance measuring system as number of waves per second (NWps) showing how often the SNS is firing; and the area under the curve (AUC) showing how forcefully the SNS is firing. An increase in these measures may be interpreted as increased activity of the SNS, demonstrating a positive correlation with other physiological and behavioral pain indicators [8],[10]-[13],[18].

The response pattern of physiological indicators to a painful event in an acute pain context starts from a baseline condition followed by reactivity to the painful stimulus, ending with the recovery period. According to Oberlander's model for studying pain [7], pain measures can be analyzed by five parameters: *intensity* or magnitude, calculated as the average and standard deviation of the score; *reactivity* (significant change of score from baseline to the stimulus); *direction* (is an increase the same as a decrease?); *regulation* (or score change from pain to recovery); and *slope* (which reflects the over-regulation or down-regulation trends, and is calculated by the linear regression coefficient) [3],[14]. *Intensity* and *reactivity* are interdependent parameters which pain measures must always meet [23]. In this sense, the *reactivity* would be inversely dependent of a baseline value, e.g., the higher the baseline value the lower the *reactivity* would be and *vice versa*. The *reactivity* parameter constitutes the basis for theoretical application of the Law of Initial Value (LIV), which states that the magnitude of a physiological response depends on the comparison from the baseline of the same individual [3],[20],[23]. On the other hand, a return to baseline (*regulation*) would occur faster [3],[23]. The *direction* would depend on the presence of a stimulus to increase or decrease the baseline value and thus the response could be ascendant or descendent. Finally, the *slope* refers to the rate at which the change occurs in the baseline score during the stimulation, which is calculated by a statistical resource, the regression coefficient. Therefore, the higher the value of the *slope*, the more significant the possibility is of the value of the period *before* predicting the value of the period *during* the procedure [9],[12].

Manuscript received April 15, 2011. This work was supported by grants from the Brazilian National Research Council (CNPq) and the Federal District Research Support Foundation (FAPDF).

J. A. L. de Jesus is with the University of Brasilia, Brasilia, DF, Brazil (e-mail: alfredo@unb.br, jalfredo.jesus@gmail.com).

R. M. Tristao is with the University of Brasilia, Brasilia, DF, Brazil (e-mail: rosana.tristao@gmail.com).

H. Storm is with Simulation Lab, Rikshospital and Ullevål University Hospital, University of Oslo, Oslo, Norway (e-mail: hanne.storm@medisin.uio.no, hanne@med-storm.no).

A. F. da Rocha is with the University of Brasilia, Gama Campus, Brasilia, DF, Brazil (corresponding author – phone: +5561-33485502; e-mail: adsonr@gmail.com).

D. Campos, Jr. is with the University of Brasilia, Brasilia, DF, Brazil (e-mail: dicampos@terra.com.br).

No reports were found comparing HRV, OSV, and SCA based on the theoretical parameters of the interpretation model of pain in newborns cited above. The criticism made of the use of these measures is that variability associated with response to the noxious stimulus, including the baseline, reactivity and recovery phases, depends on several individual conditions [2],[3],[23] and a good measure must be less sensitive to them. Hence, this study aims to verify if the HRV, OSV, and SCA fit well into Oberlander's model, especially for the five proposed parameters of pain measurement.

II. METHODS

A. Study design and participants

This is an observational study, controlled by the individual, *before*, *during*, and *after* the painful event. The sample population studied consists of 41 healthy newborns with gestational ages between 37 and 41 weeks, subjected to a heel prick for capillary blood glucose level testing within 48 hours of life. They were selected based on convenience from the neonatal care unit at the University of Brasília Hospital, Brazil. Newborns excluded were those with a five minute Apgar score less than seven, those that received any analgesia or sedation before the heel prick, those with metabolic, respiratory, cardiovascular, neurological or infectious diseases, third or fourth degree intracranial hemorrhage, those with impaired skin integrity, and those with cardiac, pulmonary, gastrointestinal, and neurological congenital malformations. Written informed consent from a parent was obtained before inclusion of the newborns in the study, which was performed in accordance with the Declaration of Helsinki [24] and the Resolution 196/1996 of the Brazilian National Commission of Ethics in Research [25], and approved by the Research Ethics Committee of the University of Brasília.

B. Procedure

The heel prick was considered as the painful event. The following clinical variables were recorded: maternal and gestational age, type of delivery and maternal anesthesia, resuscitation support of the newborn in the delivery room, birth weight, gender, breastfeeding at the last hour before the puncture, if the newborn received glucose two minutes before the procedure, postnatal age, number of previous heel pricks and number of other previous painful procedures.

All instruments were installed ten minutes before heel prick. The newborns were subjected to heel prick on the right foot with a 25x7 needle (BD Instruments). The heel lancing, squeezing and application of the compression bandage lasted for 30-60 seconds. No unpleasant procedure was carried out in the one-hour period that preceded the puncture. The newborns were observed during three periods, each of three minutes: before heel prick, considered to be the baseline or *before* period; immediately after the lancing, squeezing and application of the compression bandage (considered as the painful stimulus), the *during* period; and the remaining three minutes after this procedure being considered to be the recovery or *after* period. HRV and OSV were obtained using a pulse oximeter (DX 2515-Dixtal) videotaped by a tripod mounted digital video camera (DCR-SR47-Sony). The highest HR value and the lowest OS value for the three minutes of each period were read from these video recordings, away from the clinical setting, using a second to second stop frame technique. From the images recorded with the video camera, the behavioral state of the newborns received a score from 1 to 5, meaning from deep sleep to crying state, according to Pretchel's criteria [26].

The SCA was measured by an electric conductance meter (Skin Conductance Measure System-SCMS, Med-Storm Innovation, Oslo, Norway), using an alternating current (AC) at 60 Hz. The device emits a voltage of 50 *m*V through a system of three electrodes (Electrodes Skin Conductance Algesimeter, Med-Storm Innovation, Oslo, Norway). The first is the measurement electrode, the second, the current electrode and the third, the reference electrode, which ensures a constant voltage from the innermost layer of the cornea stratum to the measurement electrode. These electrodes were fastened to the newborn's left foot. The measurement electrode was placed on the sole of the foot, adjacent to the toes, the current electrode on the internal side of the ankle and the reference electrode on the external side of the ankle, according to Edelberg and Storm guidelines [12],[27]. The electrodes were wrapped in order to eliminate any unwanted movements. This setting is non obtrusive as OSV and HRV and can follow the infant continuously for 48 hours.

The SCMS uses a computer program that records the NWps and calculates the AUC in the periods studied. The record of the NWps corresponding to the valleys and peaks derived from the electrical impulses is defined when the derivative of the wave is zero and is observed on a monitor attached to the system in a digital graph format. The NWps correlates directly to the rate of firing in the sympathetic nerves. The AUC of the waves is measured, in μSs, by the highest value of the area huge peaks and area small peaks. The program has a basic configuration, calibration sensitivity of 0.02 μS. NWps and AUC were analyzed in time intervals of 15 seconds at the beginning of each observation period. All data was collected and stored in an electronic database by the same researcher.

C. Statistical analysis

The sample size was estimated at 26 newborns for an alpha error of 5%, a beta error of 20% and a statistical power of 80%. In order to verify significant differences of *intensity* among the periods *before*, *during* and *after* heel prick with respect to SCA, HRV, OSV, and behavioral state, the Friedman nonparametric-paired test was performed. To determine statistical significance for *reactivity* and *direction*, when comparing the differences *during-before*, the Wilcoxon nonparametric-paired test was used. To determine statistical significance for *regulation*, when comparing the differences *after-during*, the Wilcoxon nonparametric-paired test was used. Linear regression was used to verify the regression coefficient of pain indicators for the period

during (*slope* component). To verify if the clinical and demographic variables were related, two-way ANCOVA analysis was used. A level of $p< 0.05$ has been chosen as the threshold for statistical significance.

III. RESULTS

Data of two newborns for NWps and eight for AUC do not were considered due to an electronic noise attributed to the 60 Hz AC in the electrical installations of the clinical setting, compared to the 50 Hz AC used in the majority of research settings. Data of two newborns for HRV and four for OSV also do not were used on analysis due to collection failure, as there was no stabilization of the oscillations in the monitoring apparatus. The remaining data of these subjects were analyzed.

The newborns had a mean gestational age of 38.9 weeks and mean birth weight of 3184 g, 51% were male and 68.3% were delivered by cesarean section, 39% were large for gestational age, 41.5% small for date and 5.1% from diabetic mothers; 68% of the newborns were breastfed one hour before the puncture and only 9.8% received glucose orally two minutes before the procedure. The newborns' mean postnatal age at the time of procedure was of 18 hours and the mean of previous painful events before the procedure was 2.6. The neonates were awake in the period *before*, as observed from of the behavioral state analysis (mean value = 2.3) and there was a significant increase in the latter at the period *during* (mean value = 4.9, $\chi^2 = 56.66$, $p = 0.000$). No effect was found among the clinical and demographic variables and the variation between *before* and *during* the painful event by each of the measures and the entire sample data was used in the following analyses.

The analysis of the *intensity* showed that the NWps and the AUC were significantly different between the periods *before*, *during* and *after* ($\chi^2 = 13.07$, $p = 0.001$ and $\chi^2 = 7.02$, $p = 0.03$, respectively), as well for HRV and OSV ($\chi^2 = 15.17$, $p = 0.001$ and $\chi^2 = 10.97$, $p=0.004$, respectively) (Table I).

NWps, HRV and OSV variables fit the psychophysical parameters *reactivity* and *direction* of a physiologic marker. Differences *during-before* as reflecting the *reactivity* and *direction* to NWps ($Z = -4.20$, $p = 0.000$), HRV ($Z = -3.50$, $p = 0.001$) and OSV ($Z = 3.03$, $p = 0.004$) were statistically significant. The *regulation* parameter, obtained by the difference *after-during* was significant for the variables NWps ($Z = -3.48$, $p = 0.000$), AUC ($Z=-2,038$ $p=0.042$) HRV ($Z = -3.50$, $p = 0.000$) and OSV ($Z = -2.60$, $p = 0.009$). The values of all variables return to baseline level after recovery, as expected. Therefore, there was no significant difference between the periods *before* and *after* for all variables (Table II).

Regression analysis, used to evaluate the *slope* of the curve among the periods *during* and *before*, was not significant for the variables NWps ($R = 0.19$, $R^2 = 0.04$, $p = 0.22$), AUC ($R = 0.23$, $R^2 = 0.05$, $p=0.12$) and HRV ($R = 0.26$, $R^2 = 0.07$, $p = 0.102$), however it was significant for OSV ($R= 0.58$, $R^2 = 0.34$ $p = 0.000$).

TABLE I

Mean values (ranges) for SCA (number of waves per second and area under the curve of waves), HRV and OSV (maximum HR and minimum OS) for the periods *before*, *during* and *after* heel prick in 41 healthy full-term newborns

	before	*during*	*after*	*p*
NWps	0.12 (0-0.36)	0.24 (0-0.6)	0.13 (0-0.56)	0.000
AUC (μSs)	1.43 (0-6.45)	1.85 (0-9.77)	1.10 (0-10.91)	0.03
Maximum HR (bpm)	139 (112-160)	149 (119-185)	139 (119-168)	0.001
Minimum OS (%)	94 (87-98)	92 (80-98)	93 (66-98)	0.004

Notes: SCA = skin conductance activity; NWps = number of waves per second; AUC = area under the curve of waves; HRV = heart rate variability; OSV = oxygen saturation variability; μSs = microsiemens second; HR= heart rate; bpm= beats per minute; OS= oxygen saturation; (%) = percent

TABLE II

Means (ranges) for differences in SCA (number of waves per sec and area under the curve of waves), HRV and OSV (maximum HR and minimum OS) between *during* and *before*, between *after* and *during*, and between *after* and *before* periods related heel prick in 41 healthy full-term newborns

	during-before	*after-during*	*after-before*
NWps	0.12(-0.2—0.53)**	-0.11(-0.6—0.23)**	0.08(-0.2—0.3)
AUC (μSs)	0.42(-4.94—8.26)	-0.74(-9.77—8.07) *	-0.32(-5.32—9.97)
Maximum HR (bpm)	9.72(-39—52)**	-9.71(-45—21) **	0.21(-36—37)
Minimum OS (%)	-1.68(-13—4)**	1.19(-30—14)**	-0.54(-29—8)

Notes: Wilcoxon test * $p < 0.05$ ** $p < 0.01$; SCA = skin conductance activity; NWps = number of waves per sec; AUC = area under the curve of waves; HRV= heart rate variability; OSV = oxygen saturation variability; μSs = microsiemens second; HR= heart rate; bpm= beats per minute; OS=oxygen saturation; (%) = percent

IV. DISCUSSION

Three measures of pain in newborns, HRV, OSV, and SCA, were fit to the model analyzed. Measures of autonomic nervous system response to pain stimulus can also be derived from HRV and OSV, although these can suffer interference from individual and environmental variables [3],[5],[6]. Despite that SCA can vary according to gestational age, prior number of painful events and duration of stimulus, it seems to be more specific to pain response as it is not influenced by circulatory or respiratory changes or the use of cardiovascular drugs [12],[13]. Moreover, the HRV and OSV are regulated at brainstem level, different from the SCA, where higher brain areas known to be involved in pain perception are activated during painful procedures [29],[30].

In this study we found that NWps, HRV and OSV measures fit the parameters *intensity*, *reactivity* and *direction*. The parameter *regulation* was confirmed by NWps, AUC, HRV and OSV. The *slope* between the periods *before* and *during* was not satisfactory for the variables NWps, AUC and HRV. However, it was significant for OSV. The *intensity* level for both HRV and OSV was similar to those found in other studies with full term newborns, [5],[6],[13] varying significantly among the three periods observed, not suffering effects of clinical and demographic variables.

The NWps reaction is around two seconds after the stressor event and the level of these responses does not vary with gestational age and postnatal age [7],[8],[10],[11]-[13],[28],[31],[32]. This fact was confirmed in this study by a significant elevation of the NWps from 0.12 to 0.24 in a 15 second period after heel prick (Table I). These values are quite similar to those found by Hellerud and Storm that found 0.25 NWps in term newborns at one week postnatal age also subjected to heel prick [10]; and different to those by Harisson and colleagues, in newborns of several gestational ages, which showed an increase in the NWps up to 0.08 immediately after heel prick [18]. These latter values may be different due to the presence of premature conditions where there is evidence of significant increase of 0 to 0.03 NWps after heel prick in premature newborns with gestational age between 29 and 35 weeks, and with postnatal age between 1 and 25 days [31].

The *intensity* level for the NWps found by us was larger than those found by Harrison and colleagues [18] and there may be two justifications for this. The first one is the possible exposure of our sample to an intrauterine stress, because most of the newborns were either large or small for gestational age or from diabetic mothers. There is evidence that stress can affect SCA as maternal stress increases the SCA of the mother and the fetal HRV [33]; and premature newborns exposed to a greater number of punctures are more sensitive than those exposed to fewer punctures [11]. Otherwise, the small number of times that our newborns were subjected to punctures seems not to have interfered with the increase of the SCA baseline. However, none of these covariates was correlated with the variables studied here. Secondly, the behavioral state at baseline may have an effect enhancing the SCA as all the newborns were awake before heel prick, as seen by the behavioral state score of 2.1, that was almost twice as large as those found in research with premature newborns submitted to a heel prick [28]. In another study where 15 infants at rest, i.e. asleep and not moving (behavioral state 1) were observed six times during 48 hours the NWps had low values, varying between 0 and 0.04 waves per second and the mean value was 0.002 [34].

The AUC *reactivity* in the present research was not significant, although the AUC had an increment of 1.43 to 1.85 μSs between *before* and *during* the heel prick. There is a similar finding in another study with an increase in the amplitude of waves from 0 to 0.03 μS [31]. Until recently, the amplitude of waves has been used in most research works as a secondary variable for analysis of the SCA [8],[10],[11],[13],[18],[31], making a comparison with the AUC data here obtained difficult. We hypothesized that this absence of ACU *reactivity* can be attributed to short 15 second observing periods as it seems to increase in bigger intervals of 30 and 180 seconds as we could observe during the tests, but it must be tested in further studies.

The *reactivity* of HRV and OSV analyzed separately are considered an indicator of low specificity for the diagnosis of pain, due to environmental and paradoxical variations like instability in blood circulation and respiration [2],[3],[5],[6],[35]. Although there is a trend to HR increment and OS decrement during a painful procedure, the difference found between the periods *before* and *during* may not always be significant [31]. Breastfeeding or use of oral glucose before the painful event may change the HR and OS values [13],[36]. In our sample, most newborns were nursed in the last hour before the procedure and only four newborns received hypertonic sucrose orally, features that did not influence the variables studied as viewed by two-way ANCOVA analysis for clinical variables. This is in agreement with one study with premature newborns that were fed or received oral glucose, which showed reduced crying time, but not differences for HR and SCA [13]. In contrast, two studies with term and premature newborns have shown decreased HR after heel prick, being that the first group was not exposed to oral glucose and the second was exposed to oral glucose before heel prick [5],[36].

The *regulation* parameter was significant for all variables. Several articles involving SCA discuss variation in values found for the periods *during* and *after* heel prick. In our study the NWps and AUC fell significantly from 0.24 to 0.13 and from 1.85 to 1.1 μSs, respectively (Table I). All findings were consistent with those found in other research works [8],[10],[11],[13]. The difference was that the amplitude of waves rather than the AUC was used in those studies. Recent investigations, however, have worked with a new calibration of the SCMS and AUC which now appears to be an index considered more sensitive to assess how forcefully the SNS is firing [37].

The *slope* for all variables, except for OS, was not significant. The function of the *slope* is to predict the level of response during the painful event. However, when the baseline level is high, as in this study, generally the slope is low and the use of this parameter degrades in analysis. No reports were found involving *slope* and SCA. It was noted that the values for HRV and OSV in the period *after* returned to an equal or inferior level than in the period *before*, that the NWps was slightly above that found for the period *before*, and that the AUC was much lower in the *after* period than the *before* period. However, significant differences were not observed between the periods *after* and *before* for all variables. The behavior of the SCA agreed with the expected evaluation of pain through physiological indicators, where the tendency of the baseline of the *after* period to stay above that of the period *before* the painful stimulus to NWps. One explanation for the findings of HRV and OSV would be that intervals of time less than three minutes are more than sufficient to regulate the inhibitory response of the autonomic nervous system [1],[5].

V. CONCLUSION

In conclusion, NWps, HRV and OSV fit four of the psychophysical parameters of a physiologic measure being sensitive enough to show significant psychophysical change to a painful stimulation. The reactivity for AUC was not significant and needs major studies for acceptance. Our results help to state the response of the SCA as a valid physiological measure. Despite the fact that OSV was the only measure to meet the five parameters, SCA may be considered a marker related more specifically with the

painful event and reliable to be used alone in clinical contexts, suitable especially to surgery or in critically ill newborns due to its high efficacy, as both HRV and OSV show strong evidence of being also sensitive to a great influence of exogenous and endogenous factors. Thus, the HRV and OSV would only be recommended in non-emergencies contexts if adopted in combination with other physiological and behavioral methods of pain assessment [5],[6],[10].

ACKNOWLEDGMENT

This research was sponsored by grants from FAPDF, CNPq and FAHUB and received the support of the staff of the Neonatal Care Unit, University of Brasilia Hospital, Brazil. Hanne Storm is also CEO and co-owner of Med-Storm Innovation, which has developed the equipment used in this study. Special thanks to the mothers, babies, Marcos Vinicius Melo de Oliveira, Mariana Bittencourt Aflalo and NaiaraViudes for their participation and Adrian Tildesley as a native English reviewer.

REFERENCES

[1] R. D. Goldman and G. Koren, "Biologic markers of pain in the vulnerable infant," *Clin. Perinatol.*, vol. 29, no.3, pp. 415-426, Sep. 2002.

[2] R. Nader and K.D. Craig, *Infant pain expression throughout the first year of life*, Saarbrücken, VDM Verlag Dr. Müller Aktiengesellschaft & Co KG, 2008, pp.1-55.

[3] B. J. Stevens, R. R. P. Riddell, T. E. Oberlander, and S. Gibbins, "Assessment of pain in newborns and infants," in: *Pain in Neonates and Infants*, 3rd ed., K.J.S. Anand, B.J. Stevens and P.J. McGrath, Ed. Philadelphia. Elsevier, 2007, pp. 67–90.

[4] M. Walden, J. H. Penticuff, B. Stevens, M. Lotas, C. A. Kozinetz, A. Clark, *et al*, "Maturational changes in physiologic and behavioral responses of preterm newborns to pain," *Adv. Neonatal Care*, vol. 1, no. 2, pp. 94-106, Dec. 2001.

[5] A. L. S. T. Pereira, R. Guinsburg, M. F. B. Almeida, A. C. Monteiro, A. M. N. Santos, and B. I. Kopelman, "Validity of behavioral and physiologic parameters for acute pain assessment of term newborn infants," *Sao Paulo Med. J.*, vol. 117, pp. 72-80, 1999.

[6] A. B. M. Serpa, R. Guinsburg, R. C. X. Balda, A. M. N. Santos, K. C. N. Areco, and C. A. Peres, "Multidimensional pain assessment of preterm newborns at the 1st, 3rd and 7th days of life," *Sao Paulo Med. J.*, vol. 125, pp. 29-33, 2007.

[7] D. H. Crowell, C. M. Davis, B. J. Chun, and F. J. Spella, "Galvanic Skin Reflex in newborn humans," *Science*, vol. 21, no. 148, pp. 1108-1111, May 1965.

[8] M. Eriksson, H. Storm, A. Fremming, and J. Schollin, "Skin conductance compared to a combined behavioural and physiological pain measure in newborn infants," *Acta Paediatr.*, vol. 97, pp. 27-30, 2008.

[9] G. Gladman and M. L. Chiswick, "Skin conductance and arousal in the newborn infant," *Arch. Dis. Child.*, vol. 65, pp. 1063-1066, 1990.

[10] B. C. Hellerud and H. Storm, "Skin conductance and behavior during sensory stimulation of preterm and term infants," *Early Hum. Dev.*, vol. 70, pp. 35-46, 2002.

[11] H. Storm, "Skin conductance and the stress response from heel stick in preterm infants," *Arch. Dis. Child. Fetal Neonatal Ed.*, vol. 83, pp. 143-147, 2000.

[12] H. Storm, "Changes in skin conductance as a tool to monitor nociceptive stimulation and pain," *Curr. Opin. Anaesthesiol.*, vol. 21, pp. 796-804, 2008.

[13] H. Storm and A. Freming, "Food intake and oral sucrose in preterms prior to heel prick," *Acta Paediatr.*, vol. 91, pp. 555-560, 2002.

[14] T. F. Oberlander and J. P. Saul, "Methodological considerations for the use of heart rate variability as a measure of pain reactivity in vulnerable infants," *Clinics in Perinatology.*, vol. 29, no. 2, pp. 427-443, Sep. 2002.

[15] G. Bini, K. E. Hagbarth, P. Hynninen, and B. G. Wallin, "Thermoregulatory and rhythm-generating mechanisms governing the sud motor and vasoconstrictor outflow in human cutaneous nerves," *J. Physiol.*, vol. 306, pp. 537-552, Sep. 1980.

[16] K. E. Hagbarth, R. G. Hallin, A. Hongell, H. E. Torebjork, and B. G. Wallin, "General characteristics of sympathetic activity in human skin nerves," *Acta Physiol. Scand.*,vol 84, pp. 164-176, 1972.

[17] H. J. Habler, W. Janig, M. Krummel, and O. A. Peters, "Respiratory modulation of the activity in postganglionic neurons supplying skeletal muscle and skin in the rat hindlimb," *J. Neurophysiol.*, vol. 70, pp. 920-930, 1993.

[18] D. Harrison, S. Boyce, P. Loughnan, P. Dargaville, H. Storm, and L. Johnston, "Skin conductance as a measure of pain and stress in hospitalised infants," *Early Hum. Dev.*, vol. 82, no 9, pp. 603-608, September 2006.

[19] V. G. Macefield and B. G. Wallin, "The discharge behaviour of single sympathetic outflow in normotensive human sweat glands," *J. Auton. Nerv. Syst.*, vol. 14, pp. 277-286, 1996.

[20] D. Oken and H. A. Heath, "The Law of Initial Values: some further considerations," *Psychosom. Med.*, vol. 25 no. 1, pp. 3-12. January 1963.

[21] B. G. Wallin, "Recordings of impulses in unmyelinated nerve fibres in man: Sympathetic activity," *Acta Anaesthesiol. Scand*., vol. 70, pp. 130-136, Supplement, 1978.

[22] B. G. Wallin, G. Sundlöf, and W. Delius, "The effect of carotid sinus nerve stimulations on muscle and skin nerve sympathetic activity in man," *Pflugers Arch.*, vol. 358, no.2, pp. 101-110, 1975.

[23] G. G. Berntson, B. T. Uchino, J. T. Cacioppo, "Origins of baseline variance and the law of Initial Values," *Psychophysiology*, vol. 31, pp. 204-210, 1994.

[24] Declaration of Helsinki, Ethical Principles for Medical Research involving human subjects, World Medical Association. Available from: http://www.wma.net/e/policy/b3.htm**.** [last access on 2010 sep 01].

[25] Diretrizes e Normas Regulamentadoras de Pesquisa Envolvendo Seres Humanos. Resolução 196/96, do Conselho Nacional de Saúde, Brasília, Brasil. Ministério da Saúde, 1996, pp.1-19.

[26] H. F. Pretchel, "The behavioral states of the newborn infant (a review)," *Brain Res.*, vol. 76, pp. 185-212, 1974.

[27] R. Edelberg, "Electrical properties of the skin", in: *Methods in psychophysiology*, C. C. Brown, Ed. Baltimore. Williams & Wilkins, 1967, pp. 1-59.

[28] H. Storm, "The development of a software program for analyzing skin conductance changes in preterm infants," *Clinical Neurophysiology*, vol. 112, no 8, pp. 1562-1568, Aug. 2001.

[29] A. A. Dubé, M. Duquette, M. Roy, F. Lepore, G. Ducan, and P. Rainville, "Brain activity associated with the electrodermal reactivity to acute heat pain," *Neuroimage*, vol. 45, no.1, pp. 169-180, Mar. 2009.

[30] M. Fredrikson, T. Furmark, M. A. Olsson, H. Fischer, J. Andersson, and B. Långstrom, "Functional neuroanatomical correlates of electrodermal activity: A positron emission tomographic study," *Psychophysiology*, vol. 35, no. 2, pp. 179-185, Apr. 1998.

[31] A. C. Gjerstad, K. Wagner, T. Henrichsen, H. Storm, "Skin conductance as a measure of discomfort in artificial ventilated children," *Pediatrics*, vol. 122, no. 4, pp. 848-53, Oct. 2008.

[32] H. Storm, K. Myre, M. Rostrup, O. Stokland, M.D. Lien, and J.C. Raeder, "Skin conductance correlates with perioperative stress," *Acta Anaesthesiol. Scand.*,vol. 46, pp. 887-895, 2002.

[33] J. A. DiPietro, K. A. Costigan, and E. D. Gurewitsch, "Fetal response to induced maternal stress," *Early Hum. Dev.,* vol. 74, no 2, pp. 125-138, Nov. 2003.

[34] I. Roeggen, D. Harrison, L. Johnston, S. Boyce, P. Loughnan, H. Storm, *et al*, "Skin conductance variability between and within hospitalized infants at rest (Periodical style—Accepted for publication)," *Early Hum. Dev,* to be published.

[35] I. R. Landrot, F. Roche, V. Pichot, G. Teyssier, J.M. Gaspoz, J.C. Barthelemy, *et al*, "Autonomic nervous system activity in premature and full-term infants from theoretical term to 7 years," *Auton. Neurosci.,* vol. 136, pp. 105-109, Oct. 2007.

[36] N. B. Freire, J. B. S. Garcia, Z. C. Lamy, "Evaluation of analgesic effect of skin-to-skin contact compared to oral glucose in preterm neonates," *Pain*, vol. 13, no. 1, pp. 928-33, Sep. 2008.

[37] L. Pereira-da-Silva, D. Virella, I. Monteiro, S. Gomes, P. Rodrigues, H. Storm, *et al*, "Acute nociceptive responses to stimuli of different durations are better differentiated in neonates by Skin Conductance Algesimeter than by Neonatal Infant Pain Scale," (submitted for publication).