Sympathetic Nervous System Activity and Pain-Related Response Indexed by Electrodermal Activity During the Earliest Postnatal Life in Healthy Term Neonates

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Summary

Sympathetic nervous system (SNS) undergoes a prolonged period of fetal and neonatal development and maturation during which is vulnerable to a variety of influences (e.g. painful experiences). Thus, we aimed to evaluate SNS activity at rest and in response to stressful stimulus (pain) within the earliest postnatal life in healthy term neonates using electrodermal activity (EDA) measures. In twenty eutrophic healthy term neonates EDA was recorded within the first two hours after birth (measurement 1 – M1) and 72 h after birth (measurement 2 – M2) at rest and in response to pain (M1 – intramuscular K vitamin administration; M2 – heel stick). Evaluated parameters were skin conductance level (SCL), non-specific skin conductance responses (NS.SCRs), skin SCL 10 s before pain stimulus (SCL_10 before pain), skin conductance response (SCR) peak after pain stimulus, SCL 10 s after pain stimulus (SCL_10 after pain), SCR magnitude, latency, SCR rise/decline time, SCR half recovery time. SCL was significantly decreased at rest during M2 compared to M1 (p=0.010). SCL_10 before pain, SCR peak after pain, and SCL_10 after pain stimulus were significantly decreased in M2 compared to M1 (p=0.014, p=0.020, p=0.011, respectively). SCL was significantly decreased and NS.SCRs were significantly higher in the recovery period after the pain stimulus during M2 compared to M1 (p=0.015, p=0.032, respectively). Our results indicate EDA parameters sensitive to detect sympathetic changes during the earliest postnatal life reflecting its potential in early diagnosis of the autonomic maturation – linked pathological states in neonates.

Key words

Autonomic nervous system • Sympathetic nervous system • Electrodermal activity • Neonates • Pain assessment

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Introduction

The autonomic nervous system (ANS) consisting of two branches, sympathetic (SNS) and parasympathetic nervous system (PNS) is extremely important for the proper functioning of the human body and is essential in adequate fetal to neonatal transition, which represents the primary challenge of postnatal life. More specifically, at birth, neural systems including ANS centers in brainstem supported by neuroendocrine changes (predominantly catecholamine levels increase) provide more sustained support to SNS to enable brisk adaptation to postnatal life. Importantly, ANS undergoes a prolonged period of

fetal and neonatal development and maturation. In general, the development and maturation of ANS is significant within the second half of pregnancy and requires at least 37 gestational weeks to reach their accurate functioning. While SNS develops steadily throughout the gestation, PNS shows accelerated development between 25-32 weeks and also between 36-38 weeks of the gestation [1,2]. ANS continues to mature after birth with PNS modulation taking predominance in term neonates [3,4]. The normal increase in PNS activity has been repeatedly evidenced by the evaluation of heart rate variability (HRV), especially by the increased HRV in high – frequency band of spectral analysis in infants born at term [5-7]. However, little is known about the earliest postnatal changes of SNS activity. In this context, electrodermal activity (EDA) also known as skin conductance (SC) represents a useful parameter reflecting the changes in the sympathetic branch of ANS [8]. The measurement of EDA has begun over 100 years ago and refers to all active and passive electrical phenomena in the skin. In other words, SC measures the changes of the skin electrical conductivity due to the activity of eccrine sweat glands which is under the pure control of sympathetic cholinergic nervous system [9]. Moreover, in psychophysiological research EDA is commonly used to index the general states of arousal [10]. In this context, EDA measurement can be used to assess the arousal – linked SNS activity changes during early postnatal life in neonates. To the best of our knowledge, there are no studies concerning solely the issue of SNS maturation within the first postnatal days including immediate postnatal adaptation during 2 h after birth. Thus, we have focused on evaluation of SNS activity indexed by EDA in healthy term neonates during this earliest postnatal period.

Further, neonates, especially those admitted to intensive care units, commonly undergo many repeated pain experiences (e.g. medically – necessary, often invasive procedures) [11,12]. It is well-known that repeated pain stimulation during the critical period of early life can influence the development of central nervous system in the way of neurocognitive, behavioral, social or emotional impairments [13]. Importantly, pain – related changes are preventable with appropriate analgesia [14]. Therefore, an appropriate pain management (pain assessment as well as treatment) remains crucial in the prevention of short – and long – term consequences of early life pain. However, to date, no gold standard has been identified for accurate pain assessment in neonates [15]. In this context, physiological measurements such as EDA evaluation can represent potential objective reliable tool for pain discrimination as the pain experience evokes emotional sweating leading to SC increase most evident on palms or soles [16,17]. However, despite relatively good availability of EDA devices, there is still little information regarding pain stimulus – evoked EDA responses in term neonates in the critical earliest postnatal development. Thus, we also aimed to evaluate the postnatal pain-evoked sympathetic changes using EDA evaluation in healthy term neonates.

Methods

Participants

The study included 20 healthy term neonates born spontaneously (10 males, average gestational age: 39.3±1.4 weeks, average birth weight: 3488.42±519.95 g). The inclusion criteria were 38-42 weeks of gestational age at birth and physiological immediate postnatal adaptation without need for ventilation support in the delivery room (Apgar score ≥8 in the first minute and ≥9 in the fifth minute after birth). The exclusion criteria included all conditions that are proven or very likely associated with abnormal autonomic function: pathological gravidity (preterm birth, preeclampsia, intrauterine growth restriction, etc.), maternal systemic disease (including gestational and other types of diabetes), smoking during pregnancy, congenital anomaly of the neonate, signs of perinatal infection, hypoglycemia and hyperbilirubinemia requiring phototherapy during hospitalization.

This study presents preliminary data of our prospective observational study „Assessment of Autonomic Nervous Regulation During Postnatal Period in Neonates“, registered in Clinical Trials database (NCT03830424). The study was performed from January 2018 to December 2020 at the Department of Neonatology in University Hospital in Martin and was approved by the Ethical Committee of Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava (EK69/2018). All procedures performed in our study were in accordance with the 2000 Helsinki declaration of the world Medical Association. Subjects were included after meeting the inclusion criteria and with approval of their parents after signing the informed consent. All parents were carefully informed about study protocol.

Protocol

The examination was performed at the Department of Neonatology in University Hospital Martin under standard conditions with the minimization of internal and external stimuli, i.e. in quiet room, warm environment (26 °C), air humidity around 50 %, during the day between 7-12 am. Two silver – silver chloride bipolar electrodes with sampling rate 256 Hz placed on the sole of the neonate were used for the continuous EDA recording (FlexComp Infinity Biofeedback, Thought Technology Ltd., Canada) according to the recommendation of EDA biosignal measurements [18]. Neonates were all bathed with water and baby liquid washing product approximately 30 min after birth to avoid the possible influence of vernix caseosa on the EDA signal. Thus, the skin surface under the EDA electrodes was clean and dry during the examination. The examination protocol consisted of two EDA measurements: measurement 1 (M1) performed within two hours after birth and measurement 2 (M2) after 72 h of life at rest and in response to the pain stimulus which was applied as the part of routine neonatal care – intramuscular administration of K vitamin (M1) and heel stick (M2). No analgesia was administered to the subjects during the procedures. The time interval for EDA measurement was 6 min at rest and 6 min after application of pain stimulus. Recordings were initiated after the stabilization of the subjects (approximately 10 min – adaptation interval) during sleep or quiet alertness in the supine position in an incubator (M1) or neonatal cot (M2) under the same temperature conditions.

Data analysis

EDA raw records were carefully checked and rare artefacts were manually removed for exact data analysis. The tonic EDA component was extracted by the 10^{th} order low-pass finite impulse response filter [19]. Furthermore, the skin conductance level (SCL, microSiemens (μS)) – an index of tonic level of skin electrical conductivity, was evaluated as the average amplitude of the tonic EDA from 5 min of artefact – free recordings. Consequently, the non-specific skin conductance responses (NS.SCRs) were evaluated as a rate of spontaneous skin conductance response (SCR) waves without external stimuli reflecting momentary arousal with fixed threshold (0.05 μS) [20]. SCL and NS.SCRs were computed at rest and during recovery period after the pain stimulus. Further, the SC properties in response to the pain stimulus were evaluated using the following indices: SCL_10 before (μS) – SCL value 10 s before the application of the pain stimulus, latency (s) – temporal distance between the pain stimulus and the initiation of the response (typical range is between 1 and 5 s), SCR peak after pain (μS) – maximal or minimal value after the pain stimulus, SCR magnitude (μS) – absolute difference between the peak of the stimulus response and the onset of SCR rise or decline (minimum threshold magnitude is ± 0.0001 μS), SCR rise/decline time (s) – temporal distance between the initiation and the peak value, SCR half recovery time (s) – temporal distance between SCR peak after pain and the reduction of SCR to half of the amplitude, and SCL_10 after – SCL value 10 s after the application of the pain stimulus.

Statistical analysis

Statistical analysis was performed in jamovi 1.6.9 (Sydney, Australia). The data normality (Gaussian/non – Gaussian distributions) was evaluated by Shapiro – Wilk normality test. All analysed data were not normally distributed. Consequently, the Wilcoxon rank test was used for EDA – SCL, NS.SCRs, and EDA parameters related to pain stimulus – comparisons between M1 and M2. Non-parametric ANOVA (Friedman test) with Durbin – Conover *post hoc* test was used for comparisons of SCL at rest, SCL_10 before pain, SCR peak after pain, SCL_10 after pain, and SCL in the recovery during M1 and M2 measurement. EDA parameters were expressed as median and interquartile range. A value of $p<0.05$ (two – tailed) was considered statistically significant.

Results

Comparison of SCL and NS.SCRs at rest

SCL was significantly decreased during M2 compared to M1 ($p=0.010$). No significant changes were found between resting measurements of the index NS.SCRs ($p=0.850$). The significant result is summarized in Figure 1.

Comparison of EDA parameters during the pain stimulus application

SCL_10 before pain, SCR peak after pain, and SCL_10 after pain were significantly decreased during M2 compared to M1 ($p=0.014$, $p=0.020$, $p=0.011$, respectively). The other parameters were without significant changes between M1 and M2. The significant results are summarized in Figure 2.

Comparison of SCL and NS.SCRs in the recovery period after the pain stimulus

SCL was significantly decreased in the recovery period after the pain stimulus during M2 compared to M1 (p=0.015). Index NS.SCRs was significantly higher in the recovery period during M2 compared to M1 (p=0.032). The significant results are summarized in Figure 3.

Comparison of the selected EDA parameters during M1 and M2 measurements

No significant changes were found between rest SCL, SCL_10 before pain, SCR peak after pain, SLC_10 after pain, and recovery SCL in neonates during M1 neither M2 measurement.

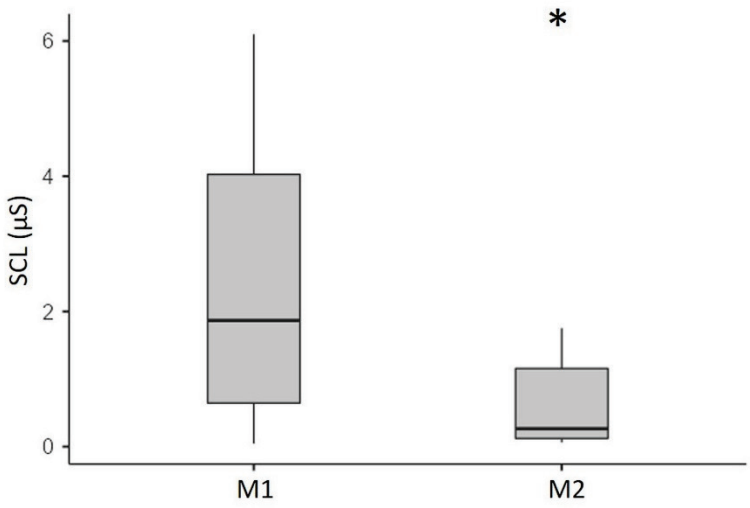

Fig. 1. Boxplots of skin conductance level (SCL, µS) during measurement within first two hours after birth (M1) and measurement after 72 h after birth (M2) at rest in newborns. * represents statistically significant difference (p<0.05) between M1 and M2.

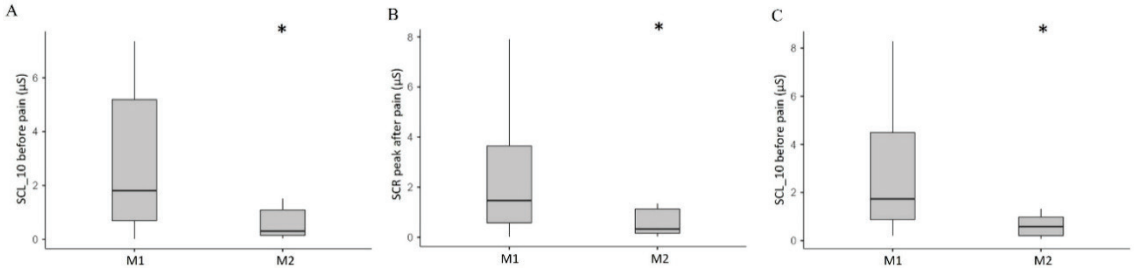

Fig. 2. Boxplots of EDA parameters of pain stimulus during measurement within first two hours after birth (M1) and measurement after 72 h after birth (M2) in newborns. (**A**) SCL_10 before pain – skin conductance level 10 s before the application of the pain stimulus; (**B**) SCR peak after pain – skin conductance response peak after the pain stimulus; (**C**) SCL_10 after pain – skin conductance level 10 s after the application of the pain stimulus. * represents statistically significant differences (p<0.05) between M1 and M2.

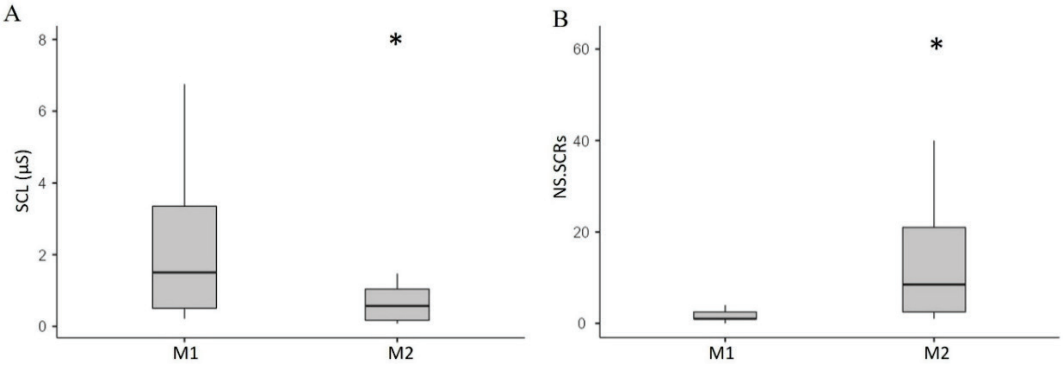

Fig. 3. Boxplots of (**A**) skin conductance level (SCL, µS), and (**B**) non-specific skin conductance responses (NS.SCRs) during measurement within first two hours after birth (M1) and measurement after 72 h after birth (M2) during recovery in newborns. * represents statistically significant differences (p<0.05) between M1 and M2.

Discussion

The major findings of this study regarding EDA evaluation during early postnatal life in term neonates are following: 1) SCL was higher within the first two hours after birth associated with significant SCL decreasing after 72 h of life reflecting a post – delivery stress-related sympathetic withdrawal after postnatal adaptation, 2) in response to pain, SCL_10 before pain, SCR peak after pain, and SCL_10 after pain were significantly higher during the first compared to the second measurement. However, it is important to note that this significant difference can be strongly influenced by the SCL baseline level (2 h compared to 72 h after birth). In this context, no significant differences were found between SC properties in response to the pain stimulus during either first and second measure indicating these pain-related procedures as not sufficiently aroused conditions for neonates to evoke immediate stimulus-related sympathetic alterations, however 3) NS.SCRs were significantly higher in the recovery period after the pain stimulus during the second compared to the first measurement reflecting increase of stress response with the number of painful procedures. Based on our results we suggest that EDA measurement may represent a sensitive tool to determine the developmental/ maturational changes in SNS during the earliest postnatal life.

Birth, i.e. transition from fetal to neonatal physiology, represents the first major life challenge associated with dynamic physiological changes, in which ANS plays a crucial role [21,22]. ANS develops in the fetal period during the development of the central nervous system and continues to mature after birth [22,23]. More specifically, the sympathetic branch of ANS matures steadily throughout the gestation and the parasympathetic division of ANS shows accelerated maturation between 25-32 weeks and also between 36-38 weeks of the gestation [2,24,25]. Before birth, the SNS outflow from central ANS structures (i.e. structures involved in the central autonomic network (CAN) [26] such as hypothalamus and forebrain) supported by neuroendocrine signalling (e.g. catecholamines) increases to enable the brisk and efficient postnatal adaptation [22]. At birth, the connectivity between higher cortical networks as well as between cortical centers and brainstem structures involved in ANS control increases [27] allowing appropriate SNS functioning. After birth, ANS constantly continues to develop with a predominant increase in the parasympathetic activity during the following two years [3,4] important for the proper regulation of increasing social interactions the infant encounters [23]. Contrary, the precise understanding of SNS development in the earliest postnatal life is limited [28]. In this context, EDA evaluation represents a non-invasive marker indexing sympathetic cholinergic activity that measures the changes in the activity of eccrine sweat glands resulting in skin conductance changes [20,29]. Moreover, EDA can reflect the nervous system readiness as well as emotional/arousal state in neonates [30]. Thus, EDA evaluation in terms of spontaneous skin conductance changes associated with arousal may represent an optimal tool to investigate SNS development/maturation. From this perspective, peripheral SNS is developed at 18 week of gestation, still continues to mature after birth during first weeks of life [30]. Similarly, the palmar and plantar sweat glands are developed in the second trimester, however, there are inconsistent findings regarding their full maturation and normal functioning. While Harpin and Rutter [31] reported functional emotional sweating occurrence in term neonates, Jorgenson *et al.* [32] suggested that eccrine sweat glands are fully matured and functional even after two postnatal weeks. Our study of the highest SCL during two hours compared to 72 h after birth confirms the functionality of eccrine sweat glands in term neonates immediately after birth. These findings can provide novel insights into the SNS regulatory mechanisms immediately after birth and during the earliest postnatal life.

Further, neonates are commonly exposed to harmful stimuli from birth, through routine heel lancing for screening tests to repeated invasive procedures in intensive care units [11]. It is widely reported that often recurring stressful and painful stimuli in neonates can result in detrimental short – term (e.g. disruption of the postnatal adaptation or of the development of parent-infant bonding) as well as long – term (e.g. altered sensitivity to pain, worsen psychological outcomes) effects on health [14,33]. As many injury – related changes can be preventable with the proper anesthetics administration [14], the understanding of the nociceptive system development to pain stimuli is crucial in the neonatal pain management. From the developmental perspective, the somatosensory system undergoes postnatal maturation at peripheral and central levels immediately after birth. Although most areas involved in pain processing are developed around birth, and thus the

term neonate´s brain should be able to process noxious information [34], when exactly the neonate brain is capable to integrate nociceptive information into a painful experience is still unknown. Nociception is transformed into pain in the complex cortical network including the frontal, parietal, somatosensory, insular, and cingulate cortices [35]. Transformation of the nociception into conscious pain perception has some similarities in infants and adults. While noxious mechanical stimuli evoke similar hemodynamic response in the somatosensory, anterior cingulate cortex, and insular cortex in both adults and children, the painful stimulus increases hemodynamic response in amygdala and orbitofrontal cortex (regions thought to be involved in sensory stimuli discrimination and identification) only in adults [36] reflecting thus an immature ability to process specific somatosensory input in neonates. On the other hand, additional brain regions such as auditory cortex, caudate, and hippocampus respond to noxious stimuli only in neonates. Importantly, these brain areas can be responsible for encoding and retention of the pain stimulus memories in infant brain [14,36-38]. In addition, excitatory and inhibitory receptive fields in the spinal cord are less "aligned" in neonates compared to adults. Diffuse and large receptive fields are likely associated with poorer discrimination between noxious and non – noxious stimuli as well as poorer spatial localization [39]. Descending inhibition is functionally immature during the first postnatal weeks [40]. More specifically, while in adults the serotoninergic projections as a major source of descending control facilitating or inhibiting nociception depend on the state of pain [41], in neonates, the serotoninergic descending system facilitates nociceptive and tactile processing. During the first postnatal weeks, there is a maturation – related switch from facilitation to predominant inhibition of nociception [42]. Altogether, as somatosensory neural circuits undergo early postnatal maturation, are highly susceptible to injuries during this critical life period. Thus, the appropriate pain management is very important to prevent the short – term and long – term consequences of early life pain on the nociceptive system development/functioning. Successful pain prevention and treatment should be preceded by a reliable pain assessment. However, as neonates are unable to quantify their pain by self-report, the exact pain assessment in neonates is still challenging for clinicians as well as researchers due to the lack of "gold standard". The majority of the developed tools in the pain assessment encompass behavioral (e.g. facial expression, body movements, cry qualities) and physiological (e.g. heart rate, blood pressure) responses or combine both into a composite measure. In this context, EDA measurement based on the SNS response to stress could represent a valuable tool to assess the pain as the pain occurrence induces stress – related sympathetic excitation and emotional sweating at palmar or plantar areas resulting in increasing of skin conductance monitored by EDA devices. In addition, EDA measurement is non – invasive, safe, easy to handle method only little influenced by other factors (e.g. circulatory changes) and EDA scores can be also easily understood by clinicians. However, relatively little research examining pain stimulus-related EDA changes exist in the period of the earliest postnatal development [43,44]. Our study revealed no significant changes in the selected pain stimulus – related EDA parameters during M1 neither M2 measurement reflecting EDA evaluation as not appropriate objective tool for pain assessment. However, EDA parameter NS.SCRs (i.e. useful measure of arousal) was significantly higher in the recovery period after the secondly applied pain stimulus during the second measurement 72 h after birth compared to firstly applied pain stimulus during the first measurement 2 h after birth indicating increased stress response after repeated pain stimulus. This finding is in accordance with the study by Storm [45] that revealed the increase of stress response with the number of heel lancing. These findings can suggest that neonates remember pain. However, further research in this area is needed to precisely elucidate this suggestion.

Conclusions

Our study revealed the highest sympathetic activity indexed by EDA early after birth (two postnatal hours) with its significant decrease after 72 h of postnatal life indicating the post – delivery stress withdrawal linked to appropriate postnatal adaptation in healthy term neonates. EDA evaluation can represent a sensitive tool to detect sympathetic changes during the earliest postnatal life reflecting its potential in early diagnosis of the autonomic maturation – linked pathological states in neonates.

Study Limitations

The sleep stage in our subjects during measurements was not specified. We have focused on

EDA evaluation in neonates in this study reflecting only the activity of sympathetic branch of ANS. In this context, simultaneous HRV evaluation (mainly in high frequency band) reflecting activity of the parasympathetic branch of ANS could bring more information about complex ANS changes during the earliest postnatal life. Further, vaginal delivery evokes a dramatic surge in vasopressin which has been shown to exert analgesic effects, thus it can influence the neonatal pain responsivity during the first hours after birth. Moreover, regarding the most precise pain assessment in neonates, the multidimensional assessment (e.g. simultaneous monitoring of several physiological parameters in association with behavioral assessment) of responses during and after pain stimuli is nowadays highly recommended. Thus, future research should take this into consideration.

Conflict of Interest

There is no conflict of interest.

References

1. Longin E, Gerstner T, Schaible T, Lenz T, König S. Maturation of the autonomic nervous system: Differences in heart rate variability in premature vs. term infants. J Perinat Med 2006;34:303-308. https://doi.org/10.1515/JPM.2006.058
2. De Rogalski Landrot I, Roche F, Pichot V, Teyssier G, Gaspoz J-M, Barthelemy J-C, Patural H. Autonomic nervous system activity in premature and full-term infants from theoretical term to 7 years. Auton Neurosci Basic Clin 2007;136:105-109. https://doi.org/10.1016/j.autneu.2007.04.008
3. Oliveira V, Von Rosenberg W, Montaldo P, Adjei T, Mendoza J, Shivamurthappa V, Mandic D, Thayyil S. Early postnatal heart rate variability in healthy newborn infants. Front Physiol 2019;10:1-12. https://doi.org/10.3389/fphys.2019.00922
4. Patural H, Pichot V, Flori S, Giraud A, Franco P, Pladys P, Beuchée A, Frédéric R, Barthelemy JC. Autonomic maturation from birth to 2 years: normative values. Heliyon 2019;5:e01300. https://doi.org/10.1016/j.heliyon.2019.e01300
5. Clairambault J, Curzi-Dascalova L, Kauffmann F, Médigue C, Leffler C. Heart rate variability in normal sleeping full-term and preterm neonates. Early Hum Dev 1992;28:169-183. https://doi.org/10.1016/0378-3782(92)90111-S
6. Fyfe KL, Odoi A, Yiallourou SR, Wong FY, Walker AM, Horne RSC. Preterm infants exhibit greater variability in cerebrovascular control than term infants. Sleep 2015;38:1411-1421. https://doi.org/10.5665/sleep.4980
7. Javorka K, Lehotska Z, Kozar M, Uhrikova Z, Kolarovszki B, Javorka M, Zibolen M. Heart rate variability in newborns. Physiol Res 2017;66(Suppl 2):S203-S214. https://doi.org/10.33549/physiolres.933676
8. Aldosky HYY, Bari DS. Electrodermal activity: Simultaneous recordings. In: *Electrochemical Impedance Spectroscopy*. EL-AZAZY M, MIN M, ANNUS P (eds), IntechOpen, London, 2019, pp 1-16.
9. Dawson ME, Schell AM, Filion DL. The electrodermal system. In: *Handbook of Psychophysiology, Fourth Edition*. CACIOPPO JT, TASSINARY LG, BERNTSON GG (eds), Cambridge University Press, Cambridge, 2016, pp 217-243. https://doi.org/10.1017/9781107415782.010
10. Critchley H, Nagai Y. Electrodermal Activity (EDA). In: *Encyclopedia of Behavioral Medicine*. GELLMAN MD, TURNER JR (eds), Springer, New York, 2013, pp 666-669.
11. Simons SHP, van Dijk M, Anand KS, Roofthooft D, van Lingen RA, Tibboel D. Do we still hurt newborn babies? Arch Pediatr Adolesc Med 2003;157:1058. https://doi.org/10.1001/archpedi.157.11.1058
12. Ohlsson A, Shah PS. Paracetamol (acetaminophen) for prevention or treatment of pain in newborns. Cochrane Database Syst Rev 2016;10:CD011219. https://doi.org/10.1002/14651858.CD011219.pub3
13. Vinall J, Grunau RE. Impact of repeated procedural pain-related stress in infants born very preterm. Pediatr Res 2014;75:584-587. https://doi.org/10.1038/pr.2014.16
14. Brewer CL, Baccei ML. The development of pain circuits and unique effects of neonatal injury. J Neural Transm 2020;127:467-479. https://doi.org/10.1007/s00702-019-02059-z
15. Roué JM, Rioualen S, Gendras J, Misery L, Gouillou M, Sizun J. Multi-modal pain assessment: Are near-infrared spectroscopy, skin conductance, salivary cortisol, physiologic parameters, and Neonatal Facial Coding System interrelated during venepuncture in healthy, term neonates? J Pain Res 2018;11:2257-2267. https://doi.org/10.2147/JPR.S165810

16. Eriksson M, Storm H, Fremming A, Schollin J. Skin conductance compared to a combined behavioural and physiological pain measure in newborn infants. Acta Paediatr 2008;97:27-30. https://doi.org/10.1111/j.1651-2227.2007.00586.x
17. Harrison D, Boyce S, Loughnan P, Dargaville P, Storm H, Johnston L. Skin conductance as a measure of pain and stress in hospitalised infants. Early Hum Dev 2006;82:603-608. https://doi.org/10.1016/j.earlhumdev.2005.12.008
18. Fowles DC. The measurement of electrodermal activity in children. In: *Developmental Psychophysiology: Theory, Systems, and Methods*. SCHMIDT LA, SEGALOWITZ SJ (eds), Cambridge University Press, Cambridge, 2007, pp 286-316. https://doi.org/10.1017/CBO9780511499791.012
19. Posada-Quintero HF, Florian JP, Orjuela-Cañón AD, Aljama-Corrales T, Charleston-Villalobos S, Chon KH. Power spectral density analysis of electrodermal activity for sympathetic function assessment. Ann Biomed Eng 2016;44:3124-3135. https://doi.org/10.1007/s10439-016-1606-6
20. Boucsein W, Fowles DC, Grimnes S, Ben-Shakhar G, Roth WT, Dawson ME, Filion DL. Publication recommendations for electrodermal measurements. Psychophysiology 2012;49:1017-1034. https://doi.org/10.1111/j.1469-8986.2012.01384.x
21. Cerritelli F, Frasch MG, Antonelli MC, Viglione C, Vecchi S, Chiera M, Manzotti A. A review on the vagus nerve and autonomic nervous system during fetal development: searching for critical windows. Front Neurosci 2021;15:721605. https://doi.org/10.3389/fnins.2021.721605
22. Mulkey SB, du Plessis A. The critical role of the central autonomic nervous system in fetal-neonatal transition. Semin Pediatr Neurol 2018;28:29-37. https://doi.org/10.1016/j.spen.2018.05.004
23. Porges SW, Furman SA. The early development of the autonomic nervous system provides a neural platform for social behaviour: a polyvagal perspective. Infant Child Dev 2011;20:106-118. https://doi.org/10.1002/icd.688
24. Schneider U, Schleussner E, Fiedler A, Jaekel S, Liehr M, Haueisen J, Hoyer D. Fetal heart rate variability reveals differential dynamics in the intrauterine development of the sympathetic and parasympathetic branches of the autonomic nervous system. Physiol Meas 2009;30:215-226. https://doi.org/10.1088/0967-3334/30/2/008
25. Fyfe KL, Yiallourou SR, Wong FY, Odoi A, Walker AM, Horne RSC. The effect of gestational age at birth on post-term maturation of heart rate variability. Sleep 2015;38:1635-1644. https://doi.org/10.5665/sleep.5064
26. Benarroch EE. The central autonomic network: functional organization, dysfunction, and perspective. Mayo Clin Proc 1993;68:988-1001. https://doi.org/10.1016/S0025-6196(12)62272-1
27. Mulkey SB, Hitchings L, Persaud R, Kota S, Maxwell GL, Baker R, du Plessis A, Govindan R. Cerebral cortical autonomic connectivity in low-risk term newborns. Clin Auton Res 2021;31:415-424. https://doi.org/10.1007/s10286-021-00793-7
28. Schneider U, Bode F, Schmidt A, Nowack S, Rudolph A, Dölker EM, Schlattmann P, Götz T, Hoyer D. Developmental milestones of the autonomic nervous system revealed via longitudinal monitoring of fetal heart rate variability. PLoS One 2018;13:e0200799. https://doi.org/10.1371/journal.pone.0200799
29. Boucsein W. *Electrodermal Activity, Second edition*. Springer, London, 2012, 636 p. https://doi.org/10.1007/978-1-4614-1126-0
30. Hernes KG. Skin conductance changes during the first year of life in full-term infants. Pediatr Res 2002;52:837-843. https://doi.org/10.1203/00006450-200212000-00005
31. Harpin VA, Rutter N. Development of emotional sweating in the newborn infant. Arch Dis Child 1982;57:691-695. https://doi.org/10.1136/adc.57.9.691
32. Jorgenson RJ, Salinas CF, Dowben JS, St John DL. A population study on the density of palmar sweat pores. Birth Defects Orig Artic Ser 1988;24:51-63.
33. Knaepen L, Pawluski JL, Patijn J, van Kleef M, Tibboel D, Joosten EA. Perinatal maternal stress and serotonin signaling: Effects on pain sensitivity in offspring. Dev Psychobiol 2014;56:885-896. https://doi.org/10.1002/dev.21184
34. Verriotis M, Chang P, Fitzgerald M, Fabrizi L. The development of the nociceptive brain. Neuroscience 2016;338:207-219. https://doi.org/10.1016/j.neuroscience.2016.07.026
35. Legrain V, Iannetti GD, Plaghki L, Mouraux A. The pain matrix reloaded: A salience detection system for the body. Prog Neurobiol 2011;93:111-124. https://doi.org/10.1016/j.pneurobio.2010.10.005

36. Goksan S, Hartley C, Emery F, Cockrill N, Poorun R, Moultrie F, Rogers R, ET AL. fMRI reveals neural activity overlap between adult and infant pain. Elife 2015;4:1-13. https://doi.org/10.7554/eLife.06356
37. Bird CM, Burgess N. The hippocampus and memory: Insights from spatial processing. Nat Rev Neurosci 2008;9:182-194. https://doi.org/10.1038/nrn2335
38. Grahn JA, Parkinson JA, Owen AM. The cognitive functions of the caudate nucleus. Prog Neurobiol 2008;86:141-155. https://doi.org/10.1016/j.pneurobio.2008.09.004
39. Fitzgerald M. Development of pain mechanisms. Br Med Bull 1991;47:667-675. https://doi.org/10.1093/oxfordjournals.bmb.a072499
40. Koch SC, Fitzgerald M. Activity-dependent development of tactile and nociceptive spinal cord circuits. Ann N Y Acad Sci 2013;1279:97-102. https://doi.org/10.1111/nyas.12033
41. Bardin L. The complex role of serotonin and 5-HT receptors in chronic pain. Behav Pharmacol 2011;22:390-404. https://doi.org/10.1097/FBP.0b013e328349aae4
42. Schwaller F, Kanellopoulos AH, Fitzgerald M. The developmental emergence of differential brainstem serotonergic control of the sensory spinal cord. Sci Rep 2017;7:1-12. https://doi.org/10.1038/s41598-017-02509-2
43. Hu J, Modanloo S, Squires JE, Harrold JA, Harrison D. The validity of skin conductance for assessing acute pain in infants: a scoping review. Clin J Pain 2019;35:713-724. https://doi.org/10.1097/AJP.0000000000000721
44. Kusumaningrum A, Rustina Y, Abuzairi T, Ibrahim N. The skin conductance-based non-invasive pain assessment instrument for infants. Sri Lanka J Child Heal 2022;51:448-455. https://doi.org/10.4038/sljch.v51i3.10249
45. Storm H. Development of emotional sweating in preterms measured by skin conductance changes. Early Hum Dev 2001;62:149-158. https://doi.org/10.1016/S0378-3782(01)00129-3