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Title:

No effect of a musical intervention on stress response to venepuncture in a neonatal population

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Music intervention and stress response in neonates.

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Abstract

Aim: To investigate the effect of a musical intervention on neonatal stress response to venepuncture as measured by salivary cortisol levels and pain profile scores.

Methods: In a randomised control crossover trial, participants were randomised to both a control arm (sucrose) and intervention arm (sucrose and music) for routine venepuncture procedures. Salivary swabs were collected at baseline, 20 minutes post-venepuncture and 4 hours post-venepuncture. Pain levels were assessed using the Premature Infant Pain Profile (PIPP). 16 preterm neonates participated in both arms to complete the study.

Results: Cortisol values were elevated at all timepoints in the intervention arm (baseline, 20 minutes, and 4 hours post-procedure) but not significantly so (p=0.056, p=0.3, and p=0.575 respectively). Median change in cortisol values from baseline was +128.48pg/ml (-47.66-517.02) at 20 minutes and +393.52pg/ml (47.88-1221.34) at 4 hours post-procedure in the control arm compared to -69.564pg/ml (-860.96-397.289) and +100.48pg/ml (-560.46-842.99) at 20 minutes and 4 hours post-procedure in the intervention arm. There was no statistically significant difference observed between groups (p=0.311 at 20 minutes, and p=0.203 at 4 hours post-procedure). PIPP scores were not significantly different between study arms.

Conclusion: Our findings did not support the additional benefit of music intervention on neonatal stress response to venepuncture in preterm infants.

Keywords: Cortisol, music intervention, neonatology

Key Notes:

- This randomised crossover trial is the first to look at both subjective and objective measures of the stress response in the ex-preterm neonate and the effect that a musical intervention has on this response.
- Our findings do not support an additional benefit of music in the neonatal stress response to procedural pain.
- Further research is recommended into music as a non-invasive and non-pharmacological intervention for the neonate.

Introduction:

Preterm infants, or sick term infants, may be subjected to long hospital stays lasting from several weeks to several months. During this period, they are often exposed to a large number of invasive diagnostic and therapeutic procedures, which can be associated with pain and can elicit a stress response as indicated by altered physiological, behavioural and biochemical changes. Research has quantified this exposure, reporting that neonates are exposed to an average of 134 painful procedures within the first two weeks of life (1), and up to 400 painful procedures during their hospital stay (2). This burden has been associated with increased oxygen demand (3), and cerebral oxygenation disturbances (4). Such exposure has been associated with neurodevelopmental morbidity later in life (5,6). It has also reported that neonates can experience prolonged negative effects from pain, with permanent changes in endocrine, immune, and behavioural reactivity to stressful events in childhood and continuing through adult life (7).

Existing guidelines for pain management in the NICU recommend a multidimensional approach, including environmental, pharmacological, and nonpharmacological measures (8). Although the potential benefits of music interventions for infants have been described as far back as 1981(9) it has not yet been established as a routine part of developmental care in the neonatal unit.

The effect of music on pain responses has been highlighted in many different domains (10,11). In a meta-analysis of randomised controlled trials, it was reported that music had significant effects on acute, procedural, and chronic pain as measured by pain scales, physiological variables and use of analgaesic medications (12). In the neonatal population, the effects of music on physiological and behavioural parameters have been studied in the context of both stressed and non-stressed infants. Some studies have reported that music was an effective intervention to decrease stress response in infants undergoing a heel lance procedure, as indicated by changes in heart rate, behavioural state, and facial expressions of pain (13, 14). However other studies have reported no significant differences in heart rate, respiratory rate, or oxygen saturations, (15) and pain scores with exposure to music (16, 17). Rossi et al recently assessed responses to three music interventions in infants undergoing Guthrie test or an intramuscular injection. They reported a significant reduction in heart rate and pain perception, and an increase in oxygen saturations compared to the control group (18). Joyce et al (19) observed that neonates receiving a music intervention had significantly higher oxygen saturations at the end of a circumcision procedure compared to control subjects. They concluded that further investigation was warranted to yield greater understanding of the potential effect of music on other indicators of neonatal pain. A systematic review concluded that there is evidence for some therapeutic effects of music intervention on physiological and behavioural parameters, but cautioned that the methodological

quality of the studies included was generally poor (20). A further review detailed how repeated stress may be associated with negative neurodevelopmental outcomes in preterm infants, and stated that while studies including music intervention reviewed have a positive measurable physiological or behavioral impact, the relative effect size remains unclear (6).

Stress typically leads to an increase in plasma cortisol levels, as well as an increase in salivary cortisol levels. Salivary cortisol levels have been demonstrated to correlate well with plasma levels in neonates (21), and have been reported as a useful indicator of stress in this population (22). There is limited evidence regarding the effect of music intervention on endogenous hormone levels. Salivary cortisol has been used as an outcome measure in a few music intervention studies. Schwilling et al observed a significant reduction in salivary cortisol levels in stable preterm infants following exposure to a live music intervention (23). Conversely, a previous pilot study found no effect of music on salivary cortisol levels in preterm infants but this was limited by a small sample size (24). Qui et al investigated the effect of combined touch and music intervention on procedural pain in preterm neonates (25). They measured β-endorphin levels and blood cortisol levels at the beginning of hospitalization and again 2 weeks later. They found no significant difference in blood cortisol concentration between groups but suggested that salivary cortisol measurement may be more accurate as it does not require a painful procedure first. The purpose of our study is to assess whether the use of music as a non-pharmacological intervention at the time of venepuncture significantly decreases the stress response of preterm neonates. The primary outcome measure of the study was the change in salivary cortisol levels at 20 minutes and 4 hours post-procedure.

Patients and Methods:

Study design

This was a randomised cross-over trial. Patients were recruited to the study from the neonatal intensive care unit and special care baby unit of a tertiary neonatal centre in Cork, Ireland. Recruitment began in March 2016. The study was approved by the Clinical Research Ethics Committee of the hospital. The trial was subsequently registered at clinical trials.gov NCT03028844.

Participants

All infants who were born at less than 32 weeks gestation were screened for inclusion in the trial. Twenty-one infants were recruited (n=21), with a final sample of 16 infants receiving both interventions (n=16). The mean birth gestational age of the enrolled infants was 26+6 (range 24+3 – 32) and the mean birth weight was 933g (range 630g – 1590g). The patient demographics are illustrated in *Table 1*.The inclusion criteria were: less than 32 weeks gestational age at birth (from 23+0 weeks' up to and including 31+6 weeks' gestational age); currently >32 weeks corrected gestational age; medically stable and due to have routine venepuncture as part of their ongoing management. The exclusion criteria were inability to obtain informed consent from parents; infants with known hearing impairment; infants receiving sedative medications; any evidence of dysfunction of the hypothalamic-pituitary-adrenal axis; infants with congenital abnormalities; and infants with known EEG abnormalities.

Procedures

When a patient was recruited to the trial, they were randomised to receive either standard sucrose-only pain management before venepuncture (Control arm), or a combination of both music intervention and sucrose (Intervention arm). They would then receive the alternative on the occasion of their second venepuncture during the trial. Allocations to either arm were determined by sealed opaque envelopes. Each patient was given a unique identifier for labelling saliva samples which was used to facilitate blinding when measuring cortisol levels. This study was conducted in conjunction with a concurrent investigation into the effects of music on electroencephalography (EEG) activity during a painful procedure, the results of which will be presented separately.

Interventions

When randomised to the Control arm of the study, infants received at least 0.3ml of Sweet-Ease Natural Sucrose Solution (24% sucrose and purified water solution) via syringe immediately prior to venepuncture and were allowed to suck a soother. In the Intervention arm, a Bluetooth speaker was placed adjacent to the infant which was pre-set to play *Brahms' Lullaby* Opus 49 No.4, as selected in line with previous similar studies (26,27). The music was delivered on a continuous

loop at a sound level between 45 and 50 decibels in line with recommendations from the American Academy of Paediatrics (AAP) regarding sound levels in the NICU. In cases where the infant was on an open-top resuscitaire for the venepuncture and the ambient noise level exceeded 50 decibels, the sound level was increased to a maximum of 58 decibels. The music was started following the initial ten-minute rest period after EEG application. The music continued from ten minutes prior to venepuncture, during venepuncture and for 5 minutes after completion of venepuncture.

Sample collection

Salivary samples were obtained using Salimetrics SalivaBio Infant's Swabs (approved for use in analysis of salivary cortisol). Baseline samples were obtained immediately prior to venepuncture, then at 20 minutes post venepuncture and at 4 hours post venepuncture in both study groups. Samples were placed in the accompanying storage tube and frozen immediately after sampling. Prior to analysis, swabs were then centrifuged for 10 min at 1000 g to extract saliva. An additional 0.5ml of blood was taken during blood sampling to assess serum cortisol levels at the time of the procedure.

Cortisol Analysis

Cortisol concentrations were determined using the Cortisol Enzyme Immunoassay Kit according to the manufacturer's instruction (Enzo® Life Sciences, UK). The assay detection limit was 0.16 nmol/l. Inter- and intra-assay coefficients of variance were 6.6% and 5.4% respectively. The researchers processing the salivary cortisol samples were blinded to the identity of the patient and to the arm of the study they were participating in at the time of sampling.

Pain scoring

Infant pain scores were measured by the Premature Infant Pain Profile (PIPP)(28), a validated scoring system for assessment of pain in preterm infants, which incorporates physiological (heart rate variability and transcutaneous oxygen saturation) and behavioural indicators (brow bulge, eye squeeze, and nasolabial furrow). It generates a score, ranging from 0-21, that is weighted for corrected gestational age and behavioural state. Data were collected using video recording equipment with a camera positioned to obtain a clear view of the infant's face for the duration of each trial, without revealing the music speaker in order to facilitate blinding. Physiological data was recorded via the EEG monitoring equipment and the Phillips bedside monitor in the NICU. The video data was later edited so that three 1-minute video extracts beginning from specific timepoints (six extracts per infant) were available for analysis:

Extract 1: Pre-procedure - Two minutes before the first salivary swab was collected

Extract 2: Intra-procedure - The instant of needle-to-skin

Extract 3: Post-procedure - Two minutes before the second salivary swab was collected

Each video extract was analysed by the same member of the research team who was blinded to the study arm, and a PIPP score was calculated. Scores were calculated for heart rate variation by calculating the difference between the mean heart rate in the first fifteen seconds of each video extract, and the maximum heart rate recorded. PIPP scores for oxygen saturation were calculated according to the lowest saturation recorded during each data extract. Repeat analysis was performed by the same researcher on a proportion of the extracts in order to assess intra-rater reliability which was calculated at kappa of 0.85. A proportion of the extracts were analysed by a second researcher in order to ascertain inter-rater reliability, which was kappa of 0.9.

Statistical Analysis

Data were analysed using IBM SPSS (Version 23). Descriptive statistics were generated to observe the demographic characteristics of the study population. For data which was normally distributed the study arms were compared using a paired samples t-test. For skewed data, the non-parametric Wilcoxin signed-rank test was used. A linear mixed model was also used with cortisol as the dependent variable and study arm, time and the interaction of group by time as fixed effects, and time and group as repeated effects. Statistical significance was defined as $p<0.05$.

Results:

Patient demographics

There were 43 patients in total screened for inclusion of which 21 patients were enrolled for participation. The remaining 22 patients did not meet inclusion criteria, or were too close to discharge to complete both arms of the study. There were11 patients randomised to the Control arm initially and 10 patients were randomised to the Intervention arm. In total, on 18 occasions patients completed the Intervention arm of the trial and 19 the Control arm (*Figure 1*)Five participants were lost to follow-up including two infants who were transferred to a different hospital, and three infants who were discharged from the NICU prior to their second procedure. Of those lost to follow-up, two were lost from the Control arm and three were lost from the Intervention arm. This resulted in crossover data available for a final sample of 16 infants.

Cortisol analysis

A total of 103 salivary samples were successfully collected and had cortisol levels measured. Sufficient volumes of saliva were available at all three time points for 14 infants in the Control arm, and 17 infants in the Intervention arm. An outlier was identified in the Intervention arm which was excluded from further analyses as it was considered to represent an error in measurement with a value (17,967.02pg/ml) far exceeding the median (1086.472pg/ml) of the remaining samples.

Absolute cortisol values were elevated in the Intervention arm compared to the Control arm at all three timepoints (baseline, 20 minutes, and 4 hours post-procedure), but not significantly so ($p=0.056$, $p=0.3$, $p=0.575$ respectively). The change (elevation or reduction) in cortisol values from baseline at 20 minutes and 4 hours post-procedure was compared. In the Control arm, median (IQR) change in cortisol values from baseline was +128.48pg/ml (-47.66-517.02) at 20 minutes and +393.52pg/ml (47.88-1221.34) 4 hours post-procedure. In the Intervention arm the median (IQR) change in cortisol values was -69.564pg/ml (-860.96-397.289) and +100.48pg/ml (-560.46-842.99) as measured from baseline at 20 minutes and 4 hours post-procedure respectively. The differences between the study arms were not statistically significant ($p=0.311$ at 20 minutes, and $p=0.203$ at 4 hours post-procedure).

In a mixed model analysis, the interaction of time by group was not statistically significant ($p=0.687$) indicating that changes over time did not differ between study arm. The main effect of time was not statistically significant ($p=0.132$) indicating that there were no changes over time. The main effect of study arm was not statistically significant ($p=0.081$) indicating that there was no difference in cortisol levels between the two study arms.

Blood cortisol values were also measured with 17 available values in the Control arm and 16 available values in the Intervention arm. Median (IQR) blood cortisol was 74nmol/L (36.5-129) in the Control arm compared to 71.5nmol/L (42-114.5) in the Intervention arm (p=0.695).

PIPP Scores

Not all infants had complete data at all time points due to movement artefacts and obscured view of the face, and missing vitals data. PIPP scores were calculated for 14 infants at all three time points during both study arms. There were 18 total PIPP scores calculated for the pre-procedure time point in both the Control arm and the Intervention arm. There were 17 total PIPP scores assigned at the intra- and post-procedure time points in both study arms. As there were no more than eight data missing at any time point, and these were not the same infants, analysis was conducted with some cases missing.

Median (IQR) baseline (pre-procedure) PIPP was 5(3.75-6) in the Control arm and 4.5(3-5.25) in the Intervention arm. Median PIPP intra-procedure was 5(4.5-10.5) in the Control arm and 7(5.5-10) in the Intervention arm. Median PIPP post-procedure was 5(3-6) in the Control arm and 5(4-5) in the Intervention arm. PIPP scores were not significantly different between study arms at any of the time points (p=0.597, p=0.438 and p=0.636 at pre-, intra-, and post-procedure respectively).

Discussion:

Studies have shown that repeated painful experiences have an adverse effect on the neurological and behavioural development of preterm infants (4-7). There has been increasing interest in non-pharmacological interventions to reduce stress and pain in the NICU. We aimed to assess the potential efficacy of a music intervention in this context. We did not observe an appreciable effect of a music intervention on the stress response of infants undergoing a painful procedure. Salivary cortisol values and pain scores were not reduced in the Intervention arm relative to the Control arm, suggesting the music intervention provided no additional benefit in reducing procedural pain for infants. Our findings are not consistent with Schwilling et al who observed significant reductions in salivary cortisol levels in response to music intervention (23). However, they did not look at response to procedural pain, but rather tested cortisol levels in stable preterm infants following exposure to music on three consecutive days. . Additionally, they took baseline samples four hours prior to the initiation of the music intervention, while our samples were taken immediately before the venepuncture was performed.

We found no difference in PIPP scores between the control and intervention arms. This is in keeping with Cardoso et al who compared PIPP scores between groups of preterm infants treated with music alone, music plus glucose, and glucose alone, and found no difference in scores (29). While multiple studies assess the effect of music interventions on physiological parameters (13-15, 18-19) and behaviour (16-17), very few use validated scores for pain assessment, making it difficult to compare our results. We did not find significant improvements in any specific physiological parameters such as heart rate or O2 saturations, but our study was not powered to do so.

We attempted to minimise the disruption to each infant's planned care by performing our testing only when the infant was already scheduled for venepuncture as part of their routine monitoring in the neonatal unit. We also attempted to avoid any disruption to the infant's schedule of feeding and routine cares when the sampling was performed. However, as previously mentioned, the environment of the neonatal unit is subject to a number of variables such as ambient sound. Where possible the infants were nursed in incubators to minimise the ambient environmental disruption, but a number of the infants were mature enough to be nursed in a standard infant cot. For these infants, the sampling was carried out on an open-top resuscitaire to facilitate monitoring, and efforts were made to ensure the staff and parents were aware of a need to minimise noise in the area. As the infants were exposed to a naturally higher environmental noise level, the decibel level of the music was increased. We maintained the sound level of the music as close to the AAP recommendations (30) for noise levels in the NICU as possible in order to avoid adverse effects. However, we noted that a number of other studies have used much higher

decibel levels for the musical interventions (14) and it is possible that our sound level may have been suboptimal. It is also possible that the duration of the music intervention may have been too short to have shown any benefit. These factors would need to be considered in any future planned trials in this area.

The crossover design of the trial served to avoid some of the potential confounding factors observed in previous studies such as the baseline health characteristics of the infant and potential medication effects. However, a number of environmental and situational factors were inherent in our design, which may have had an impact on our results and were identified as limitations. It was not practically possible to control for previous painful procedures which may have had an impact on each infant's stress response as measured by cortisol levels. On a number of occasions, a number of attempts at venepuncture were required to obtain a blood sample. This was not factored into analysis and may have had an impact on the cortisol levels measured in these instances due to increased exposure to the stressful stimulus, and relatively prolonged exposure to the music intervention. One unavoidable difficulty of performing a study on salivary cortisol in this age group is the *difficulty in obtaining samples. The natural saliva production of these preterm infants is small volume, and there are few techniques available to encourage saliva production. Although we made considerable efforts to obtain a reasonable volume of saliva, and salivettes were spun at high speed to obtain as large a volume of saliva as possible, the required saliva volume was difficult to obtain in some cases, and it was not possible to obtain a measurable cortisol result from some samples as a result. This difficulty has been acknowledged in previous studies (31) and should be given consideration when designing future studies of salivary measurement in this age group. As a result of substantial loss to follow up our study had crossover data available for only 16 infants, and affected the power of the study. While this study has generated preliminary evidence, it is difficult to generalize the results to the entire population.

In conclusion the introduction of a music intervention just prior to and during venepuncture did not provide a significant reduction in the stress response experienced by preterm infants. Future investigation would benefit from a larger sample size in order to compensate for potential loss to follow up, and improved cortisol sampling technique. Greater consideration of the type of music intervention and duration to be delivered may also be useful in future studies.

References

1. Stevens B, Johnston C, Franck L, Petryshen P, Jack A, Foster G. The efficacy of developmentally sensitive interventions and sucrose for relieving procedural pain in very low birth weight neonates. *Nurs Res* 1999;48:35-43.
2. Cignacco E, Hamers JPH, Stoffel L, van Lingen RA, Gessler P, McDougall J, et al. The efficacy of non-pharmacological interventions in the management of procedural pain in preterm and term neonates. *Eur J Pain* 2007;11:139-52.
3. Ambuel B, Hamlett KW, Marx CM, Blumer JL. Assessing distress in pediatric intensive care environments: the COMFORT scale. *J Pediatr Psychol* 1992;17:95-109.
4. Gagnon RE, Leung A, Macnab AJ. Variations in regional cerebral blood volume in neonates associated with nursery care events. *Am J Perinatol* 1999;16:7-11.
5. Perlman JM. Neurobehavioral deficits in premature graduates of intensive care--potential medical and neonatal environmental risk factors. *Pediatrics* 2001;108:1339-48.
6. Anderson, D. E. and Patel, A. D. Infants born preterm, stress, and neurodevelopment in the neonatal intensive care unit: might music have an impact? *Dev Med Child Neurol* 2018; 60: 256-266.
7. Anand KJ. Clinical importance of pain and stress in preterm neonates. *Biol Neonate* 1998;73:1-9.
8. Spence K, Henderson-Smart D, New K, Evans C, Whitelaw J, Woolnough R. Evidenced-based clinical practice guideline for management of newborn pain. *J Paediatr Child Health* 2010;46:184-92.
9. Lindsay KE. The value of music for hospitalized infants. *Child Health Care* 1981; 9:104-7.
10. Garza-Villarreal EA, Pando V, Vuust P, Parsons C.Music-induced analgaesia in chronic pain conditions: a systematic review and meta-analysis. *Pain Physician* 2017; 20:597-610.
11. Keenan A, Keithley JK. Integrative review: effects of music on cancer pain in adults. *Oncol Nurs Forum* 2015; 42:368-75.
12. Lee JH. The effects of music on pain: a meta-analysis. *J Music Ther* 2016; 53:430-77.
13. Butt ML, Kisilevsky BS. Music modulates behaviour of premature infants following heel lance. *The Canadian journal of nursing research = Revue canadienne de recherche en sciences infirmieres* 2000;31:17-39.
14. Bergomi P, Chieppi M, Maini A, Mugnos T, Spotti D, Tzialla C, et al. Nonpharmacological techniques to reduce pain in preterm infants who receive

heellance procedure: a randomized controlled trial. *Res Theor Nurs Prac* 2014;28:335-48.

15. Whipple J. The effect of music-reinforced nonnutritive sucking on state of preterm, low birthweight infants experiencing heelstick. *J Mus Ther*. 2008;45:227-72.
16. Johnston CC, Filion F, Campbell-Yeo M, Goulet C, Bell L, McNaughton K, et al. Enhanced kangaroo mother care for heel lance in preterm neonates: a crossover trial. *J Perinatol* 2009;29:51-6.
17. Zhu J, Hong-Gu H, Zhou X, Wei H, Gao Y, Ye B, et al. Pain relief effect of breast feeding and music therapy during heel lance for healthy-term neonates in China: a randomized controlled trial. *Midwifery* 2015;31:365-72.
18. Rossi A, Molinaro A, Savi E, Micheletti S, Galli J, Chirico G et al (2018). Music reduces pain perception in healthy newborns: a comparison between different music tracks and recoded heartbeat. *Early Hum Dev* 2018; 124: 7-10.
19. Joyce BA, Keck JF, Gerkensmeyer J. Evaluation of pain management interventions for neonatal circumcision pain. *J Pediatr Healthcare* 2001;15:105-14.
20. Hartling L, Shaik MS, Tjosvold L, Leicht R, Liang Y, Kumar M. Music for medical indications in the neonatal period: a systematic review of randomised controlled trials. *Arch Dis Child Fetal Neonatal Ed* 2009;94:F349-54.
21. Calixto C, Martinez FE, Jorge SM, Moreira AC, Martinelli CE, Jr. Correlation between plasma and salivary cortisol levels in preterm infants. *J Pediatr* 2002;140:116-8.
22. Morelius E, He HG, Shorey S. Salivary Cortisol Reactivity in Preterm Infants in Neonatal Intensive Care: An Integrative Review*. Int J Env Res Pub Health* 2016;13.
23. Schwilling D, Vogeser M, Kirchhoff F, Schwaiblmair F, Boulesteix AL, Schulze A, et al. Live music reduces stress levels in very low-birthweight infants. *Acta Paediatr* 2015;104:360-7.
24. Kemper KJ, Hamilton C. Live Harp Music Reduces Activity and Increases Weight Gain in Stable Premature Infants. *J Altern Complement Med* 2008;14:1185-6.
25. Qiu J, Jiang Y, Li F et al. Effect of combined music and touch intervention on pain response and β-endorphin and cortisol concentrations in late preterm infants. *BMC Pediatrics* 2017;17:38.
26. Uematsu H, Sobue I. Effect of music (Brahms lullaby) and non-nutritive sucking on heel lance in preterm infants: A randomized controlled crossover trial, *Paediatrics & Child Health* 2018; 072

27. Jabraeili M, Sabet T, MustafaGharebaghi M et al. The Effect of Recorded Mum's Lullaby and Brahm's Lullaby on Oxygen Saturation in Preterm Infants: a Randomized Double-Blind Clinical Trial. *J Caring Sci* 2016;5:85-93
28. Stevens B, Johnston C, Petryshen P, Taddio A. Premature Infant Pain Profile: development and initial validation. *Clin J Pain* 1996;12:13-22.
29. Cardoso MVLML, Farias LM, de Melo GM. Music and 25% glucose pain relief for the premature infant: a randomized clinical trial. *Revista Latino-Americana de Enfermagem.* 2014;22:810-818.
30. American Academy of Pediatrics, Committee on Environmental Health. Noise: a hazard for the fetus and newborn. *Pediatrics*. 1997;100:724–727.
31. Schmidt NA. Salivary cortisol testing in children. *Issues in comprehensive pediatric nursing* 1997;20:183-90.

List of Abbreviations:

NICU Neonatal Intensive Care Unit

HPA Hypothalamic-pituitary-adrenal

EEG Electroencephalography

AAP American Academy of Pediatrics

PIPP Premature Infant Pain Profile

IQR Interquartile range

Conflict of Interest Statement: None

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Registration: The trial was registered at clinical trials.gov NCT03028844.

Table 1. Patient demographics

	Control arm first (%)	Intervention arm first (%)
Number enrolled (n=21)	11/21 (52)	10/21 (48)
Male infants	5/11 (45)	4/10 (40)
Mean gestational age	26+3	27+4
Mean birth weight	861g	1012g
Mean weight at first sampling	2171g	1925g
Mean weight at second sampling	2885g	2616g

Table 2. Summary of salivary cortisol, blood cortisol, total PIPP scores, and PIPP scores for heart rate variability and oxygen saturation in the Control arm and the Intervention arm

	Control	Intervention	p value
Salivary Cortisol, Median (IQR), (pg/ml)			
Baseline	653.88 (273.1-1885.04)	1086.472 (542.8-2234.528)	p=0.056
20 mins post-procedure	833.23 (634.72-1930.28)	1016.9 (689.99-2190.29)	p=0.3
4 hours post-procedure	656.68 (570.65-2142.63)	915.86 (668.99-3450.02)	p=0.575
Blood Cortisol, Median (IQR), (nmol/L)	74 (36.5-129)	71.5(42-114.5)	p=0.695
Total PIPP Score, Median (IQR)			
Pre-procedure	5 (3.75-6)	4.5 (3-5.25)	p=0.597
Intra-procedure	5 (4.5-10.5)	7 (5.5-10)	p=0.438
Post-procedure	5 (3-6)	5 (4-5)	p=0.636
PIPP for HR Variation, Median (IQR)			
Pre-procedure			
Intra-procedure	1 (0-1)	1 (0-1)	p=0.713
Post-procedure	1 (0.75-2)	1 (1)	p=0.405
	0 (0-1)	1 (0.5-1)	p=0.07
PIPP for SpO2, Median(IQR)			
Pre-procedure			
Intra-procedure	0.5 (0-2.25)	0 (0-1)	p=0.086
Post-procedure	0 (0-1.25)	1 (0-2)	p=0.236
	1 (0-2)	0 (0)	p=0.037

IQR= Interquartile range

SpO2=Transcutaneous oxygen saturation;

PIPP=Premature Infant Pain Profile;HR=Heart rate;

Figure 1. Consort Flow diagram