

Full-Length Article

Efficacy of Live Lullaby Singing During Procedural Pain in Preterm and Term Neonates.

Alexandra Ullsten^{1,2}, Pernilla Hugoson^{3,4}, Malin Forsberg⁵, Lisa Forzelius⁶, Maria Klässbo⁷, Emma Olsson⁸, Ulrik Volgsten⁹, Björn Westrup¹⁰, Ulrika Ådén¹¹, Lena Bergqvist¹², Mats Eriksson¹³.

¹Department of Musicology, School of Music, Theatre and Art, Örebro University, Sweden.

²Centre for Clinical Research, Värmland County Council, Karlstad, Sweden.

³Department of Music, University of Jyväskylä, Finland.

⁴Department of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden.

⁵County Council of Dalarna, Mora Hospital, Sweden.

⁶County Council of Västernorrland, Sundsvall Hospital, Sweden.

⁷Centre for Clinical Research, Värmland County Council, Sweden.

⁸Department of Pediatrics, Faculty of Medicine and Health, Örebro University, Sweden.

⁹Department of Musicology, School of Music, Theatre and Art, Örebro University, Sweden.

¹⁰Department of Women's and Children's Health, Karolinska Institute, Karolinska University Hospital-Danderyd, Stockholm, Sweden.

¹¹Neonatal Research Unit, Department of Women's and Children's Health, Karolinska Institute, Karolinska University Hospital, Astrid Lindgren Children's Hospital, Stockholm, Sweden.

¹²Department of Clinical Science, Intervention and Technology, Unit of Pediatrics, Karolinska Institute, Karolinska University Hospital, Stockholm, Sweden.

¹³Faculty of Medicine and Health, School of Health Sciences, Örebro University, Örebro, Sweden.

Abstract

This clinical trial tested the pain relieving effect of live lullaby singing on behavioral and physiological pain responses during venepuncture in 38 preterm and full term neonates. Acute and repeated pain, as well as the use of analgesic drugs, may have long-term negative impact on infants' development and future behaviour. This emphasizes the need for complementary approaches to pain management such as music therapy.

Parent-preferred lullabies were performed live and standard care was provided for all neonates. Behavioral responses with regard to pain were assessed with Premature Infant Pain Profile-Revised (PIPP-R) and Behavioral Indicators of Infant Pain (BIIP). Heart rate, respiratory rate and oxygen saturation were measured each tenth second.

Although the live lullaby singing did not show a statistically significant effect on the infants' pain score, there was a significantly calmer breathing pattern in the lullaby intervention versus the control condition in the pre-needle stage, showing a non-significant trend towards higher oxygen saturation levels and calmer heart rate in the lullaby intervention versus the control condition in the pre-needle stage. There were non-significant indications of fewer and shorter skin punctures with lullaby singing. More research is needed to explore such positive trends in the data.

Keywords: *newborn infant, preterm infant, pain, music therapy, lullaby*

multilingual abstract | mmd.iamonline.com

Introduction

Preventing and treating neonatal pain has not improved along with the expertise to recognize and measure it. Infant pain and pain management became a strongly disputed area 30 years ago when it was established that newborn infants, both

premature and term, are capable of experiencing pain and remembering trauma [1]. Despite an increasing awareness regarding pharmacological and non-pharmacological analgesic strategies, pain still goes untreated in neonatal intensive care units [2,3]. Infants experience on average between 12 and 14 painful procedures per day exacerbated by multiple puncture attempts [2,3]. Many of the brain regions that encode pain in adults are also active in term newborns, making it likely that infants' pain experience is similar to that described by adults [4]. Infants, particularly preterm infants, are considerably more vulnerable than adults [3,5]. Repeated early procedural pain may be linked to negative impact on infants' neurocognitive development, future behavior and

PRODUCTION NOTES: Address correspondence to:

Alexandra Ullsten, E-mail: alexandra.ullsten@liv.se | COI statement: The authors declared that no financial support was given for the writing of this article. The authors have no conflict of interest to declare.

impaired brain development [6,7]. Analgesic drugs [8], also place infants at risk, which emphasizes our need for individualized non-pharmacological approaches [9].

Infants' developmental age, temperament, and previous procedural experiences, together with parental anxiety, environmental noise and other distractions, can influence the effectiveness of treatment [9,10]. Our challenge is to individualize the procedural care and avoid applying comfort strategies as a quick fix. [9] Given that infants' development involves watching and interacting with other people, a behavioristic "stimulus and response"- approach to procedural pain management is likely to fail because it oversimplifies the learning process [9]. Infants who experience pain repeatedly during the first 24-36 hours of life can develop hyperalgesia, and can anticipate pain [10,11]. Coping with painful procedures requires a learning process, which is a useful approach for older children [9,10], but perhaps for newborn infants, a more sophisticated approach may be warranted. Comfort and coping strategies, which are infant and parent-directed, may include tailored and age-appropriate preparations with biopsychosocial and cross-cultural nonverbal communication aimed toward optimizing the infant's ability to self-regulate. This can introduce the enhancement in a predictability of events and of the environment, as well as the enhancement of techniques that can empower parents- to take an active role [9]. Infant and parent-directed approaches create a reciprocal relationship between the family and the health care providers, who, in best-practice situations, will have an individual plan ready to address pain and discomforts such as anxiety, stress and fear before, during and after painful procedures [10,11]. Non-pharmacological strategies should pay equal attention to the infants' need to feel safe, surrounded by and belonging with loving, caring people [9,12].

Live lullaby singing can create an atmosphere incorporating trust and safety for vulnerable infants, their parents and staff [13]. Music, as well as pain, is a multidimensional perceptual and contextual experience. Treatment of premature infants with music therapy has shown reduced stress [14-17], inconsolable crying behaviour [18] and improved vital signs [19-26]. Interventions with recorded music have shown positive effects on behavioural pain indicators and scores. However, the sample sizes in these studies were small, with control groups with no pain treatment or only a minimum of comforting strategies [27-34]. Studies comparing live singing with recorded lullabies have demonstrated that live singing impacts physiology and behaviour more [20,35,36]. Pre-recorded music does not effectively accommodate changing arousal needs, whereas infant-directed singing can be adjusted instantaneously [35]. This flexibility gives live, parental singing a distinct advantage over recorded music as an effective medium for facilitating positive parent-infant interaction [35]. Infants' responsiveness to parental singing is well-documented

[22,35,37,38], but live infant-directed singing by a female stranger may also be more effective than listening to recorded music [35,36]. Because of the possibilities to interact with and entrain to the infant, live singing has greater potential than recorded music in addressing neonatal pain [33,39,40].

The pain-alleviating effects of non-pharmacological interventions such as breastfeeding, skin-to-skin care, facilitated tucking, swaddling, non-nutritive sucking, sweet solutions and multisensory stimulation like music, have been well-documented [29,34,41-43], and possibly explained by The Gate Control Theory [44]. In addition to the physiological effects, music also has an impact on infants' affects and amodal perception [45,46]. Music stimulates endorphins, which may activate the endogenous opioid pathways [46], as well as regions in the limbic and paralimbic systems, which are centres of affective pain [47]. When a parent is singing and humming to the newborn infant, it encourages social interaction [22,33,48], enhancing bonding and reducing parental stress and anxiety [15,19,26,49,50]. We propose, therefore, that live lullaby singing is an infant-and parent-directed non-pharmacological comfort and coping strategy. The activity can be considered age-appropriate and tailored to the individual infant's affect state, using biopsychosocial and cross-cultural nonverbal communication, which facilitates the infant's ability to self-regulate [45] and encourages the parents to participate in the pain management [13,51].

The objective of this study was to test the pain-relieving effect of live lullaby singing on behavioural and physiological pain responses during venepuncture in preterm and term neonates. This is, to our knowledge, the first clinical trial using infant-directed *live* lullaby singing entrained to the infant, pre-, during and post venepuncture.

Method

Participants

The sample size for this study was motivated by previous exploratory pain studies with neonates with a within-subjects repeated measures design or with a control group, using recorded music [27-30,34]. Sample sizes in these studies have been small, ranging from 13 up to 35, yet demonstrating significant modulations of both behavioural and physiological pain responses.

In this study, 38 infants (15 female and 23 male infants) were recruited for venepuncture on two occasions, from a level 2 neonatal ward (providing special but not intensive neonatal care) in Sweden. Infants with known congenital malformations, severe illness, respiratory support or on-going treatment with sedatives or analgesics were excluded. The study was approved by the Regional Ethical Review Board (2012/1097-31/2; 2012/1754-32). Written informed consent was acquired from each and one of the infants' parents.

Study setting

A crossover design, where the infants served as their own control, were used with randomisation of the order of the lullaby intervention and the control by the drawing of lots (Fig. 1).

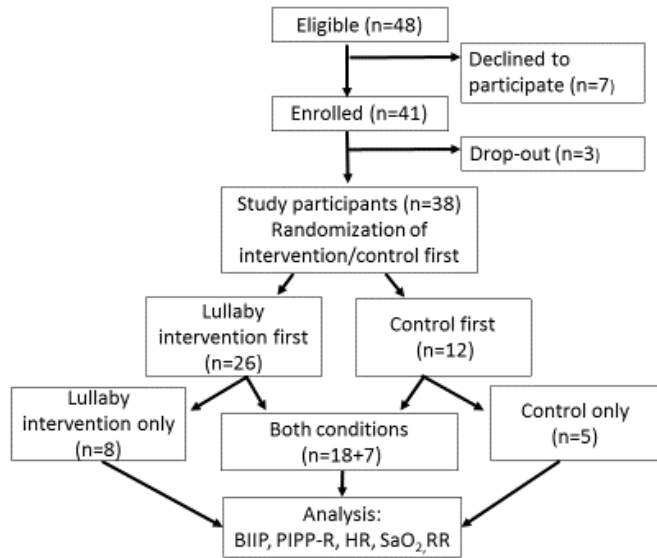


Figure 1. Flow chart showing the study protocol.

All infants were tested on two separate occasions; lullaby intervention with standard care and the silent control with standard care only (Fig. 2). Standard care entailed facilitated tucking done by a nurse or the parent, oral glucose (300 mg/ml) and the opportunity to suck on a pacifier or on a parent’s or a nurse’s plastic gloved finger [52]. The parents were invited to attend both venepuncture procedures, which were performed in the infant’s crib or on an examination bed, with a time lapse between 2 hours and 2 days.

The procedures started with a baseline period (period 1) of 2 minutes (Fig. 2). In the pre-needle stage (period 2), either lullaby intervention or control was provided during 2 minutes and 30 seconds. The lullaby singing or the control continued during the nurse’s handling of the infant and during the venepuncture/s (period 3). The time for the blood sampling varied from 1 minute and 40 seconds to up to 29 minutes and 18 seconds due to variations in the number of skin punctures to achieve the required amount of blood. In the post-needle stage (period 4), the lullaby intervention or control continued after the blood sampling for 2 minutes and 30 seconds. A 2 minute-long silent period (period 5) concluded each procedure.

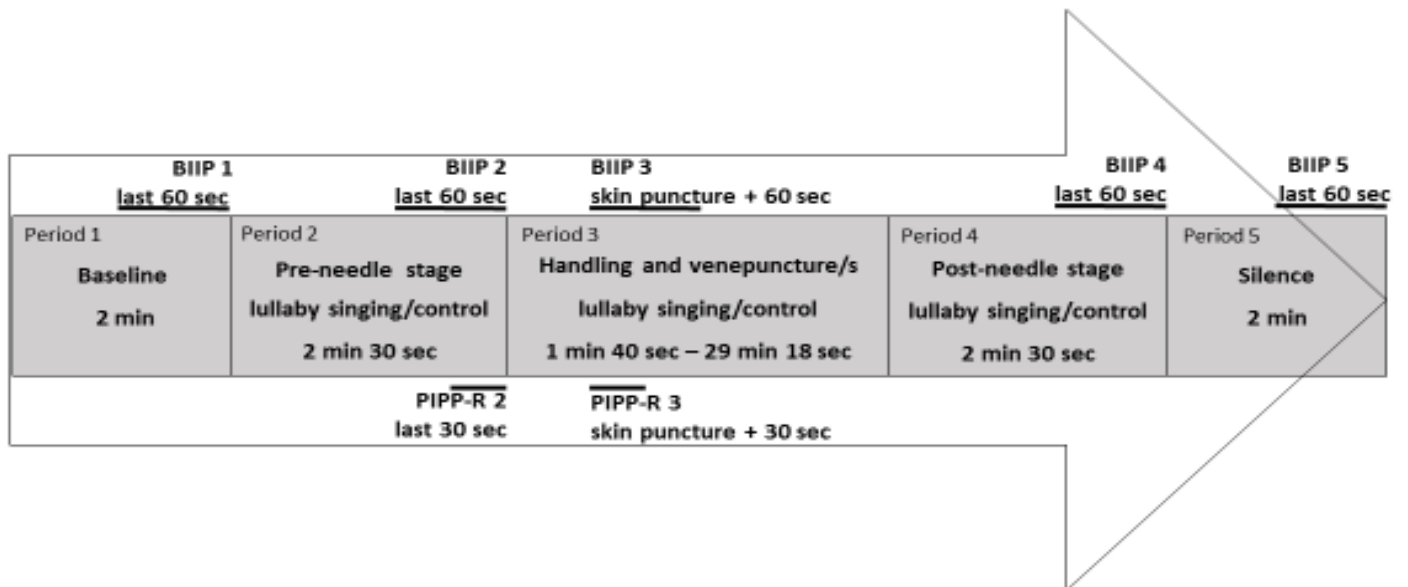


Figure 2. Overview of the two randomized venepuncture procedures in the study, lullaby intervention with standard care and control with standard care only (period 1 to period 5) and of pain assessment periods with Behavioral Indicators of Infant Pain (BIIP) and Premature Infant Pain Profile-Revised (PIPP-R). PIPP-R 2 and PIPP-R 3 are titled in parallel to the equivalent BIIP periods. According to the instructions no PIPP-R 1 was assessed.

Intervention

In order to standardize the interventions, the live lullaby-singing was performed by a student under training to become a professional music therapist (PH), according to a protocol

designed by the research team. The live lullaby singing is described in detail in a paper by Ullsten et al. [45]. The songs were parent-preferred lullabies or a traditional Swedish lullaby chosen by the music therapy student (all songs are listed in Appendix A). The singing maintained a constant sound level

between 30-60 dBA. The recommended sound level is ≤ 55 -65 dB on the A-scale [53,54].

Data collection

Oxygen saturation data (SaO₂) were collected through a saturation probe (LNOP®, Masimo Set, Infiniti Medical) on the hand or the foot. Data for heart rate (HR) and respiratory rate (RR) were obtained via three electrodes (NEOTRODE®, ConMed, New York, USA) on the infant’s chest, connected to a Dash 3000-monitor (General Electric, Canada); the same monitor for all procedures. The monitor was connected to a research computer (Toshiba, server iCentral, software Datex S/5 collect 2004), which stored the physiological information collected at an interval of ten seconds. The procedures were videotaped with a digital video camera (JVC GR-DVL 9600, Tokyo, Japan). The sound levels of the lullaby were measured 10 cm from the infant’s ear with a sound level meter (CIM390, Taipei, Taiwan) on dBA slow, A scale. Different specialist nurses and midwives performed the venepunctures because of practical schedule reasons.

Pain assessment

The infants’ behavioral responses to pain were assessed from the video films by a neonatal nurse (EO), (expert on pain assessment) who was blinded to the intervention versus control, using Behavioral Indicators of Infant Pain (BIIP) [55], and Premature Infant Pain Profile-Revised (PIPP-R) [42]. Both instruments are validated for assessing procedural pain in preterm and term infants. The BIIP evaluates the state, level of arousal, five face actions and two hand actions. The PIPP-R evaluates three behavioral facial actions, two physiological items, and two contextual items; gestational age (GA) and behavioral state. Pain assessment with BIIP was assessed in five periods, chosen by the research team (Fig. 2). PIPP-R score was assessed according to the instructions of the PIPP-R tool, in two periods during the procedures, corresponding to the second and third BIIP scoring (Fig. 2).

Statistics

Two-sample tests with the intention-to-treat (ITT) principle on the full analysis set and all test situations, were performed, conservative due to the positive dependence within individuals. Complementary secondary-paired analyses on the per protocol population (PP) were performed for those infants fulfilling both the song and the quiet test situation. Continuous variables are presented with both mean and standard deviation (SD), and median with min-max values.

For comparison between the lullaby intervention and the control in the two-sample ITT-analysis, the Mantel-Haenszel chi square test was used for ordered categorical variables and the Mann-Whitney U-test for continuous. For comparison between the lullaby intervention and control in the PP-analysis, the Wilcoxon signed rank test was used for continuous variables and Sign test for ordered categorical. Period adjusted p-value was calculated by analysing change from period 1 to period 2 - between treatment during period 1 (standard cross over analysing method) using the Mann-Whitney U-test, Mantel-Haenszel Chi Square test. For comparison over time, a linear Non-Parametric Permutation Test for paired observations was used for continuous variables and Sign test for categorical. All significance tests were two-tailed and conducted at the 5% significance level. Statistical analyses were performed using SAS version 9.3 (Cary, NC).

Results

Of the 48 infants who were eligible for entering the study, 41 were enrolled. Three of those were excluded because of incomplete data, such as equipment malfunction or blood sample collection failure. 38 infants were included (Table 1).

Table 1. Characteristics of the included 38 infants.

	Median (Min-Max)	Mean (SD)
Gestational age, weeks+days	34+4 (25+0 - 42 +0)	34+6 (4+3)
Birth weight, grams	2 460 (736-4 245)	2 519 (1 019)
Post-natal age at first blood test, days	3 (1-100)	13 (24)
Female sex, n (%)	15 (40)	

All comparisons were between groups. For the primary efficacy variable in the ITT-analysis, the mean PIPP-R score, no statistically significant difference (p=0.97) was found between lullaby intervention 3.76 (SD 3.44) and control 3.83 (SD 3.33), and no statistically significant changes in the physiological variables from baseline to the post-needle period immediately following the skin puncture (Table 2).

The BIIP pain score at skin puncture was 2.3 (SD 2.8) for the lullaby intervention versus 2.1 (SD 3.1) for the control (p=0.35), (Table 2). In the lullaby intervention, enough blood was collected during the first skin puncture in 73 % of the cases, versus in 57 % in the control (p=0.18), (Table 2). No mean difference in duration of the puncture stage was found between the lullaby intervention 6.5 (SD 4.0) minutes, and control 8.0 (SD 6.5) minutes (p=0.58), (Table 2).

Table 2. Primary and secondary outcome variables during the lullaby intervention and control in the Intention-to-Treat population.

Variables	Lullaby intervention (n=33)	Control (n=30)	p-value	Difference between groups Mean (95% CI)
Primary variable				
PIPP-R score at skin puncture	3.76 (3.44) 4.00 (0.00; 12.00)	3.83 (3.33) 4.00 (0.00; 12.00)	0.97 ^b	-0.076 (-1.733; 1.567)
Secondary variables				
Change in mean HR from Baseline to Skin puncture and Post-needle stage	8.93 (11.30) 6.53 (-12.07; 52.52)	7.09 (13.08) 7.78 (-35.50; 39.56)	0.95 ^b	1.84 (-3.99; 7.89)
Change in mean SaO2 from Baseline to Skin puncture and Post-needle stage	-1.27 (2.26) -0.71 (-9.92; 0.86)	-0.652 (4.082) -0.302 (-18.808; 9.571)	0.15 ^b	-0.613 (-2.143; 1.156)
Change in mean RR from Baseline to Skin puncture and Post-needle stage	1.46 (10.18) -0.22 (-15.80; 33.56)	-2.65 (9.62) -3.41 (-20.40; 18.52)	0.079 ^b	4.10 (-0.63; 8.93)
BIIP score at skin puncture	2.30 (2.77) 1.00 (0.00; 8.00)	2.13 (3.14) 0.00 (0.00; 9.00)	0.35 ^b	0.170 (-1.315; 1.612)
Change in mean HR from Baseline to Skin puncture	14.9 (12.3) 13.9 (-17.9; 46.6)	11.8 (21.8) 15.9 (-69.6; 43.6)	0.97 ^b	3.12 (-5.13; 12.35)
Change in mean SaO2 from Baseline to Skin puncture	-2.08 (3.94) -0.86 (-15.86; 1.57)	-1.49 (3.90) -1.14 (-15.14; 9.43)	1.00 ^b	-0.587 (-2.535; 1.337)
Change in mean RR from Baseline to Skin puncture	0.367 (12.433) -0.429 (-22.857; 30.286)	-6.33 (10.92) -5.64 (-27.50; 16.43)	0.065 ^b	6.70 (1.25; 12.59)
Total duration of the intervention/control, from the start of period 1 until the end of period 5 (minutes)	15.7 (4.1) 15.0 (10.9; 25.3)	17.3 (6.5) 16.0 (11.2; 38.5)	0.46 ^b	-1.61 (-4.40; 1.00)
Change in mean HR from Baseline to last 60 seconds of Post-needle stage	2.76 (13.65) 0.29 (-19.29; 42.29)	-0.603 (12.443) -1.000 (-33.952; 37.400)	0.54 ^b	3.36 (-2.86; 9.77)
Change in mean SaO2 from Baseline to last 60 seconds of Post-needle stage	-0.604 (1.236) -0.429 (-4.000; 2.286)	-0.005 (2.695) -0.143 (-6.429; 9.857)	0.26 ^b	-0.599 (-1.671; 0.424)
Change in mean RR from Baseline to last 60 seconds of Post-needle stage	0.833 (11.064) 0.857 (-31.000; 26.286)	-1.72 (11.46) -2.46 (-24.71; 29.71)	0.20 ^b	2.55 (-3.13; 7.92)
BIIP score at last 60 seconds of Post-needle stage	0.576 (1.347) 0.000 (0.000; 7.000)	0.300 (0.702) 0.000 (0.000; 3.000)	0.45 ^b	0.276 (-0.194; 0.833)
Duration of handling before 1st skin puncture (minutes)	2.09 (2.09) 1.35 (0.47; 9.00)	2.07 (1.56) 1.67 (0.33; 7.33)	0.61 ^b	0.013 (-0.854; 0.916)
Number of skin punctures				
1	24 (72.7%)	17 (56.7%)		
2	8 (24.2%)	11 (36.7%)		
3	1 (3.0%)	2 (6.7%)	0.18 ^a	
Duration of skin puncture period (minutes)	6.53 (4.02) 5.67 (1.67; 16.00)	8.04 (6.54) 6.59 (1.98; 29.13)	0.58 ^b	-1.52 (-4.30; 1.09)

For categorical variables n (%) is presented. For continuous variables Mean (SD) / Median (Min; Max) is presented.

For comparison between groups the Mantel-Haenszel Chi Square test was used for ordered categorical variables (= a).

The Mann-Whitney U-test was used for continuous variables (=b).

Calculation of confidence interval (CI) for continuous variables is based on bootstrapping of 10 000 replicates picking the 2.5 and 97.5 percentiles of the 10 000 mean differences as confidence interval. PIPP-R = Premature Infant Pain Profile-Revised, BIIP = Behavioral Indicator of Infant Pain, HR = Heart rate, SaO2 = Oxygen saturation, RR= Respiratory rate.

There was a significantly calmer breathing pattern in the lullaby intervention versus control in the pre-needle stage ($p=0.028$), (Table 3), a non-significant trend towards calmer breathing in the lullaby intervention in the puncture stage as well as in the post-needle and silence stages (Fig. 3). The same pattern was seen with the SaO₂ (Table 3). There was also a

trend towards calmer HR ($p=0.69$), in the pre-needle stage with lullaby singing versus control (Table 3). The HR decreased after the puncture stage during lullaby singing but there was a non-significant trend in HR being slightly higher in the lullaby intervention from the puncture stage through the silence stage (Table 3).

Table 3. Physiological variables during the lullaby intervention and control in the Intention-to-Treat population. For continuous variables mean (SD), median (min; max) is presented.

Situation	Variables	Lullaby intervention (n=33)	Control (n=30)	p-value
Baseline (period 1)				
	HR	138.0 (18.9) 138.2 (98.1; 175.5)	139.6 (20.9) 136.9 (99.9; 198.5)	0.93
	RR	49.2 (11.8) 47.3 (29.8; 68.9)	53.8 (11.6) 54.9 (28.8; 73.8)	0.10
	SaO ₂	98.4 (1.4) 98.4 (95.3; 100.0)	97.2 (2.6) 97.5 (87.3; 100.0)	0.060
Pre-needle stage (period 2)				
	HR	138.7 (17.0) 136.9 (105.9; 174.9)	140.6 (16.9) 138.6 (103.8; 193.6)	0.69
	RR	47.2 (11.9) 45.1 (27.6; 80.8)	52.7 (9.1) 52.0 (35.5; 69.8)	0.028
	SaO ₂	97.8 (1.7) 97.8 (93.6; 100.0)	96.6 (3.3) 97.1 (84.9; 100.0)	0.10
Puncture stage (period 3)				
	HR	148.8 (17.4) 151.4 (108.5; 187.1)	148.4 (18.9) 150.3 (113.8; 192.0)	0.91
	RR	49.3 (13.5) 46.8 (24.8; 90.6)	50.9 (10.9) 49.6 (30.7; 80.9)	0.54
	SaO ₂	96.7 (3.5) 97.7 (85.4; 100.0)	95.9 (5.7) 97.4 (69.9; 100.0)	0.61
Post-needle stage (period 4)				
	HR	143.4 (17.7) 146.2 (107.4; 177.9)	141.2 (16.4) 143.9 (98.7; 178.5)	0.50
	RR	50.7 (12.8) 48.2 (25.1; 87.8)	51.9 (11.8) 50.1 (28.4; 78.9)	0.65
	SaO ₂	97.5 (1.7) 97.7 (93.2; 100.0)	96.7 (3.9) 97.8 (80.8; 100.0)	0.79
Silence stage (period 5)				
	HR	138.7 (18.8) 137.3 (108.2; 174.6)	136.4 (14.0) 137.7 (102.8; 166.8)	0.63
	RR	48.0 (10.0) 48.8 (22.2; 67.0)	51.3 (10.5) 51.6 (29.4; 76.6)	0.30
	SaO ₂	97.5 (1.7) 97.7 (93.2; 100.0)	96.4 (2.9) 97.3 (86.9; 100.0)	0.18

The mean value is calculated for all observations within each period for each subjects.
For comparison between groups the Mann-Whitney U-test was used for continuous variables.

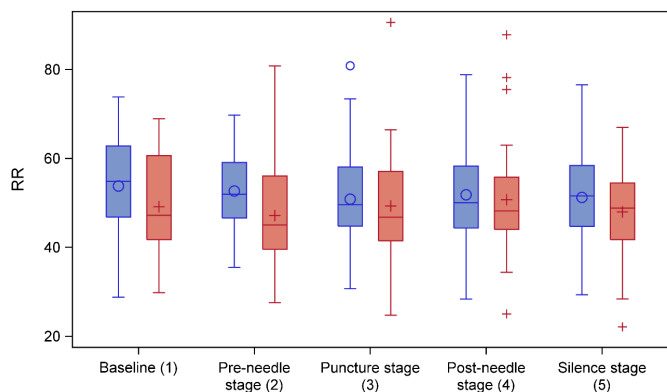


Figure 3. Box-plots showing distribution of respiratory rate (RR) during five periods (1-5) in intent-to-treat population (ITT). ■ mean control ■ mean lullaby intervention. Puncture stage (3) starts at the time of the first puncture. The top of the rectangle shows the third quartile (Q3), the horizontal line near the middle shows the median, the bottom shows the first quartile (Q1). The vertical line extending from the top of the rectangle shows the maximum value within 1.5 * IQR and the vertical line from the bottom indicates the minimum value within 1.5 * IQR. ○ and + = scores more than 1.5 times the interquartile range (IQR) below Q1 or above Q3.

The results of the complementary PP-analysis are shown in Table 4. These results are consistent with the results from the ITT-analysis. It should be noted that the mean difference in duration of the puncture stage, was non-significantly lower in the PP population in the lullaby intervention; 6,47 (SD 4.2) minutes, and control 8,45 (SD 6.79) minutes (p=0.16), (Table 4). The mean difference in the duration of the total procedure, was non-significantly lower in the PP population in the lullaby intervention; 15.6 (SD 4.2) minutes, and control 17.7 (SD 6.8) minutes (p=0.13), (Table 4).

Table 4. Primary and secondary outcome variables during the lullaby intervention and control in the completed Per Protocol population.

Variable	Lullaby intervention (n=25)	Control (n=25)	Difference between Lullaby intervention and Control		Period adjusted p-value
				p-value	
Primary variable					
PIPP score at skin puncture	4.04 (3.18) 4.00 (0.00; 10.00) (2.84; 5.24)	3.52 (3.11) 3.00 (0.00; 10.00) (2.36; 4.76)	-0.520 (3.393) 0.000 (-7.000; 8.000) (-1.810; 0.844)	0.49	0.52
Secondary variables					
Change in mean HR from Baseline to Skin puncture and post-needle stage	9.13 (12.66) 6.64 (-12.07; 52.52) (4.68; 14.41)	6.56 (12.23) 8.55 (-35.50; 21.81) (1.56; 10.73)	-2.58 (16.90) 3.12 (-46.20; 26.65) (-9.55; 3.62)	0.46	0.79
Change in mean SaO2 from Baseline to Skin puncture and post-needle stage	-1.30 (2.28) -0.59 (-9.92; 0.60) (-2.28; -0.55)	-0.670 (4.452) -0.290 (-18.808; 9.571) (-2.650; 0.842)	0.628 (4.591) 0.563 (-17.693; 10.026) (-1.277; 2.200)	0.057*	0.085
Change in mean RR from Baseline to Skin puncture and post-needle stage	0.959 (7.922) 1.630 (-15.804; 14.043) (-2.112; 3.953)	-3.33 (9.80) -3.48 (-20.40; 18.52) (-6.91; 0.55)	-4.28 (12.34) -4.53 (-31.66; 20.49) (-9.09; 0.54)	0.096	0.12

BIIP score at skin puncture	2.44 (2.75) 1.00 (0.00; 8.00) (1.44; 3.52)	2.00 (3.14) 0.00 (0.00; 9.00) (0.88; 3.24)	-0.440 (3.583) 0.000 (-7.000; 7.000) (-1.836; 1.000)	0.58	0.38
Change in mean HR from Baseline to Skin puncture	15.7 (12.1) 13.9 (-16.0; 46.6) (11.1; 20.4)	15.0 (16.5) 17.3 (-34.4; 43.6) (8.5; 20.9)	-0.698 (18.946) 2.714 (-56.429; 47.000) (-8.421; 6.438)	0.80*	0.98
Change in mean SaO2 from Baseline to Skin puncture	-1.92 (3.75) -0.71 (-15.86; 1.57) (-3.56; -0.71)	-1.34 (4.11) -1.14 (-15.14; 9.43) (-3.04; 0.17)	0.576 (4.677) 0.143 (-14.829; 10.571) (-1.297; 2.331)	0.44*	0.57
Change in mean RR from Baseline to Skin puncture	-0.086 (11.205) -0.143 (-22.857; 30.286) (-4.326; 4.330)	-7.35 (11.22) -6.43 (-27.50; 16.43) (-11.61; -3.10)	-7.26 (12.55) -7.43 (-41.43; 11.82) (-12.37; -2.47)	0.0074	0.037
Total duration (minutes) of the intervention/control, from the start of period 1 until the end of period 5	15.6 (4.2) 15.0 (10.9; 25.3) (14.1; 17.3)	17.7 (6.8) 16.0 (11.2; 38.5) (15.3; 20.5)	2.11 (6.72) 1.00 (-10.82; 15.65) (-0.43; 4.73)	0.13	0.33
Change in mean HR from Baseline to last 60 seconds of post-needle stage	2.69 (15.43) 0.14 (-19.29; 42.29) (-2.84; 8.90)	-1.71 (10.47) -0.86 (-33.95; 12.86) (-5.87; 2.02)	-4.39 (17.73) -3.71 (-43.86; 26.86) (-11.46; 2.26)	0.23	0.33
Change in mean SaO2 from Baseline to last 60 seconds of post-needle stage	-0.600 (1.225) -0.429 (-4.000; 2.286) (-1.080; -0.143)	0.103 (2.392) -0.143 (-4.286; 9.857) (-0.697; 1.117)	0.703 (2.457) 0.286 (-3.000; 9.857) (-0.135; 1.741)	0.26*	0.28
Change in mean RR from Baseline to last 60 seconds of post-needle stage	1.28 (11.42) 2.71 (-31.00; 26.29) (-3.21; 5.57)	-3.04 (11.42) -3.43 (-24.71; 29.71) (-7.19; 1.65)	-4.32 (16.62) -6.43 (-36.21; 33.81) (-10.49; 2.38)	0.20	0.15
BIIP score at last 60 seconds of post-needle stage	0.640 (1.497) 0.000 (0.000; 7.000) (0.200; 1.320)	0.320 (0.748) 0.000 (0.000; 3.000) (0.080; 0.640)	-0.320 (1.626) 0.000 (-7.000; 2.000) (-1.000; 0.217)	0.50	1.00
Duration (minutes) of handling before 1st skin puncture	2.01 (1.96) 1.33 (0.47; 9.00) (1.35; 2.85)	2.15 (1.64) 1.67 (0.33; 7.33) (1.57; 2.83)	0.142 (1.713) -0.017 (-4.483; 3.833) (-0.543; 0.803)	0.69	0.59
Duration (minutes) of skin puncture period	6.47 (4.15) 5.67 (1.67; 16.00) (4.92; 8.13)	8.45 (6.79) 6.67 (1.98; 29.13) (5.97; 11.24)	1.97 (6.75) 0.80 (-11.17; 15.63) (-0.58; 4.61)	0.16	0.41

For categorical variables n (%) is presented.

For continuous variables Mean (SD) / Median (Min; Max) / (Bootstrapped (10 000 replicates) 95% CI for Mean) / n= is presented.

*) The isher Non Parametric Permutation Test for Paired observations failed to approximate the p-value so Wilcoxon Signed Rank Test was used instead.

There was a change in mean RR from baseline to skin puncture in the PP population in the lullaby intervention -7.26 (SD 12.55), (p=0.0074), (Table 4). The non-significant trends in the physiological variables described earlier in the ITT-

analysis were noticeable in the paired PP-analysis (Fig. 4, Fig. 5 and Fig. 6).

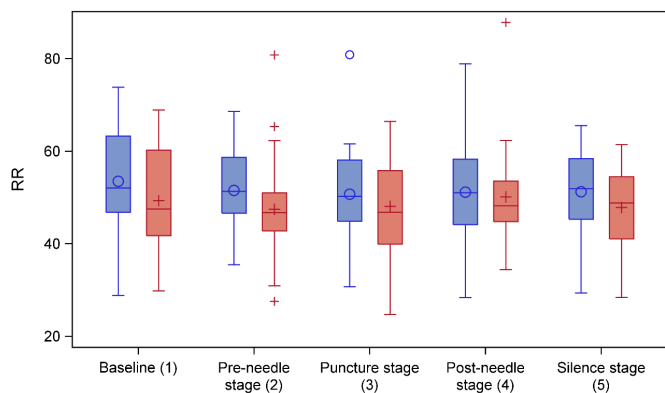


Fig 4. Box-plots showing distribution of respiratory rate (RR) during five periods (1-5) in per protocol population (PP). ■ mean control ■ mean lullaby intervention, Puncture stage (3) starts at the time of the first puncture. The top of the rectangle shows the third quartile (Q3), the horizontal line near the middle shows the median, the bottom shows the first quartile (Q1). The vertical line extending from the top of the rectangle shows the maximum value within 1.5 * IQR and the vertical line from the bottom indicates the minimum value within 1.5 * IQR. ○ and + = scores more than 1.5 times the interquartile range (IQR) below Q1 or above Q3.

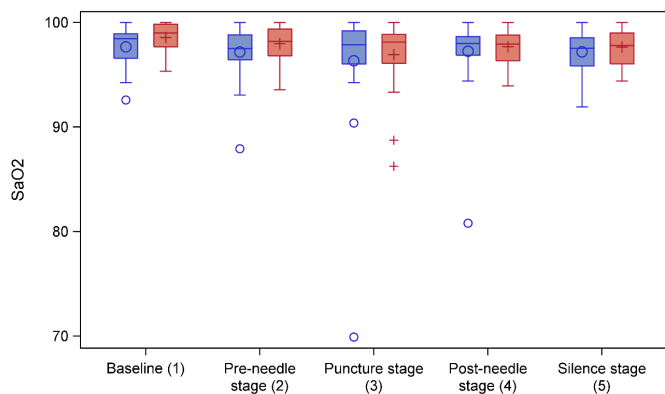


Fig 5. Box-plots showing distribution of oxygen saturation (SaO₂) during five periods (1-5) in per protocol population (PP). ■ mean control ■ mean lullaby intervention, Puncture stage (3) starts at the time of the first puncture.

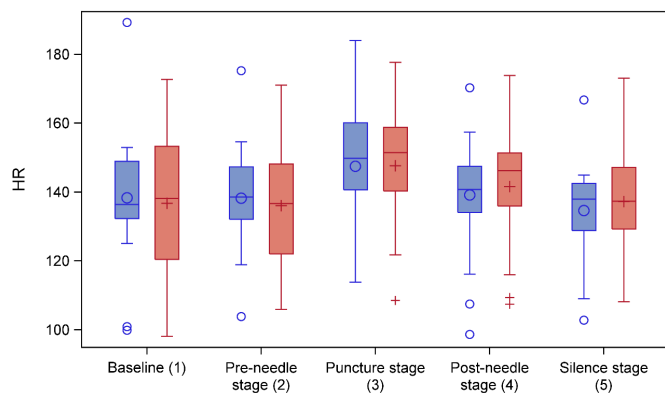


Fig 6. Box-plots showing distribution of heart rate (HR) during five periods (1-5) in per protocol population (PP). ■ mean control ■ mean lullaby intervention, Puncture stage (3) starts at the time of the first puncture.

Discussion

This is to our knowledge the first clinical trial measuring the pain-relieving effects of live lullaby singing on behavioral and physiological pain responses during venipuncture in preterm and term neonates. Considering this, we have sought to improve upon and develop existing literature in the area. Despite a comprehensive and accomplished study, our choice of design did not show any significant pain-alleviating effects of live lullaby singing on preterm and term infants' pain response assessed with PIPP-R or BIIP. Nor do the results indicate that live lullaby singing is harmful during venipuncture. Previously performed pain studies on infants with recorded lullaby music have used a no treatment control [27-30]. In contrast, our live lullaby pain study ensured the ethical conduct of neonatal pain trials [56] by providing standard pain management for all involved infants with parental presence, facilitated tucking, oral glucose and the opportunity to suck on a pacifier or on a parent's or nurse's plastic gloved finger, hence only additive effects of the live lullaby singing could be assessed. A larger number of participants might have resulted in more robust differences.

Our findings are consistent with previous observations that lullaby music may improve vital parameters like RR, HR and SaO₂ in infants in a non-painful situation [19-21,23-26,36]. In the pre-needle stage (period 2) in our study, there was a significant calmer breathing pattern in the lullaby intervention versus control, both in the ITT-analysis and the paired PP-analysis. This is an effect that probably carries clinical significance as an indicator of a relaxation response, thus suggesting that the live lullaby singing lowers stress and contributes to clinical stability [19]. There was also a non-significant trend towards a more stable HR and SaO₂ during the lullaby intervention in the pre-needle stage (period 2), which is also consistent with a relaxation response (Table 3).

The trend with improved RR and SaO₂ during lullaby intervention, was maintained during skin puncture (period 3), in the post-needle stage (period 4) and in the silent period 5. Studies using recorded music stimulation report a significant decreased HR after heel lance compared to the no-music control condition, indicating a more rapid modulation of the stress response from the heel lance procedure in the recovery period with music [27,28,30,34]. Some infants were even soothed to lower HR levels of arousal compared to baseline [28]. Comparably with these earlier findings HR in our study also decreased after venepuncture during live lullaby singing in the post-needle stage, period 4 (Table 3), surprisingly not significantly lower than the HR levels in the control condition (Fig. 6). The infants' non-significant greater levels of arousal in the lullaby intervention during period 4, could be related to the live vocal stimulation and an increase in the proportion of quiet alert state associated with increased HR response [22,51,57]. The quiet alert state, commonly considered as a positive state, can enhance the bonding between the parent and the infant [22,23,51]. However the quiet alert state is more often induced by infant-directed speech with its attention-eliciting properties for newborn infants, whereas infant-directed singing tends to maintain the infant in an active sleep state [22].

Because of the risk for overstimulation with auditory interventions, live singing must be introduced and monitored carefully in a painful setting. The microanalysis of the live lullaby singing, performed for 2 infants in this study [45], demonstrated the importance of constant assessment of the infant's vital signs and behavioral responses during the live vocal communication. The vocal performance should be more audible and soothing using elongated breathy vowels instead of a closed humming [23], and the singing should be more predictable in the temporal, shape and intensity structures from the start of the live intervention. As previous research has discussed [17], maybe the combination of vocal interaction and the painful stimuli became over stimulating during skin puncture, which suggests that live vocal communication is more beneficial in the pre- and post-needle stage.

In this study we did not assess the heart rate variability (HRV), which might have provided a clearer picture of the efficacy of live singing. Previous observations of HRV have shown that live maternal singing during kangaroo care produced changes in HRV that indicated a better autonomic stability in stable preterm infants and had a positive effect on the mothers' anxiety response [49].

Though the pain burden was not significantly lower in the lullaby intervention versus control, the non-significant trend in the present study towards shorter handling and blood sampling periods with live singing along with a non-significant trend of fewer skin punctures (Table 2 and 4), are important aspects of the live singing in that they may lessen the total burden of the painful procedure, allowing neonates to

conserve energy for growth and healing. Live singing with newborn infants is a communicative interaction, which may optimize the homeostatic mechanisms of the infant during painful procedures [45]. Assisting infants in coping with painful procedures requires age-appropriate pre-procedural preparation [9]. Infant-directed singing is a form of age-appropriate communication, which is comprehensible for infants [58]. An emotionally stable parent would, through the lullaby prosody [59], be able to communicate love and comfort before the skin puncture, safety during the pain and reassurance after. The predictable pulse of lullaby singing is ideal for emotional coordination between caregiver and infant, calming both the infant and the caregiver [49,51,60]. Due to practical reasons it was not possible to invite parents to sing in this study. Since previous studies have shown it to be likewise effective in a non-painful setting, we chose a female stranger to sing in the lullaby interventions [26,35]. If we had chosen to coach and train the parents to sing for their infant during venepuncture, the outcome might have been more differentiated. The unique prosodic qualities of infant-directed singing may effectively encourage a reciprocal relationship between parents and infants [35,60], and may provide a way for parents to become intimately involved in the treatment of their infant and convey love and safety during painful procedures, while the voice of a stranger cannot facilitate this capacity [45].

Other limitations of this study were confounding variables, with respect to differences among the specialist nurses' blood sampling preparation routines, parents being present or not, and different environmental circumstances. The problem with the PIPP-R and the BIIP, is that it is difficult to discriminate between stress caused by pain and stress related to other causes. Background noise was an issue in all situations in this study, with loud conversations in the background, as well as high-pitched sounds from monitors and phones. Non-pharmacological comfort and coping strategies like live lullaby singing applied in painful and stressful conditions, while other distracting events are occurring in the environment, are unlikely to be effective [9]. Future studies may find it useful to consider the soundscape of the NICU; eliminate background noise if possible and keep the noise levels consistent [27].

Conclusion

The additive effect of live lullaby singing has not been shown in clinical trials to necessarily alleviate infants' behavioural pain responses during venepuncture; however nor has it been shown to be harmful. The absence of significant differences do not necessarily indicate evidence of no effect, but lead to the clear need that further research is needed. Since music, as well as pain, is a multidimensional perceptual and contextual experience, research *in vivo* on these complex interactions is challenging and might require a qualitative methodology

instead of traditional quantitative approaches [9]. Studying live lullaby singing, with qualitative approaches, will enhance the nuances in the infant and parent-directed non-pharmacological comfort and coping perspective [9,45].

The loss of exposure to live vocal relationship between the parent and the infant during the first weeks of life in the NICU, has been overlooked in current research [22]. There are other advantages with musical communicative dialogues with infants, especially before and after a painful procedure. The use of live singing may encourage compassion, which is an important part of an infant and parent-directed non-pharmacological comfort and coping strategy. A research area of great future interest is how live lullaby singing can be further individualized to empower the newborn infant during painful procedures and encourage parents to participate more actively in the pain management of their infant.

Acknowledgements

Thanks to parents and staff in the NICU at Danderyd-Karolinska University Hospital in Stockholm, Sweden, to Louis Armstrong Center for Music and Medicine, New York, USA and to Nils-Gunnar Pehrsson and Mattias Molin, Statistiska Konsultgruppen, Sweden. This work was supported by Värmland County Council, Sweden; Queen Silvia's Jubilee Fund, Sweden; Karin and Erik Gerdéns Foundation, Sweden; Berit and Carl-Johan Wettergrens Foundation, Sweden; and Svensk Barnsmärtförening, Sweden.

References

- Anand KJS & Hickey PR. Pain and its effects in the human neonate and fetus. *The New England Journal of Medicine*. 1987;317(21): 1321-1329.
- Carbajal R, Rousset A, Danan C, et al. Epidemiology and treatment of painful procedures in neonates in intensive care units. *Jama*. 2008;300(1): 60-70.
- Simons SH, van Dijk M, Anand KJS, Roofthoof D, van Lingen RA & Tibboel D. Do we still hurt newborn babies?: A prospective study of procedural pain and analgesia in neonates. *Archives of Pediatrics & Adolescent Medicine*. 2003;157(11): 1058-1064. doi:10.1001/archpedi.157.11.1058.
- Goksan J, Hartley C, Emery F, et al. fMRI reveals neural activity overlap between adult and infant pain. *eLife*. 2015; 4: e06356.
- Grunau RE. Neonatal pain in very preterm infants: long-term effects on brain, neurodevelopment and pain reactivity. *Rambam Maimonides Med J*. 2013; 4(4): e0025.
- Brummelte S, Grunau RE, Chau, V, et al. Procedural pain and brain development in premature newborns. *Annals of Neurology*. 2012;71(3): 385-396.
- Doesburg SM, Chau CM, Cheung TP, et al. Neonatal pain-related stress, functional cortical activity and visual-perceptual abilities in school-age children born at extremely low gestational age. *PAIN®*. 2013;154(10): 1946-1952.
- de Graaf J, van Lingen RA, Simons SH, et al. Long-term effects of routine morphine infusion in mechanically ventilated neonates on children's functioning: five-year follow-up of a randomized controlled trial. *PAIN*. 2011;152(6): 1391-1397.
- Leroy PL, Costa LR, Emmanouil D, van Beukering A, Franck LS. Beyond the drugs: nonpharmacologic strategies to optimize procedural care in children. *Curr Opin Anaesthesiol*. 2016;29 (Suppl 1):S1-S13. doi: 10.1097/ACO.0000000000000312.
- Franck LS, Greenberg CS, Stevens B. Pain assessment in infants and children. *Pediatric Clinics of North America*. 2000;47(3): 487-512.
- Czarnecki ML, Turner HN, Collins, PM, Doellman D, Wrona S, Reynolds J. Procedural pain management: A position statement with clinical practice recommendations. *Pain Management Nursing*. 2011;12(2): 95-111.
- Anand KJS, Hall RW. Love, pain, and intensive care. *Pediatrics*. 2008;121(4): 825-7.
- Loewy, J. The use of music psychotherapy in the treatment of pediatric pain. In: Dileo, C, Ed. *Music therapy and medicine: theoretical and clinical applications*. Silver Spring, MD: American Music Therapy Association; 1999:189-206.
- Haslbeck FB. Music therapy for premature infants and their parents: An integrative review. *Nordic Journal of Music Therapy*. 2012a;21(3): 203-226.
- Lai HL, Chen CJ, Peng TC, et al. Randomized controlled trial of music during kangaroo care on maternal state anxiety and preterm infants' responses. *International Journal of Nursing Studies*. 2006;43(2): 139-146.
- Schwilling D, Vogeser M, Kirchhoff F, et al. Live music reduces stress levels in very low birth weight infants. *Acta Paediatrica*. 2015;104(4): 360-367.
- Whipple J. The effect of music-reinforced nonnutritive sucking on state of preterm, low birthweight infants experiencing heelstick. *Journal of Music Therapy*. 2008;45.3:227-272.
- Keith DR, Kendra R, Weaver BS. The effects of music listening on inconsolable crying in premature infants. *Journal of Music Therapy*. 2009;46.3: 191-203.
- Bieleninik L, Ghetti C, Gold C. Music Therapy for Preterm Infants and Their Parents: A Meta-analysis. *Pediatrics*; 2016;138(3):e20160971
- Arnon S, Shapsa A, Forman L. et al. Live music is beneficial to preterm infants in the neonatal intensive care unit environment. *Birth*. 2006;33.2: 131-136.
- Cassidy JW, Standley JM. The effect of music listening on physiological responses of premature infants in the NICU. *Journal of Music Therapy*; 1995;32(4): 208-227.
- Filippa M, Devouche E, Arioni C, Imberty M, Gratier M. Live maternal speech and singing have beneficial effects on hospitalized preterm infants. *Acta Paediatrica*. 2013; 102(10):1017-1020.
- Loewy J, Stewart K, Dassler AM, Telsey A, Homel P. The effects of music therapy on vital signs, feeding, and sleep in premature infants. *Pediatrics*. 2013;131(5): 902-918.
- Rand K, Lahav A. Maternal sounds elicit lower heart rate in preterm newborns in the first month of life. *Early Human Development*. 2014;90.10: 679-683.
- Standley JM, Moore RS. Therapeutic effects of music and mother's voice on premature infants. *Pediatric Nursing*. 1995;21(6): 509-512.
- Teckenberg-Jansson P, Huotilainen M, Pölkki T, Lipsanen J, Järvenpää AL. Rapid effects of neonatal music therapy combined with kangaroo care on prematurely-born infants. *Nordic Journal of Music Therapy*. 2011;20(1): 22-42.
- Bo LK, Callaghan P. Soothing pain-elicited distress in Chinese neonates. *Pediatrics*. 2000;105(4): e49-e49.
- Butt ML, Kisilevsky BS. Music modulates behaviour of premature infants following heel lance. *The Canadian Journal of Nursing Research*. 2000;31(4): 17-39.

29. Bergomi P, Chieppi M, Maini A, Mugnos T, Spotti D, Tzialla C, Scudeller L. Nonpharmacological Techniques to Reduce Pain in Preterm Infants Who Receive Heel-Lance Procedure: A Randomized Controlled Trial. *Research and Theory for Nursing Practice*. 2014;28(4): 335-348.
30. Shabani F, Nayeri ND, Karimi R, Zarei K, Chehrazi M. Effects of music therapy on pain responses induced by blood sampling in premature infants: A randomized cross-over trial. *Iranian Journal of Nursing and Midwifery Research*. 2016;21(4): 391-396.
31. Chou LL, Wang RH, Chen SJ, Pai L. Effects of music therapy on oxygen saturation in premature infants receiving endotracheal suctioning. *Journal of Nursing Research*. 2003; 11(3): 209-216.
32. Ghetti CM. Music therapy as procedural support for invasive medical procedures: toward the development of music therapy theory. *Nordic Journal of Music Therapy*. 2012;21(1): 3-35.
33. Pölkki T, Korhonen A. The effectiveness of music on pain among preterm infants in the NICU: A systematic review. *The JBI Database of Systematic Reviews and Implementation Reports*. 2014;12(4): 354-373.
34. Tramo MJ, Lense M, Van Ness C, Kagan J, Settle MD, Cronin JH. Effects of Music on Physiological and Behavioral Indices of Acute Pain and Stress in Premature Infants Clinical Trial and Literature Review. *Music and Medicine*. 2011;3(2):72-83.
35. de L'Etoile SK. Infant behavioral responses to infant-directed singing and other maternal interactions. *Infant Behavior and Development*. 2006;29(3):456-470.
36. Garunkstiene R, Buinauskiene J, Uloziene I, Markuniene E. Controlled trial of live versus recorded lullabies in preterm infants. *Nordic Journal of Music Therapy*. 2014;23(1):71-88.
37. Nöcker-Ribaupierre M. Ed. *Music Therapy for Premature and Newborn Infants*. Gilsum: Barcelona Books; 2004.
38. O'Neill CT, Trainor LJ, Trehub SE. Infants' responsiveness to fathers' singing. *Music Perception*. 2001;18(4): 409-425.
39. Loewy J, MacGregor B, Richards K, Rodriguez J. Music therapy pediatric pain management: Assessing and attending to the sounds of hurt, fear and anxiety. In: Loewy J, Ed, *Music therapy and pediatric pain*. New Jersey: Jeffrey Books;1997: 45-56.
40. Turry AE. The use of clinical improvisation to alleviate procedural distress in young children. In: Loewy J, Ed., *Music therapy and pediatric pain*. New Jersey: Jeffrey Books;1997: 89-96.
41. Fernandes A, Campbell-Yeo M, Johnston CC. Procedural pain management for neonates using nonpharmacological strategies: Part 1: sensorial interventions. *Advances in Neonatal Care*. 2011;11.4: 235-241.
42. Stevens BJ, Gibbins S, Yamada J, et al. The premature infant pain profile-revised (PIPP-R): initial validation and feasibility. *The Clinical Journal of Pain*. 2014;30(3): 238-243.
43. Olsson E, Ahlsén G, Eriksson M. Skin-to-skin contact reduces near-infrared spectroscopy pain responses in premature infants during blood sampling. *Acta Paediatrica*. 2015:1-5.
44. Melzack R, Wall PD. Pain mechanisms: A new theory. *Science*. 1965;150:971-979.
45. Ullsten A, Eriksson M, Klässbo M, Volgsten U. Live music therapy with lullaby singing as affective support during painful procedures: A case study with microanalysis. *Nordic Journal of Music Therapy*. 2016:1-25.
46. Mondanaro J, Sara GA. *Music and medicine: Integrative models in the treatment of pain*. New York: Satchnote Press; 2013.
47. Bernatzky G, Presch M, Anderson S, Panksepp J. Emotional foundations of music as a non-pharmacological pain management tool in modern medicine. *Neuroscience & Biobehavioral Reviews*. 2011;35(9):1989-1999.
48. Shoemark H. Contingent singing: The musicality of companionship with the hospitalized newborn infant. In: Baker F, Uhlig S, Eds., *Voicework in music therapy. Research and practice*. London: Jessica Kingsley Publishers; 2011;231-251.
49. Arnon S, Diamant C, Bauer S, Regev R, Sirota G, Litmanovitz I. Maternal singing during kangaroo care led to autonomic stability in preterm infants and reduced maternal anxiety. *Acta Paediatrica*. 2014;103(10): 1039-1044.
50. Schlez A, Litmanovitz I, Bauer S, Dolfin T, Regev R, Arnon S. Combining kangaroo care and live harp music therapy in the neonatal intensive care unit setting. *The Israel Medical Association Journal: IMAJ*. 2011;13(6): 354-358.
51. Loewy J. NICU music therapy: song of kin as critical lullaby in research and practice. *Annals of the New York Academy of Sciences*. 2015;1337: 178-185.
52. Axelin A, Eriksson M, Gradin M. Smärta [Pain]. In Jackson K, Wigert H. Eds., *Familjecentrerad neonatalvård*. Lund: Studentlitteratur; 2013: 111-119.
53. Neal D, Lindeke L. Music as a nursing intervention for preterm infants in the NICU. *Neonatal Network*. 2008;27.5: 319-327.
54. Philbin MK. The influence of auditory experience on the behavior of preterm newborns. *Journal of perinatology: official Journal of the California Perinatal Association*. 2000;20(8 Pt 2): 77-87.
55. Holsti L, Grunau RE. Initial validation of the Behavioral Indicators of Infant Pain (BIIP). *Pain*. 2007;132(3): 264-272.
56. Campbell-Yeo M. 'First, do no harm' – the use of analgesia or placebo as control for babies in painful clinical trials. *Acta Paediatrica*. 2016;105.2: 119-120.
57. Rock AML, Trainor LJ, Addison TL. Distinctive messages in infant-directed lullabies and play songs. *Developmental Psychology*. 1999;35(2): 527-534.
58. Fernald A. Intonation and communicative intent in mothers' speech to infants: Is the melody the message? *Child Development*. 1989:1497-1510.
59. Bergeson T.R, Trehub SE. Absolute pitch and tempo in mothers' songs to infants. *Psychological Science*. 2002;13(1): 72-75.
60. Nakata T, Trehub SE. Infants' responsiveness to maternal speech and singing. *Infant Behavior and Development*. 2004;27(4): 455-464.

Appendix A

Lullabies used in the lullaby intervention and how often used in the study (%).

- 40 % Trollmors vaggång (Holmberg, M., 1943, Swedish)
- 27 % Byssan lull (Taube, E., 1919, Swedish)
- 24 % Vyssa, lulla litet barn (trad., 1842, Swedish)
- 6 % Ro ro barnet (Tegnér, A. 1892, Swedish)
- 3 % Videvisan (Tegnér, A. & Topelius, Z., 1895, Swedish)

Biographical Statements

Alexandra Ullsten, PhD candidate at Örebro University and a member of the PEARL (Pain in Early Life) research group, is a pioneer in Sweden in the field of neonatal music therapy.

Pernilla Hugoson is a PhD-student in Music Therapy at University of Jyväskylä, Finland and affiliated to Karolinska Institute, Sweden.

Malin Forsberg, is a medical intern at Mora hospital.

Lisa Forzelius, is a former medical student at the Karolinska Institute and a current medical intern at Sundsvall hospital.

Maria Klässbo is a PhD and RPT working since 2003 as a research tutor at Centre for Clinical Research at Värmland County Council.

Emma Olsson, PhD, is working as a nurse at the Neonatal Intensive Care Unit at the University Hospital in Örebro.

Ulrik Volgsten PhD, is Professor in Musicology, whose research includes studies on the role of affect attunement for the perception of music, from historical, ethnological and philosophical perspectives.

Björn Westrup MD, PhD. Danderyd Karolinska Institute, Astrid Lindgren Children's Hospital, Karolinska University Hospital, Stockholm, Sweden.

Ulrika Ådén MD, PhD. Neonatal Research Unit, Department of Women's and Children's Health, Karolinska Institute, Karolinska University Hospital, Astrid Lindgren Children's Hospital, Stockholm, Sweden

Lena Bergqvist MD, PhD, is a clinical doctor in pediatrics at the child clinic and delivery ward at Södertälje hospital and post doc. in clinical research on neonates at Karolinska Institutet.

Mats Eriksson PhD, is Professor in Nursing Science and leader of the PEARL (Pain in Early Life) research group.