Full-Length Article

The Effects of Live Singing on the Biophysiological Functions of Preterm Infants Hospitalized in a Neonatal Intensive Care Unit in Greece: *A Pilot Study.*

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Abstract

The aim of the study was to investigate the effects of live singing on the biophysiological functions, weight gain, head circumference, body length, and duration of hospitalization of preterm infants hospitalized in a neonatal intensive care unit (NICU) in Greece. Forty-one preterm infants 25-33 weeks + 6 days of PCA took part in the study. 14 infants were assigned to the Mother Singing group (MS) in which mothers sang to their infants for 15 minutes every day during the fourteen-day intervention, 13 to the Music Therapist Singing group (MTS) in which a music therapist sang selected songs to the infants for 15 minutes every day during the 14-day intervention, and 14 in the control group (CG) which did not receive any additional intervention. Infants of both MS and MTS presented statistically significant improvements in heart rate and blood oxygen saturation, compared to CG. No significant differences between groups were found in weight, head circumference, body length, and duration of hospitalization. The use of live singing by mothers or music therapists appears to elicit positive effects on vital biophysiological functions of premature infants hospitalized in NICU in Greece. Further investigation of the role of live singing in the NICUs in Greece is warranted.

Keywords: ***singing, NICU, preterm infant, music therapy, mother***

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Introduction

Globally, 1 in 10 newborns is born prematurely, before the 37th week of gestation [1,2]. Due to functional and anatomic immaturity, preterm infants are at high risk of severe short- and long-term health problems and disabilities [3-5]. As a result, immediate management after birth in the Neonatal Intensive Care Units (NICU) is considered vital, to maximize the possibility of intact survival.

NICUs typically have been noisy and stressful environment due to the architecture that permitted a great number of infants receiving care in a co-joint space. Although this has changed in some cases in the Western world, most NICUs globally and certainly within Greece accommodate many infants in the same room, which may present psychophysiological and physiological challenges in premature infants. This includes noise which might evoke irritability and crying, or stress related to fatigue, overstimulation, oxygen desaturation, increased heartrate and/or irregular breathing [6-9]. Additional concerns regarding the premature infants' untimely separation from the mother's body, which in addition to the lack of family-centered care in NICUs may increase the risk of short- and long- term emotional and developmental problems [10-11]. The application of supplementary interventions and music therapy strategies to reduce the impact of the often, unfavorable elements of the infants' experiences of the NICU environment and to address medical problems experienced during hospitalization [12-15] have not been well-researched and are not readily implemented in the NICUs in Greece.

Previous studies have suggested that music may be a useful complementary therapeutic technique for the NICU and proposed the use of music and music therapy interventions, such as live or recorded lullabies and lullaby-like singing, 'songs of kin' with calm and continuous rhythms that are culturally indicated, with simple melodies [16-19], and/or specific instrumental music [20-21] or soundscapes that resemble the sounds heard by the fetus in the womb [16, 22], with sound intensity outside the incubator ranging no

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higher than 65 - 70db on the A scale basis [23]. Indeed, studies have highlighted the positive effects of a variety of music therapy interventions in NICU on infants' heartbeat, blood oxygen saturation, breathing, caloric intake, weight gain, emotional stress, pain management, quality of sleep [6, 14-16, 18, 24-29] and on length of hospital stay [25, 30]. Some scholars have argued that live music is preferable than recorded music, as it can more easily guarantee the desired stability, continuity, calmness, and relaxation of the infant, and continuity of care and bonding post hospitalization [16, 24]. Given the infants' familiarity with the mother's voice [6, 16], mother's singing is often considered to be more effective compared to other voices [6, 11, 31, 32, 35], especially when using lullabies, which are simple and predictable in melodic structure [5, 6, 8, 16, 17, 33, 34, 35] and can be fostered to imbue the cultural heritage of the family. This can strengthen the aspect of familiarity and recognition for the infant-parent bond and can foster the probability of continuity of care at home, easing the transition from hospital to home [16].

The present pilot study aimed to investigate the effects of live singing by the mother or the music therapist on the biophysiological functions and development of preterm infants (≤25-33 weeks +6 days of PCA) hospitalized in a NICU in Greece. To the best of our knowledge, this is the first published study of this kind in Greece. Although there is ample research internationally on the effects of music interventions on pre-term infants, we consider this culturally specific research to be important in identifying whether there are issues that are culturally defined or that can verify the universal findings on the effects of music interventions on the biophysiological functions of preterm infants. The primary objective of the study was to investigate the impact of live singing songs (Appendix I) using the mother's voice or the voice of a female music therapist to regulate the infants' heart rate. The secondary objectives of the study were to investigate: a) whether the two treatment modalities were more effective than standard treatment alone in eliciting positive changes in blood oxygen saturation (SpO2), caloric intake, weight gain, and duration of hospitalization of preterm infants, and b) whether the two music interventions differed regarding their effect on the primary and secondary outcomes of the study.

Materials and Methods

The study was conducted from June 2017 to February 2018 at the Neonatology Department of a major university public hospital in Greece. The study was approved by the Scientific Committee of the hospital and the General Assembly of the university department with which the primary researchers were affiliated and which according to the internal regulations of the university, entailed no further required permission.

Inclusion Criteria

Participants in the study met the following inclusion criteria at the point of entry to the study: (a) born between 25 – 33 weeks+6 days of pregnancy; (b) hospitalized in Level I, II and III at the same NICU; (c) weighed 1000 grams or more, (d) reached their original birth weight; (e) their health was stable and there was no risk of fatality; (e) were not on repression medication; (f) had a prospective hospital stay in the NICU for at least one month. According to the study protocol, an infant's participation would be terminated in the following cases: (a) when facing necrotizing enterocolitis, as this could be threatening for the life of the infant and further necessitated milk feeding to stop, affecting food intake which was a secondary outcome of the study; (b) severe aggravation of an infant's health as this would increase the duration of hospital stay which was a secondary outcome of the study; (c) transfer of the infant to a different NICU for operation due to severe aggravation of health; and (d) unexpected death of the infant.

Study groups

Due to the practical implications of mothers' participation in the study, it was not possible to randomize the infants within the 3 study groups. Consequently, this being a pilot project, it made allowances for the 41 infant-participants to be assigned to one of the study groups as follows: 14 infants whose mothers were willing and available on a daily basis to deliver live singing for their babies were assigned to the Mother Singing group (MS); 13 infants whose mothers were willing to participate but were not available on a daily basis were assigned to the Music Therapist Singing group (MTS). The 14 infants whose mothers did not want them to receive any of the interventions, were assigned to the control group (CG). CG infants received the standard medical treatment for hospitalized preterm infants but no music treatment. Except from the music intervention that was absent in the CG, the mothers and nurses caring for the CG infants were allowed to interact with the babies in the same way that mothers and nurses of the other two groups did. No special instructions were given to mothers and nurses of the CG. Consent forms were signed by parents of the 41 infants who met the inclusion criteria.

Music interventions

The intervention period was comprised of 14 consecutive daily sessions for each infant of the MS and MTS groups. Although, a music intervention would be unlikely to take place in a format of 14 consecutive days in a clinical NICU setting, this decision was made because of the restrictions of the NICU for hosting this research study and the need to control for the number of preterm infants participating in the study in the course of the 8-month research period. The intervention for both groups lasted 15 minutes and took place before the afternoon feeding process, as this is the time mothers are permitted to enter the NICU settings in Greece and no medical interventions are performed during this time, except in the cases of emergency.

The MS and MTS interventions took place in the form of live singing of lullabies or lullaby-like melodies by the voice of the mother or a female music therapist respectively. The song form was guided by the principles of Song of Kin [16]. The researcher suggested to mothers to sing songs of their own preference ('song of kin' employs parent-selected meaningful songs) [16], and informed them of the main features of appropriate music for NICU environment, i.e. simple melodic line, gentle rhythm with flow, a cappella and soft voice timbre [28], so that these would be taken into consideration in their choices. The music therapist used her own 'songs of kin', with characteristics suitable for NICU, in the MTS group cohort. (Appendix I) The tempo of the songs was suggested to be approximately at 65-75 bpm, which corresponded to the heartbeat of most of the women during pregnancy, as we considered that this tempo would be less likely to cause over-stimulation or stress to the infant. We considered that it was important to leave the choice of songs to the mother, as we valued the emotional context that the mother would bring to the treatment through her singing. All songs sung by the mother and the music therapist were sung acapella. Both mothers of the MS group and the MTS were encouraged to make small pauses in singing, to offer space for the infant to respond, and which would propel the MT and mother to examine the infant's responses and adjust communicative singing. The music therapist who implemented the intervention protocol of the study was trained in the Rhythm, Breath and Lullaby music therapy approach for NICU [36] by attending relevant training courses over the past few years.

During both types of intervention, infants were in the incubators with the incubator doors left open. The intensity of sound reaching the incubator was repeatedly measured via a Terrasonde sound level meter prior to the beginning of the study. The measurements confirmed that a 65-75dbA sound intensity outside the incubator was heard as 60dbA inside the incubator, as also shown in previous studies [37]. Similarly, mothers of the MS group were guided to control the sound intensity of their singing so that it would range between 65 and 75dbA.

After consulting the NICU medical staff, it was determined that MS and MTS sessions would be interrupted if infants exhibited one of the following signs or symptoms and would resume only if the medical staff considered it appropriate: (a) severe changes in physiology, such as low blood oxygen saturation, low heart rate, low respiratory rates, apnea or bradycardia; (b) subtle disengagement cues such as hiccoughs, grimace, clinched eyes, eyes averted, tong posture, finger splay, struggling movement; and (c) potent discharge cues such as crying, whining, fussing, cry face, spitting/vomiting, and hand in halt position [38]. For the second and third case, it was predetermined that the mothers and music therapist would initially reduce the volume and tempo of their singing and if the cues persisted, the sessions would be stopped.

Outcome measurements

The outcome measurements of the study comprised: (a) daily heart rate and SpO2 measurements taken from the NICU medical monitors via pulse oximeters; (b) daily measurements of food intake provided by the nursing stuff via TPN-CALCUL medical software, which calculated fluid (ml/kg/day) and nutritional (kcal/kg/day) intake based on the total amount and type of milk consumed; (c) daily weight measurements provided by the nursing stuff; and (d) weekly measurements of head circumference and body length, made by a neonatologist. The total number of days of hospitalization was also recorded for each infant. Heart rate and SpO2 measurements in MS and MTS were taken 10 minutes before and after the beginning of each music intervention, as well as 10 minutes after the end of each intervention. The same measurements at the same hours of the day were also taken for the infants of CG.

Results

All data were analyzed using the SPSS statistic software, version 23.

Descriptive analysis of the sample

The results of the descriptive analysis concerning the socio-demographic data of the mothers and the pregnancy period and birth are summarized in Tables 1a, 1b and 1c (*see appendix*). Of the 27 infants that received one of the two interventions, 3 infants participated in 12 sessions, 3 infants in 13 sessions, and 2 infants took part in 11 sessions, all due to earlier exit from the NICU.

Treatment outcomes

A mixed-design ANOVA with Tukey's post-hoc tests was carried out to investigate the within- and between-subjects effects and time by group interaction regarding the pre-session to post-session impact of the interventions on the heart rate and SpO2 of the infants, as well as the effects of the interventions on their overall physical development (head circumference, height, weight) and nutritional intake (kcal and fluid) during the study. A one-way ANOVA was also carried out to observe potential differences between groups with respect to length of hospital stay. The results are summarized in Tables 2a, 2b and 2c (*see appendix)*. Normality and outlier tests were carried out and all results were reported within the accepted ranges for the dependent variables of the study.

Analysis of changes in heart rate showed that that there was a significant difference in time, F (1, 39) = 522.52, p < 0.001, $\eta 2$= 0.931. Between-group comparisons revealed statistically significant differences, F (2, 39) = 59.47, p < 0.001, $\eta 2$= 0.753, while a significant interaction between time and group was also observed F (2, 39) = 313.01, p < 0.001, $\eta 2$= 0.941. Further to this, this interaction indicated that the mean pre- session scores of MS group were higher than those of MTS, p < 0.001. Statistically significant decreases were observed within both MS, p < 0.001, and MTS, p < 0.001, while significant increases were observed within the control group, p < 0.001 (see Fig. 1). Both MS and MTS groups presented a statistically significant improvement compared to the control group, while MS appeared to induce a significantly stronger effect than MTS.

Figure 1

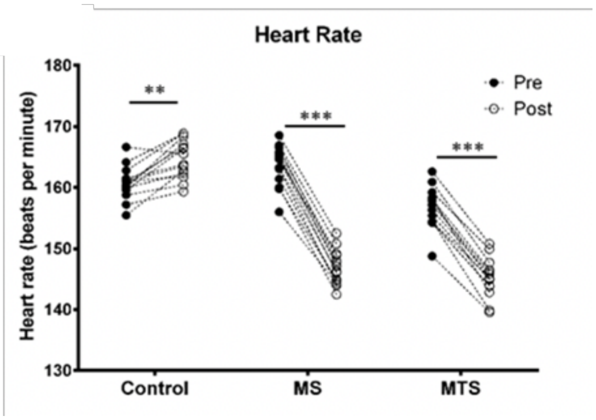


Figure 1. *Presentation of changes in heart rate within the 3 groups during the study. This figure presents the pre-session to post-session mean changes exhibited within each group. *** p < 0.001, ** p < 0.01.*

The results for changes in blood oxygen saturation revealed that that there was a significant difference in time, F (1, 39) = 20.49, p < 0.001, $\eta 2$= 0.344, and significant differences between groups, F (2, 39) = 39.97, p < 0.001, $\eta 2$= 0.672. A significant interaction between time and group was also observed F (2, 39) = 28.4, p < 0.001, $\eta 2$= 0.593. Further to this, the mean pre-session scores of both MS and MTS groups were higher than those of the control group, p < 0.001 and p = 0.001 respectively. Statistically significant increases were observed within both MS, p < 0.001, and MTS, p < 0.001, while a statistically significant decrease was observed within the control group, p = 0.001 (see Fig. 2). Both MS and MTS groups presented a statistically significant improvement compared to the control group, but no significant differences between them.

Figure 2

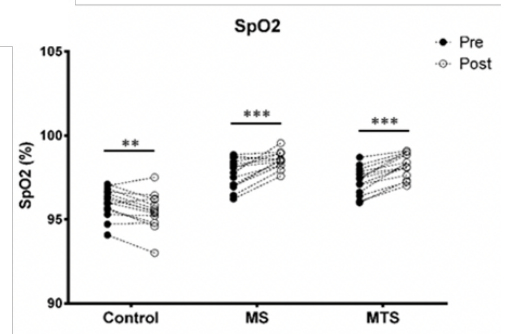


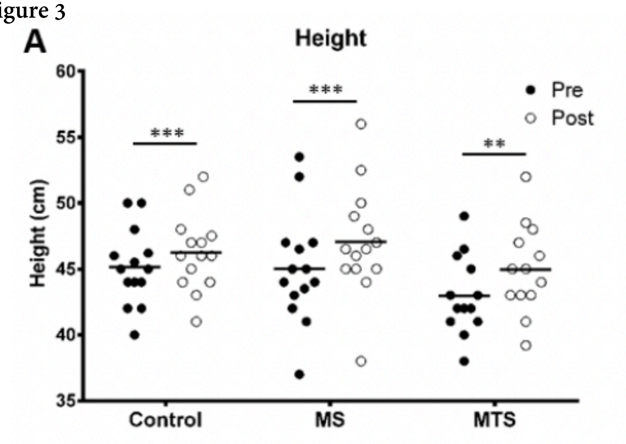
Figure 2. *Presentation of changes in blood oxygen saturation within the three groups during the study. The figure presents the pre-session to post- session mean changes exhibited within each group. *** p < 0.001, ** p < 0.01.*

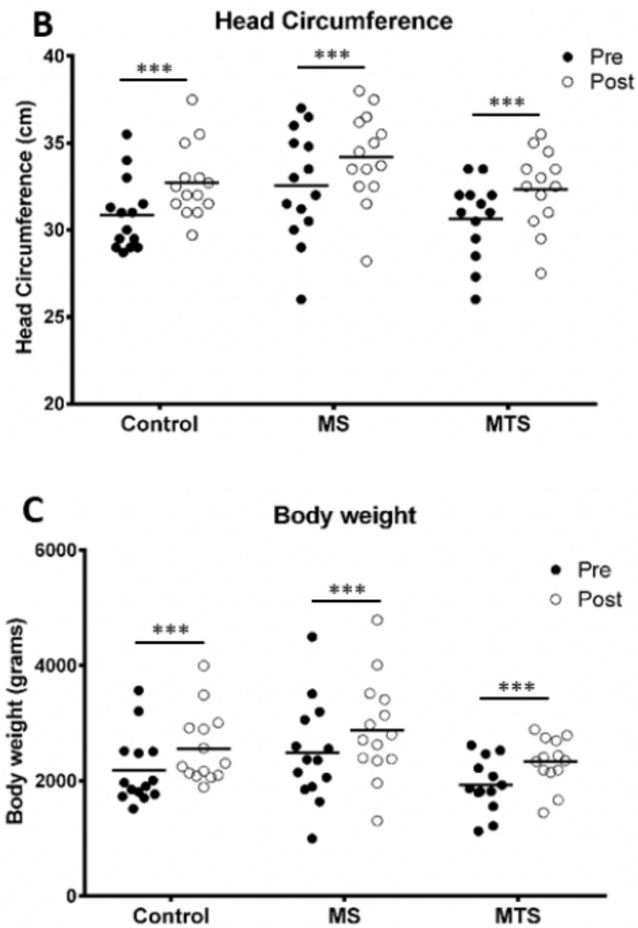
Analysis on changes in body length revealed that that there was a significant difference in time, F (1, 38) = 67.43, p < 0.001, $\eta 2$= 0.64, but no significant differences between groups, F (2, 38) = 1.4, p = 0.26, $\eta 2$= 0.069, or interaction between time and group, F (2, 38) = 1.99, p = 0.15, $\eta 2$= 0.095. Post-hoc analysis did not show any significant differences at baseline and revealed similar increases within all three groups (see Fig. 3a).

With respect to changes in head circumference, analysis revealed a significant difference in time, F (1, 38) = 212.73, p < 0.001, $\eta 2$= 0.848, but no significant differences between groups, F (2, 38) = 2.45, p = 0.1, $\eta 2$= 0.114, or interaction between time and group, F (2, 38) = 0.35, p = 0.71, $\eta 2$= 0.018. Post-hoc analysis showed that were no significant differences between groups at baseline and showed similar increases within all groups (see Fig. 3b).

Similarly, the results on changes in weight gain revealed that that there was a significant difference in time, F (1, 38) = 447.6, p < 0.001, $\eta 2$= 0.922, but no significant differences between groups, F (2, 38) = 2.33, p = 0.11, $\eta 2$= 0.109, or interaction between time and group, F (2, 38) = 0.165, p = 0.85, $\eta 2$= 0.009. Post-hoc analysis showed that the groups did not significantly differ at baseline and that all groups experienced statistically significant increases in weight during the study (p < 0.001) which did not differ between groups (see Fig. 3c).

Figure 3

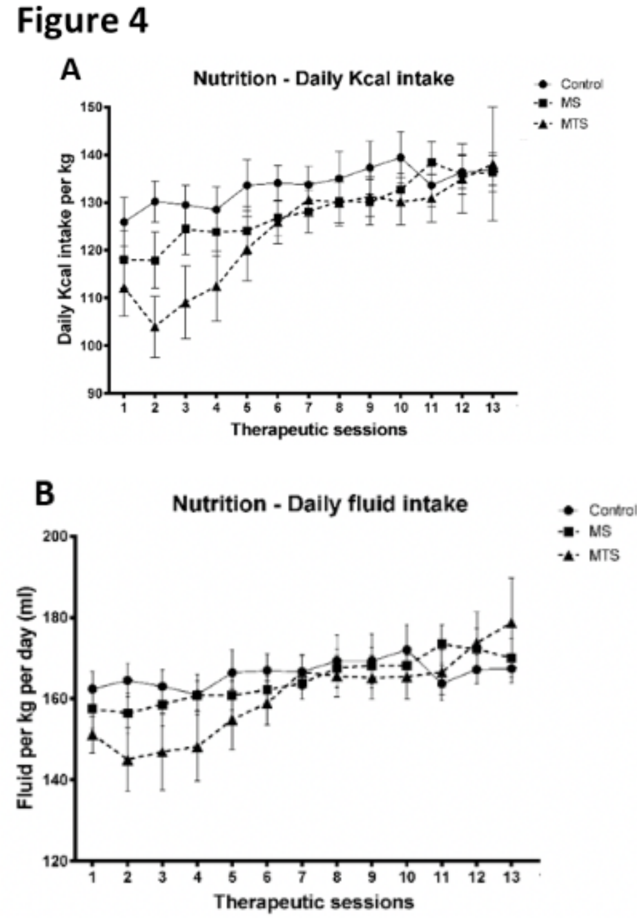




Figures 3a, 3b, 3c. *Presentation of changes in height (figure 3a), head circumference (figure 3b) and body weight (figure 3c) of the infants during their participation in the study (baseline to end of study). *** p < 0.001, ** p < 0.01.*

Analysis on nutrition intake (Kcal/Kg per day) showed that that there was a significant difference in time, $F\ (1, 30) = 7.9$, $p = 0.009$, $\eta 2 = 0.208$, but no significant differences between groups, $F\ (2, 30) = 0.6$, $p = 0.56$, $\eta 2 = 0.038$, or interaction between time and group, $F\ (2, 30) = 0.484$, $p = 0.62$, $\eta 2 = 0.031$. Post-hoc analysis showed that the groups did not significantly differ at baseline and that MS and MTS presented increases that approached statistical significance, $p = 0.057$ and $p = 0.061$ respectively, as opposed to CG which did not exhibit statistically significant changes (see Fig. 4a). No significant differences between groups were found.

Regarding fluid intake (ml/Kg per day), analysis revealed that there was a significant difference in time, $F\ (1, 30) = 6.75$, $p = 0.014$, $\eta 2 = 0.184$, but no significant differences between groups, $F\ (2, 30) = 0.295$, $p = 0.75$, $\eta 2 = 0.019$, or interaction between time and group, $F\ (2, 30) = 1.205$, $p = 0.31$, $\eta 2 = 0.074$. Post-hoc analysis did not reveal any significant differences at baseline. Statistically significant increases were found only in the MTS, $p = 0.032$, while MS and CG increases did not reach statistical significance, $p = 0.086$ and $p = 0.729$ respectively (see Fig. 4b). No significant differences were observed between groups. Finally, analysis did not show any significant effect of group on the duration of the infants' overall length of hospital stay, $F\ (2, 38) = 0.62$, $p = 0.54$.



Figures 4a, 4b. *Presentation of changes in nutrition and fluid intake of the infants during the study (session 1 to session 13). Figure 4a presents the course of nutrition intake (Kcal/Kgr per day) and figure 4b presents the course of fluid intake (ml/Kgr per day) within each group.*

Discussion

Consistent with the literature [8, 15, 24, 27], the findings of the study confirmed our hypothesis about the capacity of live singing and song of kin institution to regulate heart rate in premature infants, as both music interventions elicited significant improvements compared to CG, with MS appearing to be superior than MTS. Regarding the latter, the higher pre- session scores of MS compared to MTS group, which may be due to infants' hyperactivity in anticipation to their mother's visit, may partly explain the presented differences and do not allow for definitive conclusions to be drawn about the potential superiority of live singing by the mother over live singing by the music therapist.

Our study also confirmed that live singing, and in particular the implementation of adopting the mothers' song of kin, can be a useful intervention for SpO2 regulation in NICU [5-6, 15, 24, 27, 34, 35]. Despite the higher pre-session scores of both intervention groups compared to CG, the infants of both MS and MTS groups presented significant amelioration of SpO2 levels compared to the infants of the control group. Although we cannot acquire insight into the reasons behind the pre-session differences between groups, the improvements elicited by the singing interventions indicate that live singing may facilitate breathing functions [27, 37], which could further be associated with the respective heart rate regulation in MS and MTS infants.

We did not find any significant differences between groups in weight gain, as previously indicated by other studies [8, 18, 27]. We did observe, however, that both MS and MTS presented considerable increases in nutritional and fluid intake, which were either significant or approached statistical significance. This indicates that longer periods of music interventions could potentially have a positive impact on these variables and further lead to weight gain.

We did not observe any significant differences between groups regarding infants' body length and head circumference, or length of hospital stay. It is possible that a different study protocol with longer intervention sessions and periods could be more indicative of the potential benefits of music interventions on these variables, as indicated in previous studies [25, 30].

The limitations of our study, i.e. the non-randomization of the sample due to the complexity of the study and the NICU environment, and the relatively small sample size, do not allow for strong conclusions to be drawn. However, as the first pilot study in Greece to be published on the subject, this sets the path for further research on the effects of live singing in Greek NICUs, so as to promote good practice and further investigate whether there are country-specific factors that may influence the effectiveness of the intervention, as well as if there are findings that seem to be universal. For instance, although there seems to be a consensus on good practice in NICU environments across many European countries and policies, NICUs in Greece seem to be hosting a higher number of infants in what can be considered as more noisy environments. This is mainly due to the burden imposed by economy-related funding cuts on major functional and operational aspects of NICUs.

Similarly, there is a lack of family-centered care, and parents can only visit their infants twice per day. Furthermore, the effects of 'song of kin' which imbue meaningful themes and carry culturally-defined meanings on the heart rate and SpO2 levels of premature infants hospitalized in NICU in Greece, is also worthy of further investigation. To this end, perhaps using the same 'song of kin' in each of the test groups, and also logging the vocal range of the mother's voice might render some further descript results.

In conclusion, given the variation in NICU settings and treatment and care protocols in Greece, further investigation on the therapeutic benefits of live singing for premature infants has been both confirmed and warranted as a significant first step toward promoting good practice in the country. We hope this may be a beginning toward identifying significant culture-related factors that may influence the therapeutic process.

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Appendix I: Table 1. Presentation of the descriptive analysis of the sociodemographic data and information on pregnancy/birth within the study sample.

MS

Mother's Age			**Education**			**Family status**			**Single-parent family**			**Working mother**		
	Frequency	Percent		Frequency	Percent		Frequency	Percent		Frequency	Percent		Frequency	Percent
<18	1	7,1	Primary school	1	7,1	Single	5	35,7	Yes	2	14,3	Yes	5	35,7
18-25	2	14,3												
26-35	7	50,0	High school	6	42,9							No	8	57,1
36-45	3	21,4	Vocational training	4	28,6	Married	9	64,3	No	12	85,7	Not answered	1	7,2
>45	1	7,1	University	3	21,4	Total	14	100,0	Total	14	100,0	Total	14	100,0
Total	14	100,0	Total	14	100,0									
Child gender			**Labour method**			**Gestation**			**Number of sessions attended**			**Birth week**		
	Frequency	Percent		Frequency	Percent		Frequency	Percent		Frequency	Percent		Frequency	Percent
Male	8	57,1	Natural	2	14,3	Single	11	78,6	14	12	85,8	<28	3	21,4
									11	1	7,1	28-31+6	6	42,9
Female	6	42,9	C-section	12	85,7	Twins	3	21,4	13	1	7,1	32-33+6	5	35,7
Total	14	100,0	Total	14	100,0	Total	14	100,0	Total	14	100,0	Total	14	100,0

MTS

Mother's Age			**Education**			**Family status**			**Single-parent family**			**Working mother**		
	Frequency	Percent		Frequency	Percent		Frequency	Percent		Frequency	Percent		Frequency	Percent
18-25	1	7,7	High School	6	46,2							Yes	5	38,5
26-35	6	46,2	Vocational training	1	7,7	Single	2	15,4	Yes	0	0,0	No	6	46,2
36-45	3	23,1												
>45	1	7,7	University	4	30,8	Married	11	84,6	No	13	100,0	Not answered	2	15,4
Not answered	2	15,4	Not answered	2	15,4	Total	13	100,0	Total	13	100,0	Total	13	100,0
Total	13	100,0	Total	13	100,0									
Child gender			**Labour method**			**Gestation**			**Number of sessions attended**			**Birth week**		

	Frequency	Percent
Male	9	69,2
Female	4	30,8
Total	13	100,0

	Frequency	Percent
Natural	2	15,4
C-section	11	84,6
Total	13	100,0

	Frequency	Percent
Single	10	76,9
Twins	3	23,1
Total	13	100,0

	Frequency	Percent
14	7	53,8
11	1	7,7
12	3	23,1
13	2	15,4
Total	13	100,0

	Frequency	Percent
<28	1	7,7
28-31+6	7	53,8
32-33+6	5	38,5
Total	13	100,0

CG

Mother's Age	Frequency	Percent
<18	1	7,1
18-25	0	0,0
26-35	8	57,1
36-45	4	28,6
>45	1	7,1
Total	14	100,0

Education	Frequency	Percent
Primary school	1	7,1
High school	3	21,4
Vocational training	1	7,1
University	5	35,7
Not answered	4	28,6
Total	14	100,0

Family status	Frequency	Percent
Single	3	21,4
Married	11	78,6
Total	14	100,0

Single-parent family	Frequency	Percent
Yes	1	7,1
No	13	92,9
Total	14	100,0

Working mother	Frequency	Percent
Yes	7	50,0
No	7	50,0
Total	14	100,0

Child gender	Frequency	Percent
Male	6	42.9
Female	8	57.1
Total	14	100,0

Labour method	Frequency	Percent
Natural	2	14.3
C-section	12	85.7
Total	14	100,0

Gestation	Frequency	Percent
Single	8	57.1
Twins	6	42.9
Total	14	100,0

Session measurements	Frequency	Percent
8	1	7,1
10	1	7,1
13	1	7,1
14	11	78,7
Total	14	100,0

Birth week	Frequency	Percent
<28	3	21,4
28-31+6	7	50,0
32-33+6	4	28,6
Total	14	100,0

Appendix II: Table 2. Presentation of all the data gathered for MS (n =14), MTS (n=13) and control group (n =14) during the study (95% confidence interval). The table includes the results of the mixed factorial analysis of variance and one-way analysis of variance with Tukey's post-hoc tests, as well as comparisons of the descriptive data of the study groups.

	Mixed-design ANOVA		
Heart rate	Within-time: F (1, 39) = 522.52, p < 0.001, η^2= 0.931 Between-groups: F (2, 39) = 59.47, p < 0.001, η^2= 0.753 Time x Group: F (2, 39) = 313.01, p < 0.001, η^2= 0.941		
Within group	**MS**	**MTS**	**CG**
(*Mean ± Std. Dev, p*)	163.65 ± 3.36 **vs** 146.92 ± 2.8, $p < 0.001$	156. 56 ± 3.42 **vs** 145.15 ± 3.18, $p < 0.001$	160. 68 ± 2.74 **vs** 164.7 ± 3.13, $p < 0.001$
Before the sessions	**MS vs. MTS**	**MS vs. CG**	**MTS vs. CG**
(*Mean ± Std. Deviation, p*)	163.65 ± 3.36 **vs** 156. 56 ± 3.42, $p < 0.001$	163.65 ± 3.36 **vs** 160. 68 ± 2.74, $p = 0.055$	156. 56 ± 3.42 **vs** 160. 68 ± 2.74, $p = 0.005$
After the sessions	**MS vs. MTS**	**MS vs. CG**	**MTS vs. CG**
(*Mean ± Std. Deviation, p*)	146.92 ± 2.8 **vs** 145.15 ± 3.18, $p = 0.39$	146.92 ± 2.8 **vs** 164.7 ± 3.13, $p < 0.001$	145.15 ± 3.18 **vs** 164.7 ± 3.13, $p < 0.001$
Group by time comparisons (*p*)	**MS vs. MTS** $p = 0.001$	**MS vs. CG** $p < 0.001$	**MTS vs. CG** $p < 0.001$
Blood oxygen saturation	Within-time: F (1, 39) = 20.49, p < 0.001, η^2= 0.344 Between-groups: F (2, 39) = 39.97, p < 0.001, η^2= 0.672 Time x Group: F (2, 39) = 28.4, p < 0.001, η^2= 0.593		
Within group	**MS**	**MTS**	**CG**
(*Mean ± Std. Dev, p*)	97.73 ± 0.9 **vs** 98.53 ± 0.48, $p < 0.001$	97.29 ± 0.8 **vs** 98.22 ± 0.72, $p < 0.001$	95.99 ± 0.86 **vs** 95.46 ± 1.04, $p = 0.001$
Before the sessions	**MS vs. MTS**	**MS vs. CG**	**MTS vs. CG**
(*Mean ± Std. Deviation, p*)	97.73 ± 0.9 **vs** 97.29 ± 0.8, $p = 0.55$	97.73 ± 0.9 **vs** 95.99 ± 0.86, $p < 0.001$	97.29 ± 0.8 **vs** 95.99 ± 0.86, $p = 0.001$
After the sessions	**MS vs. MTS**	**MS vs. CG**	**MTS vs. CG**
(*Mean ± Std. Deviation, p*)	98.53 ± 0.48 **vs** 98.22 ± 0.72, $p = 0.91$	98.53 ± 0.48 **vs** 95.46 ± 1.04, $p < 0.001$	98.22 ± 0.72 **vs** 95.46 ± 1.04, $p < 0.001$
Group by time comparisons (*p*)	**MS vs. MTS** $p = 0.41$	**MS vs. CG** $p < 0.001$	**MTS vs. CG** $p < 0.001$
Weight	Within-time: F (1, 38) = 447.6, p < 0.001, η^2= 0.922 Between-groups: F (2, 38) = 2.33, p = 0.11, η^2= 0.109 Time x Group: F (2, 38) = 0.165, p = 0.85, η^2= 0.009		
Within group	**MS**	**MTS**	**CG**
(*Mean ± Std. Dev, p*)	2482.86 ± 875.21 **vs** 2885 ± 871.04, $p < 0.001$	1970.7 ± 471.1 **vs** 2380.7 ± 444.2, $p < 0.001$	2010.51 ± 810.8 **vs** 2355.8 ± 889.8, $p < 0.001$
Before the study	**MS vs. MTS**	**MS vs. CG**	**MTS vs. CG**
(*Mean ± Std. Deviation, p*)	2482.86 ± 875.21 **vs** 1970.7 ± 471.08, $p = 0.23$	2482.86 ± 875.1 **vs** 2010.5 ± 810.8, $p = 0.3$	1970.71 ± 471.1 **vs** 2010.5 ± 810.8, $p = 0.98$
After the study	**MS vs. MTS**	**MS vs. CG**	**MTS vs. CG**
(*Mean ± Std. Deviation, p*)	2885 ± 871.04 **vs** 2380.71 ± 444.24, $p = 0.27$	2885 ± 871.04 **vs** 2355.8 ± 889.84, $p = 0.22$	2380.71 ± 444.24 **vs** 2355.8 ± 889.84, $p = 0.99$

Group by time comparisons (*p*)	**MS vs. MTS** $p = 0.19$	**MS vs. CG** $p = 0.19$	**MTS vs. CG** $p = 0.99$
Height	Within-time: $F(1, 38) = 67.43, p < 0.001, \eta^2 = 0.64$ Between-groups: $F(2, 38) = 1.4, p = 0.26, \eta^2 = 0.069$ Time x Group: $F(2, 38) = 1.99, p = 0.15, \eta^2 = 0.095$		
Within group (*Mean ± Std. Dev, p*)	**MS** 45.04 ± 4.2 **vs** 47.04 ± 4.19, $p < 0.001$	**MTS** 43.04 ± 2.88 **vs** 45.05 ± 3.27, $p < 0.001$	**CG** 48.91 ± 14.16 **vs** 49.84 ± 13.66, $p = 0.014$
Before the study (*Mean ± Std. Deviation, p*)	**MS vs. MTS** 45.04 ± 4.2 **vs** 43.04 ± 2.88, $p = 0.98$	**MS vs. CG** 45.04 ± 4.2 **vs** 48.91 ± 14.16, $p = 0.73$	**MTS vs. CG** 43.04 ± 2.88 **vs** 48.91 ± 14.16, $p = 0.24$
After the study (*Mean ± Std. Deviation, p*)	**MS vs. MTS** 47.04 ± 4.19 **vs** 45.05 ± 3.27, $p = 0.99$	**MS vs. CG** 47.04 ± 4.19 **vs** 49.84 ± 13.66, $p = 0.98$	**MTS vs. CG** 45.05 ± 3.27 **vs** 49.84 ± 13.66, $p = 0.43$
Group by time comparisons (*p*)	**MS vs. MTS** $p = 0.81$	**MS vs. CG** $p = 0.56$	**MTS vs. CG** $p = 0.24$
Head circumference	Within-time: $F(1, 38) = 212.73, p < 0.001, \eta^2 = 0.848$ Between-groups: $F(2, 38) = 2.45, p = 0.1, \eta^2 = 0.114$ Time x Group: $F(2, 38) = 0.35, p = 0.71, \eta^2 = 0.018$		
Within group (*Mean ± Std. Dev, p*)	**MS** 32.57 ± 3.15 **vs** 34.19 ± 2.59, $p < 0.001$	**MTS** 30.81 ± 2.26 **vs** 32.54 ± 2.29, $p < 0.001$	**CG** 35.44 ± 17.86 **vs** 37.08 ± 17.13, $p < 0.001$
Before the study (*Mean ± Std. Deviation, p*)	**MS vs. MTS** 32.57 ± 3.15 **vs** 30.81 ± 2.26, $p = 0.98$	**MS vs. CG** 32.57 ± 3.15 **vs** 35.44 ± 17.86, $p = 0.99$	**MTS vs. CG** 30.81 ± 2.26 **vs** 35.44 ± 17.86, $p = 0.76$
After the study (*Mean ± Std. Deviation, p*)	**MS vs. MTS** 34.19 ± 2.59 **vs** 32.54 ± 2.29, $p = 0.99$	**MS vs. CG** 34.19 ± 2.59 **vs** 37.08 ± 17.13, $p = 0.99$	**MTS vs. CG** 32.54 ± 2.29 **vs** 37.08 ± 17.13, $p = 0.72$
Group by time comparisons (*p*)	**MS vs. MTS** $p = 0.9$	**MS vs. CG** $p = 0.74$	**MTS vs. CG** $p = 0.47$
Nutrition intake* **(Kcal/Kg per day)**	Within-time: $F(1, 30) = 7.9, p = 0.009, \eta2 = 0.208$ Between-groups: $F(2, 30) = 0.6, p = 0.56, \eta2 = 0.038$ Time x Group: $F(2, 30) = 0.484, p = 0.62, \eta2 = 0.031$		
Within group (*Mean ± Std. Dev, p*)	**MS** 119.1 ± 22.87 **vs** 136.28 ± 14.15, $p = 0.057$	**MTS** 116.58 ± 21.67 **vs** 138.05 ± 33.71, $p = 0.061$	**CG** 128.55 ± 18.13 **vs** 136.8 ± 10.77, $p = 0.368$
Before the study (*Mean ± Std. Deviation, p*)	**MS vs. MTS** 119.1 ± 22.87 **vs** 116.58 ± 21.67, $p = 0.998$	**MS vs. CG** 119.1 ± 22.87 **vs** 128.55 ± 18.13, $p = 0.805$	**MTS vs. CG** 116.58 ± 21.67 **vs** 128.55 ± 18.13, $p = 0.661$

After the study	**MS vs. MTS**	**MS vs. CG**	**MTS vs. CG**
(*Mean ± Std. Deviation, p)*	136.28 ± 14.15 **vs** 138.05 ± 33.71, *p* = .	136.28 ± 14.15 **vs** 136.8 ± 10.77, *p* = .	138.05 ± 33.71 **vs** 136.8 ± 10.77, *p* = .
Group by time comparisons (*p*)	**MS vs. MTS** *p* = 0.999	**MS vs. CG** *p* = 0.999	**MTS vs. CG** *p* = 0.999

Fluid intake* (ml/Kg per day)	Within-time: F (1, 30) = 6.75, p = 0.014, η2= 0.184 Between-groups: F (2, 30) = 0.295, p = 0.75, η2= 0.019 Time x Group: F (2, 30) = 1.205, p = 0.31, η2= 0.074		
Within group	**MS**	**MTS**	**CG**
(*Mean ± Std. Dev, p)*	157.57 ± 18.58 **vs** 170.01 ± 16.92, *p* = 0.086	158.56 ± 19.76 **vs** 178.66 ± 31.09, *p* = 0.032	164.89 ± 16.14 **vs** 167.44 ± 11.98, *p* = 0.729
Before the study	**MS vs. MTS**	**MS vs. CG**	**MTS vs. CG**
(*Mean ± Std. Deviation, p)*	157.57 ± 18.58 **vs** 158.56 ± 19.76, *p* = .0.999	157.57 ± 18.58 **vs** 164.89 ± 16.14, *p* = 0.956	158.56 ± 19.76 **vs** 164.89 ± 16.14, *p* = 0.998
After the study	**MS vs. MTS**	**MS vs. CG**	**MTS vs. CG**
(*Mean ± Std. Deviation, p)*	170.01 ± 16.92 **vs** 178.66 ± 31.09, *p* = 0.999	170.01 ± 16.92 **vs** 167.44 ± 11.98, *p* = 0.999	178.66 ± 31.09 **vs** 167.44 ± 11.98, *p* = 0.674
Group by time comparisons (*p*)	**MS vs. MTS** *p* = 0.73	**MS vs. CG** *p* = 0.907	**MTS vs. CG** *p* = 0.924

	One-Way ANOVA		
Duration of hospital stay	**MS vs. MTS**	**MS vs. CG**	**MTS vs. CG**
F (2, 38) = 0.62, p = 0.54	66.93 ± 48.25 **vs** 52.38 ± 29.6, *p* = 0.71	66.93 ± 48.25 **vs** 72.07 ± 58, *p* = 0.95	52.38 ± 29.6 **vs** 72.07 ± 58, *p* = 0.53

**Nutrition and fluid intake analysis corresponds to comparisons between session 1 and session 13 as it was not possible to acquire the respective data for more than half of the MTS infants in session 14.*

Appendix III: Songs selected and sung by the Music Therapists

1. Lullaby "Kimisou Angeloudi Mou" - 1962, Music: Theodorakis M. Lyrics: Virvos K.
2. Lullaby "Tha kentiso" - 1994, Lyrics: Lefteris Papadopoulos, Music: Manos Loizos
3. Lullaby "Asimia Koudounia"- 1974, Lyrics: Lefteris Papadopoulos, Music: Christos Leontis
4. To Minore tis Augis" - 1936, Lyrics: Minos Machas, Music: Spyros Peristeris
5. "Nani to Moro Mou" Lyrics: D. Savvopoulos, Beatrice Kantzola-Sabatakou, Music: D. Savvopoulos
6. Asteri Mou Fengari Mou / Fedra" - 1962, Lyrics: Giannis Theodorakis, Music: Mikis Theodorakis
7. Omorfi poli/ tha ginis dikia mou" - 1966, Lyrics: Giannis Theodorakis, Music: Mikis Theodorakis
8. "Chartino to Fengaraki" - 1958, Lyrics: Nikos Gatsos, Music: Manos Hadjidakis
9. Lullaby "Nani to pedi mou nani" - 1965, Lyrics: Nikos Gatsos, Music: Manos Hadjidakis

Songs selected and sung by Mothers

1. Lullaby "Twinkle Twinkle Little Star" (Fengaraki you labro) Traditional folk Melody

2 Lullaby "Tha Kentiso" - 1994, Lyrics: Lefteris Papadopoulos, Music: Manos Loizos

3. "Chartino to fengaraki" - 1958, Lyrics: Nikos Gatsos, Music: Manos Hadjidakis

4. "Apopse" – 1998, Music/Lyrics: Papakonstantinou Thanasis

5. Lullaby "Hypne Pou Pernis ta Pedia", Lyrics: Unknown, Music: Kipourgos Nikos.

6. Lullaby "Cla Hypne Ke Pare To", Lyrics & Music: unknown

7. "To Minore tis Augis" - 1936, Lyrics: Minos Machas, Music: Spyros Peristeris

8. "Chartino to fengaraki" - 1958, Lyrics: Nikos Gatsos, Music: Manos Hadijdakis

9. "Lullaby", Lyrics/music: Selitsaniotis M. Sideri Is.