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ORIGINAL ARTICLE

Preterm infants with severe brain injury demonstrate unstable physiological responses during maternal singing with music therapy: a randomized controlled study

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Abstract

Preterm infants with severe brain injury are at high risk for poor outcomes and, therefore, may benefit from developmental care modalities such as music therapy (MT). In this prospective, randomized intervention, preterm infants with severe brain injury (grade 3 or 4 intraventricular hemorrhage or periventricular leukomalacia) who underwent skin-to-skin contact (SSC) with or without maternal singing during MT were evaluated for physiological responses, including autonomic nervous system stability (low frequency (LF)/high frequency (HF) power), heart rate, respiratory rate, oxygen saturation, and behavioral state. Maternal anxiety state and physiological data were also evaluated. A total of 35 preterm infants with severe brain injuries were included in the study analysis. Higher mean ± standard deviation (SD) LF/HF ratio (1.8 ± 0.7 vs. 1.1 ± 0.25, $p = 0.01$), higher mean ± SD heart rate (145 ± 15 vs. 132 ± 12 beats per minute, $p = 0.04$), higher median (interquartile range) infant behavioral state (NIDCAP manual for naturalistic observation and the Brazelton Neonatal Behavioral Assessment) score (3 (2–5) vs. 1 (1–3), $p = 0.03$), and higher mean ± SD maternal anxiety (state-trait anxiety inventory) score (39.1 ± 10.4 vs. 31.5 ± 7.3, $p = 0.04$) were documented in SSC combined with maternal singing during MT, as compared to SSC alone.

Conclusion: Maternal singing during MT for preterm infants with severe brain injury induces physiological and behavioral instability and increases maternal anxiety during NICU hospitalization. A unique MT intervention should be designed for preterm infants with severe brain injury and their mothers.

What is Known:
- *Preterm infants with severe brain injury are at high risk for poor outcomes.*
- *Music therapy benefits brain development of preterm infants without severe brain injury, however it is unknown whether maternal singing during music therapy for preterm infants with severe brain injury is beneficial.*

What is New:
- *Maternal singing during music therapy for preterm infants with severe brain injury induces physiological and behavioral instability and increases maternal anxiety during NICU hospitalization.*
- *A unique music therapy intervention should be designed for preterm infants with severe brain injury and their mothers.*

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Keywords Intraventricular hemorrhage · Music therapy · Maternal singing · Periventricular leukomalacia · Preterm infants · Skin-to-skin contact

Abbreviations

HF	High frequency
HRV	Heart rate variability
IVH	Intraventricular hemorrhage
LF	Low frequency
MT	Music therapy
NICU	Neonatal intensive care unit
NIDCAP	Neonatal Individualized Developmental Care and Assessment Program
PVL	Periventricular leukomalacia
SD	Standard deviation
SSC	Skin-to-Skin contact
STAI	State-trait anxiety inventory

Introduction

Preterm infants, born before 32 weeks of gestation, are at increased risk of brain injury and may have reduced white- and grey-matter volumes [1]. Severe brain injuries such as intraventricular hemorrhage (IVH, grades 3 or 4) and periventricular leukomalacia (PVL) are associated with significant morbidity and long-term neurodevelopmental impairments, including motor dysfunction, cerebral palsy, cognitive, and behavioral problems. Therapeutic options are limited, especially during the post-natal period [2]. Furthermore, preterm birth increases parental stress and causes symptoms consistent with post-traumatic stress, anxiety, and depression, with short- and long-term consequences [3–6]. All of these may affect the parent-infant bonding process [7], which further affects neurodevelopmental outcomes. Preterm infants with brain injury are frequently exposed to noxious stimuli in the NICU that disrupt and shorten their sleep periods. Sleep disruption may have a negative effect on clinical outcomes, growth, and development and may also delay hospital discharge. Increasing evidence suggests that sleep quality is critical for brain development and synaptic plasticity and is associated with long-term neurodevelopmental outcomes [8].

Therefore, studies have evaluated developmental care modalities that may attenuate the severity of neurodevelopmental outcomes and improve sleep quality and bonding [8, 9]. Two common modalities in the neonatal intensive care unit (NICU) are skin-to-skin contact (SSC) and music therapy (MT). SSC was found to facilitate physiological stability, increase immunity, and improve breastfeeding and parent-infant bonding, hence decreasing mortality and morbidity [10]. MT in general is the "professional use of music and its elements as an intervention in medical, educational, and everyday environments with individuals, groups, families, or communities who seek to optimize their quality of life and improve their physical, social, communicative, emotional, intellectual, and spiritual health and wellbeing" [11]. Guidelines for music-based interventions were published to ensure transparent reporting of interventions while considering the uniqueness and varsity of these interventions [12]. MT and music stimulation have been shown to be beneficial for preterm infants and their families [13–19]. MT used with preterm infants is based on the intrauterine acoustic environment, such as uterine sounds, maternal voice, and breathing sounds [17]. However, a distinction should be made between music therapy performed by a music therapist with specific training involving a therapeutic relationship with the mother- and father-infant dyad or triad and music stimulation techniques. In MT, using an individual approach adapted to the demands and characteristics of each dyad, live music is adjusted to the infant's behavioral and physiological signs. A trained NICU music therapist ensures that the music implementation is at a safe decibel level and appropriately monitors the infant for signs of overstimulation [15]. In contrast, music stimulation performed by other health professionals usually employs recorded songs in a non-individualized approach, lacks an interaction between a trained music therapist and the patient, and is conducted in the absence of a treatment plan with nonmusical, therapeutic goals [16]. MT supports parent and infant bonding and stabilizes the physiological and emotional consequences of stress triggers in the NICU [17–19]. MT with or without SSC benefits brain development among preterm infants without severe brain injury [14]. The rationale for using maternal singing during music therapy in this study was based on our experience with this type of MT intervention, which empowers the mother as the central caregiver who delivers the intervention to the infant with full support from a therapist who is sensitive to her feelings and desires. This intervention, fully attuned to the mother's needs and NICU regulations, was adjusted to the surroundings and was well-known to our music therapist, who gained experience from our previous study [18]. Evidence suggests that maternal singing that uses lullabies, song of kin, or improvised vocalizations; favors development of self-regulation; prolongs active sleep and overall development; and promotes the well-being of mothers and the quality of mother-infant interactions [15, 19].

Our hypothesis was that the combined effect of maternal singing with MT delivered by a trained music therapist, during SSC, as compared to SSC alone on stable, preterm (< 32 weeks' gestation) infants who had severe brain injury (IVH grades 3 or 4, or PVL), would have beneficial effects on achieving a steady physiological state and improve sleep quality with fewer signs of discomfort and decrease maternal anxiety. Our primary outcome was autonomic nervous system stability, measured by low frequency/high frequency (LF/HF) power during the interventions. Secondary outcomes were physiological parameters including infants' heart rate, respiratory rate, oxygen saturation, maternal heart rate and oxygen saturation, and neonatal behavioral score, as well as self-reported maternal anxiety levels.

Methods

Design

This single-center, prospective, randomized intervention used a within-subject, crossover, repeated measures design with participants acting as their own controls. The study was conducted at Meir Medical Center in Kfar Saba, Israel, in a tertiary level NICU with Neonatal Individualized Developmental Care and Assessment Program (NIDCAP) certification.

Participants

All preterm infants born before 32 weeks' gestation, admitted to the NICU during 2014–2018 who developed IVH grades 3 or 4 and/or PVL diagnosed by brain ultrasound, were eligible for the study and were recruited consecutively after their clinical state stabilized, regardless of their corrected age at study entry. A clinically stable infant at the time of enrollment was defined as one without supplemental oxygen demands during enrollment, no significant apnea requiring stimulation during the 3 days before enrollment, with or without methylxanthines, and no documented seizure episode or anticonvulsive treatment a week before study enrollment. All infants had their hearing confirmed by distortion product otoacoustic emissions. Due to the national parental rights of birth and preterm birth in Israel, where fathers are entitled to a very short paternity leave, only mothers who were able to attend all the sessions participated in the study.

Baseline data were recorded from electronic medical records and included ethnic origin, maternal age, and educational level, as well as infant's sex, post-menstrual age, weight at birth and at study entry, grades of IVH and PVL, and other comorbidities such as bronchopulmonary dysplasia, necrotizing enterocolitis, retinopathy of prematurity, and sepsis. If it became necessary for medical reasons to discontinue an infant's participation in the study for a period shorter than 2 weeks, participation recommenced when the criteria for clinical stability were again met.

Interventions and procedures

During hospitalization, each mother-infant dyad participated in three sessions of maternal singing during MT combined with SSC, which were compared to three sessions of SSC without MT. The allocation for the first session (either SSC or SSC with maternal singing during MT) was decided using a random number table and presented to the researcher in a sealed, opaque envelope. The two types of treatment were administered in alternating order during the remaining five sessions. Each therapy session began 30 min after completion of feeding, two to three times per week. All SSC and/or maternal singing during MT sessions were offered about 3–5 days after the previous session, according to mothers' ability and presence in the NICU and the infants' length of hospitalization. Only infants who completed all sessions were included in the analysis. Participants could not be blinded due to the nature of the intervention. The primary outcome was based on objective measurements and was unlikely to be affected by subjective bias. The outcome assessor (IL) was blinded to treatment allocation and arranged physiological data of infants and mothers, STAI score, and behavioral scale according to the sequence of the intervention, without knowing whether the intervention was SSC or maternal singing with MT.

During all sessions, the mother-infant dyad was in SSC position, as described previously [18]. Before each session, surrounding sound volumes were tested with a sound analyzer, and those higher than the recommended 50 dB(A) [20] were avoided. All study sessions took place in a quiet environment, in a closed room, with lights dimmed, in the NICU. Sessions began with 10 min of SSC alone (baseline phase), followed by either SSC for 20 min alone or the intervention of combined SSC and maternal singing during MT (intervention phase). The sessions ended with another 10 min of SSC alone (recovery phase).

The combined SSC and maternal singing during MT intervention was part of the protocol and designed according to our previous study [18]. It fulfilled the guidelines for reporting studies involving music-based interventions [12]. A certified music therapist delivered the intervention. During the intervention, the mother was guided by the therapist to sing her chosen songs/lullabies using a repetitive, soothing tone, softly, simply, and with a slow tempo. According to the mother's choice, the music therapist provided vocal or instrumental support for the mother using a nylon string guitar. As both the mother and music therapist were women, the vocal range during the intervention was the average female vocal range (G3 to C5). The music therapist helped the mother attune the live music to the infant's breathing patterns, movements, vocals, or other viewed reactions. According to the infant's reactions, the mother and music therapist decided whether to continue with the same rhythm, to change the tempo and vocals, or pause. After the singing, the session ended with a few moments of silence and reorganization of breathing. Then, the mother was encouraged to reflect on her experience.

A second NIDCAP-certified research assistant, familiar with study protocol, sat near the mother and recorded the infant's physiological and behavioral data and maternal heart rate and oxygen saturation every 5 min, during baseline, intervention, and recovery phases, using a wall-mounted clock. If the infant exhibited crying, signs of hunger, or persistent signs of stress, the interventions were paused until baseline behavioral state was achieved, but study parameters were still recorded. Standard SSC as described is considered "treatment as usual" and is recommended to all families admitted to the NICU. During SSC, vital signs are recorded continuously as per department policy, but maternal vital signs were recorded only for study purposes. No therapeutic conversation, music playing, or singing occurred during the SSC only sessions. During SSC, a research assistant sat near the mother without interfering with the intimacy between the dyads. The approved IRB protocol stated that the intervention would be discontinued should the infant experience bradycardic or apneic events during the intervention that require medical intervention and that these occurrences would be reported as adverse events.

Study parameters

The primary outcome was autonomic nervous system stability, as measured by the mean heart rate variability (HRV) sympathetic and parasympathetic response during all sessions, namely, low frequency/high frequency (LF/HF) power during interventions. The assumption underlying the LF/HF ratio is that LF power may be generated by the sympathetic nervous system, while HF power is produced by the parasympathetic nervous system. In this model, a low LF/HF ratio reflects parasympathetic dominance. This is seen when we conserve energy and engage in tend-and-befriend behaviors. In contrast, a high LF/HF ratio indicates sympathetic dominance, which occurs when we engage in fight-or-flight behaviors or parasympathetic withdrawal [18]. The analogue electrocardiogram signal from a cardiorespiratory monitor was fed into a computer containing the HRV software (ANSR1000 System, Ansar, Inc., Philadelphia, PA, USA). The analogue electrocardiogram signal was converted to digital values reflecting cyclic changes in the RR interval of the electrocardiography. The data were transformed into a waveform across a spectrum of various frequencies. Frequencies were measured in Hertz (1 Hz = 1 cycle/s). Applied to HRV, sympathetic influences appear at frequencies < 0.15 Hz, representing the LF power spectrum. The HF power spectrum is in the range of > 0.15–1.80 Hz, which is predominately influenced by parasympathetic inputs. The HRV indices were summarized for SSC with and without MT with maternal singing. Given that the HRV indices represent changes in heart rate over time, the geometric mean was used for these data. Heart rate and respiratory activity graphs display the 128-s (512 points) segment required to produce the heart rate and respiratory activity spectra. Movement and artifacts were eliminated by comparing the amplitude (height) of the R wave to be included with the amplitude for the last acceptable R wave.

Additional secondary outcomes included the mothers' anxiety levels and physiological vital signs and the infants' physiological vital signs and behavioral states during all sessions. Maternal state anxiety levels were measured using the state-trait anxiety inventory (STAI). This is a validated, self-report questionnaire with 20 statements describing present anxiety symptoms. Each item is scored on a scale from 1 (not at all) to 4 (very much), leading to total scores ranging from 20 to 80, with higher scores indicating more anxiety. A cut-off point of 39–40 has been suggested to detect clinically significant symptoms of anxiety [21]. A reliable, validated Hebrew version was used [22]. After each session, mothers were asked to complete the STAI questionnaire and return it in a sealed envelope. Additional physiological measures of the preterm infants included heart rate, oxygen saturation, and respiratory rate. For mothers, heart rate and oxygen saturation were measured with a Masimo monitor (Masimo Corp., Irvine, CA). Lower heart and respiratory rates and higher oxygen saturation were considered indicators of better physiologic stability.

The behavioral state of the preterm infants during intervention sessions was evaluated using the criteria of Als et al. [23], based on the NIDCAP manual for naturalistic observation and the Brazelton Neonatal Behavioral Assessment Scale [24]. It is used to assess and differentiate between 7 behavioral states: (1) deep sleep, with obligatory regular breathing, relaxed facial expression; (2) diffuse light sleep with eyes closed, low activity level, respirations are regular, mild sucking; (3) diffusely drowsy, fussy, and/or little discharge of vocalization, whimpers, facial grimacing; (4) hyper-alertness, eyes wide open, giving the impression of panic, fear or being overwhelmed; (5) actively awake and aroused, may also be clearly fussy without crying robustly; (6) highly aroused, agitated, upset and/or crying; and (7) removal from the state continuum, a prolonged respiratory pause beyond 8 s. Lower behavioral states indicate higher sleep quality with fewer signs of discomfort and, hence, greater stability. The music therapy was provided by a trained, NIDCAP-certified therapist. The research assistant was familiar with physiological parameter recordings and the Als Behavioral Scale and recorded this information every 5 min during each session.

Power calculation and sample size justification

The minimal clinically significant difference for LF/HF power in infants is unknown. The power calculation for the primary outcome was informed by a previous study [18] that found mean LF/HF power values of 1 (SD = 0.8) during MT combined with SSC, compared to 3.4 (SD = 1.6) during SSC alone. Therefore, the study was powered to detect a substantial effect on LF/HF (d = 0.80). In an individually randomized trial, with a 2-sided 5% significance level and 80% power, a sample size of 35 infants was required.

Data analysis plan

Continuous data are presented as mean (SD) or median (interquartile range, IQR) as appropriate, with numbers and percentages for nominal variables. The normality of continuous data was assessed with the Kolmogorov-Smirnov test. Comparisons between the two interventions (SSC alone or SSC and maternal singing during MT), for the LF/HF ratio, our primary outcome, were analyzed using general linear model with analysis of covariance (ANCOVA). The covariates entered into the multivariate analysis were sex, birth weight, post-menstrual age at birth, post-menstrual age at study entry, and IVH or PVL. The main effect was the type of intervention (SSC or maternal singing during MT). The secondary outcomes, infant's heart rate, respiratory rate, oxygen saturation and maternal heart rate, oxygen saturation, and STAI scores from all sessions were compared using paired *t*-test for normally distributed variables and the Wilcoxon signed-rank test for non-normally distributed paired data (behavioral score). $P < 0.05$ was considered significant. Analyses were conducted using SPSS for Windows version 14 (SPSS, Inc., Chicago. IL, USA).

Results

Forty preterm infants with severe brain injury (IVH grades 3 or 4, or PVL) were enrolled in the study group. Three did not complete all sessions, and two were excluded due to prolonged illnesses during their hospitalizations that interrupted the timing between study sessions for more than 2 weeks. Therefore, 35 preterm infants were included in the final analysis (Fig. 1). The characteristics of the study population are depicted in Table 1. The intervention groups were similar in post-menstrual age at study entry and in duration of study participation from the first intervention to the last intervention. Most infants were AGA (75%), of Jewish origin (54%), and the mothers had an average of 12 years of education. Using paired *t*-test comparing physiological data of infant and mother dyads from the two interventions showed higher infant heart rates and respiratory rates, lower oxygen saturation, higher behavioral state scores, and higher maternal anxiety (STAI) scores during SSC combined with maternal singing during MT, as compared to SSC alone (Table 2). For our primary outcome, a significantly higher mean LF/HF was found for SSC combined with maternal singing during MT compared to SSC alone ($F(1,30) = 8.47$, $p = 0.01$, partial $\eta^2 = 0.27$). This difference was significant after controlling for covariates such as sex, birth weight, post-menstrual ages at birth and at study entry, IVH, or PVL. Notably, the most obvious LF/HF difference was during intervention (2.8 ± 1.1 vs. 1.0 ± 0.5) (Table 3). Unintended effects were documented in two infants in the SSC group: oxygen saturation < 80%, which lasted for a few seconds, and an episode of food reflux that also lasted for a few seconds. In both cases, the sessions continued as planned. No adverse events as defined by the IRB protocol occurred.

Fig. 1. Trial Profile

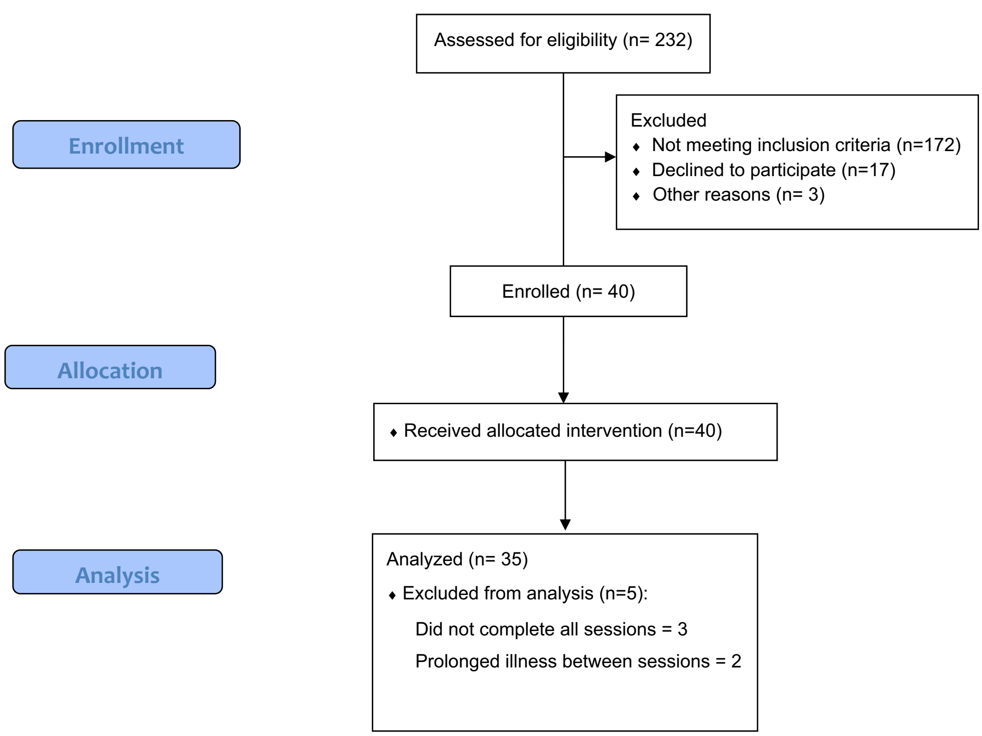

Table 1 Characteristics of the study population ($n = 35$)

Characteristic	Number ($n = 35$)
Infants	
Male#	21 (60%)
Post-menstrual age at birth (weeks)*	27 ± 2.5
Post-menstrual age at study entry (weeks)*$ (SSC group)	31 ± 2.7
Post-menstrual age at study entry (weeks)*$ (SSC + MT group)	32 ± 3.5
Duration of study time from the first session (randomly allocated) to the final session (days) (SSC group)*&	18 ± 3.2
Duration of study time from the first session (randomly allocated) to the final session (days) (SSC + MT group)*&	20 ± 2.1
Birth weight (grams)*	823 ± 213
Weight at study entry (grams)*	1180 ± 357
AGA/SGA#	27 (77)
Intraventricular hemorrhage grade 3#	16 (45)
Intraventricular hemorrhage grade 4#	10 (29)
Periventricular leukomalacia#	9 (26)
Bronchopulmonary dysplasia#	18 (51)
Necrotizing enterocolitis#	1 (3)
Retinopathy of prematurity#	
Stages 1–2	3 (8)
Stages 3–4	1(3)
Proven sepsis episodes#	3 (8)
Mothers	
Jewish ethnic origin#	19 (54)
Age, years (range)**	32 (24–43)
Education (years)**	12 (10–20)

AGA appropriate for gestational age, *SGA* small for gestational age, *SSC* skin-to-skin contact, *MT* music therapy. Data are presented as * mean ± SD, ** median (interquartile range), # count (percentage), $ $p = 0.25$, & $p = 0.31$ compared to SSC alone, based on paired *t*-test

Table 2 The effect of the interventions on mother-infant dyads#

Variable	SSC alone	SSC + MT	*p* Value
Heart rate (BPM)#			
Infants	132 ± 12	145 ± 15*	0.04
Mothers	65 ± 9	79 ± 15	0.07
O_2 saturation#			
Infants	95 ± 2	91 ± 6*	0.04
Mothers	98 ± 1	96 ± 2	0.15
Infants			
Respiratory rate (number per minute)*	51 ± 7	63 ± 9*	0.04
Behavioral state$	1 (1–3)	3 (2–5)*	0.04
Mothers			
STAI score#	31.5 ± 7.3	39.1 ± 10.4*	0.04

BPM beats per minute, *SSC* skin-to-skin contact, *STAI* state-trait anxiety inventory

Mean ± SD

*$p < 0.05$ compared to SSC alone. Compared with paired *t*-test for all variables except behavioral score, where Wilcoxon signed-rank test was used

$ Behavioral state on a 7-point score adopted from ALS criteria [23] in both modes of therapy, median (IQR)

Table 3 Heart rate variability spectral power in skin-to-skin contact with and without maternal singing and music therapy

Interval	Skin-to-skin contact (SSC)			SSC and MT		
	LF power (ms^2/Hz)	HF power (ms^2/Hz)	LF/HF ratio	LF power (ms^2/Hz)	HF power (ms^2/Hz)	LF/HF ratio
Baseline	26.8 ± 6.2	26.7 ± 7.1	1.0 ± 0.5	32.8 ± 11.1	25.3 ± 16.8	1.3 ± 0.7
Intervention	22.7 ± 8.5	21.1 ± 15.8	1.0 ± 0.5	49.3 ± 14.2	17.9 ± 10.6	2.8 ± 1.1
Recovery	30.1 ± 7.9	22.8 ± 11.4	1.3 ± 0.7	28.8 ± 6.3	23.7 ± 13.3	1.2 ± 0.5
Overall			1.1 ± 0.25			1.8 ± 0.7*

HF high frequency, *LF* low frequency, *SSC* skin-to-skin contact, *MT* music therapy; * $p = 0.01$ compared to SSC alone, based on ANCOVA, after controlling for the covariates sex, birth weight, post-menstrual age at birth, post-menstrual age at study entry, and IVH or PVL. The statistical analysis did not include the "interval" variable, and the means are presented here for reference only

Discussion

Numerous studies have demonstrated the positive effects of developmental interventions in the NICU on brain structure, motor organization, and development, as well as longer-term cognitive and behavior outcomes [10, 25]. We hypothesized that because interventions within the framework of MT are beneficial for preterm infants [17, 19, 26], they would be even more useful for preterm infants with severe brain injury. Our study results indicated that SSC combined with maternal singing during MT for preterm infants with severe brain injury led to physiological and behavioral instability for both the preterm infants and their mothers, as documented by higher mean LF/HF ratio, heart rate, respiratory rate, infant behavioral state scores, and higher maternal anxiety score. When the same infants experienced SSC alone (control group), physiological and behavioral stability were maintained among the infants and their mothers, during all sessions. No adverse events requiring medical intervention occurred during maternal singing during MT combined with SSC.

Interventions that involve sensory stimuli for preterm infants are designed to facilitate neurobehavioral development and improve behavioral state organization and feeding progression, as well as decrease length of hospitalization [27]. SSC and interventions within MT support sensory regulation and stress reduction. They also include parents in their infant's care, support parents' emotional state, and enhance the parent-infant attachment process [10, 17, 19, 28–31]. However, the neuroanatomical and physiological backgrounds regarding the beneficial effects of MT remain poorly understood. Recent MRI and fMRI studies on the effects of MT on preterm infants have shown improvement in brain connectivity and functional activity. MT also improved thalamocortical processing, prefrontal, supplementary motor, and temporal brain regions and was associated with higher-order cognitive, socio-emotional, and motor functions [13, 14]. Infants with severe brain injuries were excluded from these studies. Other studies evaluating the effect of MT on short-term physiological and behavioral parameters and long-term neurodevelopmental outcomes also excluded infants with severe brain injury from the study populations [18, 32, 33]. Infants with severe brain injury might have been excluded because it was not clear how they would process music stimulation. Sensory processing disorder is common among infants born before 30 weeks' gestation [34, 35]. Preterm infants with brain injury suffer from impaired brain connectivity in regions associated with sensory information processing [36] and present with sensation avoiding patterns and heightened responses to low levels of stimulation [37]. Specifically, moderate-to-severe white matter abnormalities such as PVL were shown to be associated with delayed maturation of the autonomic nervous system and increased risk of decelerative HR changes and associated clinical compromise with various stimuli [38]. Recommendations within research are that infants diagnosed with PVL and IVH should be closely monitored during procedures or interventions that may be stressful, such as auditory stimuli or the combination of a few developmental care modalities [39–41]. This information may support our findings that the auditory stimulation component of maternal singing during MT resulted in instability of the autonomic nervous system and physiological variables, with the infants' unstable behavioral state leading to maternal anxiety during MT.

Another possible explanation for our findings might be the severe illness of preterm infants with brain injury, which exposes them to more procedures that cause pain. This exposure might lead to changes in the organization of the preterm infant's nervous system, which result in overreaction to other stimuli, even if they are less painful [42, 43].

As physiological measurements (specifically heart rate and respiratory rate) are highly variable in preterm infants, we used a sensitive marker of physiological function, such as HRV, to better determine the impact of MT on the dynamic biological systems of our study population. Furthermore, the behavioral state scores of preterm infants with maternal singing during MT were clinically and statistically higher than during the SSC intervention alone, demonstrating instability and not just arousal state.

A recent systematic review suggested that MT targeted studies evaluating autonomic nervous system stability based on HRV are encouraged [44]. Our findings of higher HR and LF/HF ratios are in agreement with previous research showing that the sympathovagal imbalance was increased in infants diagnosed with PVL [38]. The findings support the notion that preterm infants with PVL might be more vulnerable to stress than their healthy counterparts are and that PVL may affect autonomic nervous system maturation and associated recommendations for intervention [39].

Our study results agree with those of Harrison et al. [40] who showed that motor system stress correlates with the physiological stress indicated by oxygen saturation and heart rate and hence by the autonomic nervous system, as expressed by LF and HF power.

Another interesting finding of the current study concerns the mothers' heightened anxiety levels. We speculate that as the infants in maternal singing during MT were not in a quiet sleep, as the mothers experienced during SSC, that increased the mothers' anxiety. Another explanation is that since 13 of the mothers had not used music with their infants previously, the request to sing in the presence of a music therapist with their baby in the NICU might have created a stressful reaction. Further explanation for the mothers' elevated anxiety level could be worry about their infants' health and outcomes, inhibiting their ability to sing freely and increasing their anxiety level while singing. A mother's emotional state has a direct effect on her ability to synchronize and attune to her infant, which may led to the infant's increased arousal and difficulty regulating [45]. It might be argued that although our findings of the differences in STAI scores between the SSC and the SSC combined with maternal singing during MT is statistically significant, the clinical relevance is doubtful due to high variability. However, a cut-off point of 39–40, which we used, has been suggested to detect clinically significant symptoms of anxiety [21].

We choose a specific mode of intervention, namely, maternal singing during MT, led by a music therapist specializing in therapy for preterm infants during their stay in the NICU. We choose this method because we found beneficial effects using the same method in a previous study [18]. In addition, our team of music therapists was familiar with this method. Of note is the physiologic stability that was achieved with SSC and MT in our previous study, as compared to SSC alone [18], in contrast to the current study. However, the main differences between the two studies were the exclusion of infants with severe brain injury in the previous study and the music therapist's active involvement in the current study. A music therapist is helpful for conducting MT intervention in preterm infants without severe brain injury, but might be overstimulating for infants with brain injury and for their mothers.

A limitation of this study might be that our treatment schedules were 3–5 days apart; this may have caused a wash-out bias between interventions. However, we assumed that this period created a balance between wash-out effect and maternal familiarity with interventions and that due to the different nature of these interventions, this bias was minimal. In addition, we could not differentiate the reactions to maternal singing during MT between infants with grade 3,4 IVH or PVL, as 26 of the 35 infants had IVH. However, our results indicate that infants with IVH demonstrate overstimulation towards certain stimuli, as do preterm infants with PVL, unlike the stable reactions of preterm infants without severe brain injury to stimuli [26].

In conclusion, maternal singing during MT added to SSC might induce physiological and behavioral instability in preterm infants with IVH and PVL and in their mothers. This group might require guided MT intervention that has been modified to meet their unique circumstances. Music therapists should closely monitor infants diagnosed with IVH and/or PVL during procedures that may be stressful or require handling. Monitoring strategies may include assessing behavioral and physiological responses. Additional considerations and research are needed to determine appropriate, multisensory interventions specifically tailored for infants with IVH and PVL.

Authors' contributions Shulamit Epstein contributed to the study conception, design, material preparation and data collection. Sofia Bauer contributed to the study conception. Orly Levkovits Stern contributed to the study design. Ita Litmanovitz contributed to the study methodology and outcome assessments. Cochavit Elefant contributed to the study conception and wrote the first draft. Dana Yakobson contributed to the study methodology and conducted the music therapy intervention. Shmuel Arnon contributed to the study design, data collection and analysis and manuscript preparation. All authors read and approved the final manuscript.

Data availability Data will be made available upon request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval The study was approved by the Institutional Review Board of Meir Medical Center and conducted in accordance with the ethical and humane principles of research established by the Declaration of Helsinki (trial registration number NCT 01427894).

Consent to participate Written informed consent was obtained from all parents.

Consent for publication Not applicable.

Code availability Not applicable.

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