Full-Length Article

Neonatal music therapy and cerebral oxygenation in extremely and very preterm infants: *A pilot study*

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Abstract

Objectives: Music therapy is a novel intervention that may minimize neonatal stress. The mechanism of action is still largely unknown. We hypothesized that one mechanism of action regards altered brain oxygenation (either due to altered cerebral perfusion or altered cerebral oxygen consumption).

Study design: Pilot study

Methods: We measured cerebral oxygenation before, during and after music therapy sessions using Near-Infrared Spectroscopy (NIRS). We extracted data on cerebral oxygen saturation (r_cSO_2) and calculated cerebral fractional tissue oxygen extraction ($_c$FTOE). In addition, we measured heart rate.

Results: We included 20 infants, receiving 44 music therapy sessions. Median gestational age was 27 weeks, the majority were males. We identified two distinct reactions: in one group r_cSO_2 increased and $_c$FTOE decreased during therapy compared with before therapy, whereas in the other group r_cSO_2 decreased and $_c$FTOE increased during therapy compared with before therapy. The first may indicate a sedative effect, whereas the second may reflect a hyperalert state. The observed changes in heart rate may contribute to these observations through altered cerebral perfusion.

Conclusion: Our findings suggest that there are two distinct reactions in cerebral oxygenation to music therapy in extremely and very preterm infants. The clinical significance of these two reactions for music processing and future neurological functioning in these infants warrants further investigation.

Keywords: Near-Infrared Spectroscopy; Cerebral Oxygenation; Neonatal Hemodynamics; Prematurity

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The neonatal intensive care unit (NICU) offers life-saving medical treatments for infants born extremely and very preterm (gestational age <32 weeks). Although lifesaving, the NICU environment can be intense and full of unnatural experiences, such as unpredictable noises, sleep interruptions, invasive procedures and parental separation, causing neonatal stress [1,2]. Neonatal stress may have profound effects on growth and development [3,4]. Currently, a variety of interventions exist that aim to minimize this neonatal stress [5]. One of these interventions is live-performed music therapy, defined as "the clinical and evidence-based use of music interventions to accomplish individual goals within a therapeutic relationship by a credentialed professional who has completed an approved music therapy program" [6]. The live, Rhythm, Breath and Lullaby music therapy approach, implements tailored ocean-disc (womb-like sounds) and lullaby interventions to soothe the infants, and has been associated with positive effects on the cardiovascular-pulmonary system and behavior of preterm neonates [7,8].

As of yet, the mechanism of action underlying the potential effects of music therapy is largely unknown. A couple of theories have been proposed, including music being stress-relieving [9], impacting metabolic processing [10], or impacting regulatory behavior [11,12]. We hypothesize that a potential mechanism of action concerns altered brain oxygenation, resulting from either altered brain activity (i.e. oxygen consumption of the brain), or from altered brain perfusion (i.e. from an altered cardiac output). Brain oxygenation can be non-invasively and continuously assessed at the cot-side using near-infrared spectroscopy (NIRS). Near-infrared light is emitted through the skull into the brain, where it is partly absorbed and partly reflected by hemoglobin and oxygenated hemoglobin. Reflected light is detected by the NIRS sensor and the relative amount of oxygenated

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hemoglobin is calculated, resulting in in oxygen saturation of underlying regional brain tissue (r_cSO_2). With this study, we aimed to elucidate mechanisms that might be triggered by music therapy, by investigating whether live-performed music therapy would have an effect on cerebral oxygenation markers (r_cSO_2 and fractional tissue oxygen extraction of the brain [$_c$FTOE]) measured using NIRS in extremely and very preterm infants.

Methods

Setting and population

We included infants born between August 2019 and August 2020 with a gestational age of less than 30 weeks, who were admitted to the level III-IV NICU of the University Medical Center Groningen. This pilot study is a sub-analysis from a larger ongoing music therapy trial, which concerns a randomized-controlled cross-over study, where infants are randomly assigned to either the intervention of music therapy or a waitlist for a period of two weeks and cross-over after this period. Infants were included within the first week after birth and were randomized to start either the intervention or the waitlist period from the second week after birth onwards. All parents of the eligible infants were asked to participate in this trial. Both parents provided written informed consent. The study was approved by the Ethical Review Board of the University Medical Center Groningen (METc 2019/093) and was registered online (ISRCTN94562698).

Measures and procedure

In this study, the intervention regarded music therapy and the outcome regarded cerebral oxygenation measured using NIRS.

Music Therapy

Within the music therapy trial, all therapy sessions were aimed at relaxation and stress reduction. A full description of the music therapy procedure was previously described [13]. Summarizing, therapy was provided three times a week for approximately 15 minutes by a certified music therapist who specialized in NICU music therapy. Individual appointments were made with parents, thus times of the sessions on a particular day could therefore slightly vary. Sessions were provided on an open bay ward. Therapy interventions were based on the "rhythm, breath, and lullaby" method and consisted of improvised music using the Remo Lullaby Ocean Disk, guitar-arpeggios and voice [14]. One instrument (either the Ocean Disc or the guitar) and/or voice were used in in each session. The first two sessions mainly involved the Ocean Disc. From the third session onwards, parents were asked to provide their song-of-kin [14], which was incorporated in the therapy sessions. Song-of-kin suggestions were translated into lullabies in 6/8 patterns. If a song-of-kin was not provided, the melody of "Twinkle Twinkle Little Star" (Dutch equivalent: "Olifantje In Het Bos") was the melody of choice because of its familiarity in many families. The sessions were all individualized and tailored to the behavioral state of the infants. The music therapist applied music that met the pace of the respiratory patterns of the child and calmed it to a slower tempo applying musical entrainment to realize a calm behavioral state for the infant [15]. If necessary and feasible the music therapist incorporated environmental sounds to help achieve this goal.

Cerebral oxygenation

A non-invasive method to measure cerebral oxygenation is Near-Infrared Spectroscopy (NIRS) [16,17]. NIRS uses near-infrared light which is scattered, absorbed, and reflected by chromophores, such as hemoglobin. Because oxygenated hemoglobin and deoxygenated hemoglobin have a distinct absorption spectrum, the regional oxygen saturation of the underlying brain tissue (r_cSO_2) can be determined. Taking the arterial oxygen saturation into account, measured using pulse oximetry (SpO_2), the fractional tissue oxygen extraction of brain tissue ($_c$FTOE) can be calculated according to the following equation: $_c\text{FTOE} = (SpO_2 - r_cSO_2) / SpO_2$. The $_c$FTOE reflects the balance between oxygen supply to the brain and consumption of oxygen by the brain. A *decreased* $_c$FTOE may indicate an increased oxygen supply, i.e. due to increased cerebral blood flow (while cerebral oxygen consumption remains constant), but it could also indicate a decreased oxygen extraction due to reduced metabolism in the cerebral tissue. The latter is often observed when infants are being medically sedated. Likewise, an *increased* $_c$FTOE may reflect a decreased cerebral oxygen supply (while cerebral oxygen consumption remains constant) or an increased oxygen consumption by the brain. As cerebral oxygen supply depends largely on the infants' cardiac output [18,19], a change in cardiac output would affect $_c$FTOE. Heart rate is an important determinant for cardiac output and is regulated by the autonomic nervous system [20,21].

We routinely measured r_cSO_2 using the INVOS™ 5100c near-infrared spectrometers (Medtronic, Dublin, Ireland), with neonatal SomaSensors. NIRS measurements are standard of care in the extremely and very preterm population for at least one week after birth and may be continued thereafter based on clinical condition. The neonatal sensor was placed on the frontoparietal side of the head, either left or right. To attach the sensor while protecting the underlying skin, we used Mepitel® strips (Mölnlycke, Sweden), or elastic bandages. We simultaneously measured the transcutaneous arterial oxygen saturation (SpO_2) using pulse oximetry (Massimo, Medtronic, Dublin, Ireland), to be able to calculate the $_c$FTOE. Data was stored every 5 seconds. Mean r_cSO_2 and $_c$FTOE values were calculated in the hour before music therapy, during the 15 minutes of music therapy and in the hour after music therapy. Data were visually inspected for artefacts (that is non-physiological changes in r_cSO_2 values or absent r_cSO_2

values due to misplaced sensors) by two authors (NvD and EK). These data were either deleted in case of short periods, or the session was excluded in total in case of long periods.

Clinical characteristics

Clinical characteristics of the infants included gestational age, postmenstrual age, birth weight, sex, small-for-gestational age status, multiple pregnancy, Apgar scores, respiratory support, and neonatal morbidities with onset during the two-week musical intervention period. The morbidities included intraventricular hemorrhages, convulsions, necrotizing enterocolitis or spontaneous intestinal perforation, sepsis, neonatal anemia requiring red blood cell transfusions, hyperbilirubinemia requiring phototherapy, hemodynamically significant patent ductus arteriosus and circulatory insufficiency requiring treatment. Small-for-gestational age status was defined as a birth weight for gestational age below the 10th percentile of the Dutch reference growth charts [22]. Presence and grading of intraventricular hemorrhages was determined with serial cranial ultrasounds, routinely performed every week. Presence of intestinal pathologies was based on clinical and radiographic examinations. Presence of a hemodynamically significant persistent ductus arteriosus was determined by cardiac ultrasound. Circulatory insufficiency was defined as requiring fluid therapy and/or treatment with inotropic agents such as dopamine or dobutamine. All these included clinical characteristics were obtained from a medical chart review.

In addition, we extracted data on heart rate from bedside monitors. Mean heart rates were calculated for the periods before, during and after the music therapy sessions.

Statistical analyses

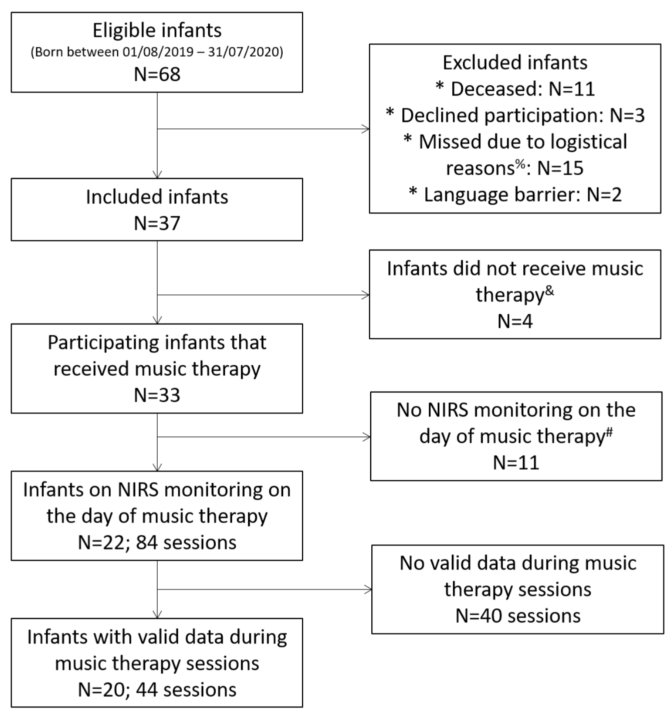
First, we described clinical characteristics, and we checked the data for normality using Q-Q plots and the Shapiro-Wilk test. As all data were not normally distributed, we used non-parametric tests throughout the study. Next, we tested differences in mean r_cSO_2 before, during and after music therapy for all sessions using the Wilcoxon signed rank test. Upon visual inspection of the data of each individual session, we identified two distinct reactions to music therapy and we subsequently divided all sessions in two groups: (1) increase in r_cSO_2 during as compared with before therapy and (2) decrease in r_cSO_2 during as compared with before therapy. Between these two groups we next tested differences in $_c$FTOE and heart rate before, during and after therapy using the Wilcoxon signed rank test. We also tested whether clinical characteristics, musical instrument(s) used and postnatal age differed between these two groups using the Mann-Whitney-U, Chi-square or Kruskal-Wallis tests where appropriate. Analyses were performed using SPSS for Windows (IBM, NY, USA), version 26. Results with P<0.05 were considered statistically significant. As this was a hypothesis generating pilot study, we chose not to correct for multiple testing.

Results

Participant characteristics

The flow of participants is presented in **Figure 1**. In total, 68 infants were eligible to participate in the study. Of these, 37 were included in the study. Four infants did not receive music therapy before discharge, because they were waitlisted first. Eleven infants did not receive NIRS monitoring on the days of their music therapy sessions, for various reasons. We could therefore include NIRS monitoring of 84 sessions of music therapy, provided to 22 infants. We excluded 40 sessions because of missing NIRS data during music therapy or too many artefacts. We were eventually able to analyze NIRS monitoring of 44 sessions of music therapy, provided to 20 infants. These 20 infants had a median gestational age of 27 weeks and the majority were males. Two of the infants deceased after the study period, both due to sepsis and subsequent circulatory failure. Analyzed therapy sessions were provided on median postnatal day 24.5 (interquartile range 16.5-29.8), at a postmenstrual age of median 30.3 days (interquartile range 29.4-31.1). We provide a summary of all participant characteristics in **Table 1**.

Figure 1. *Flow of participants*



% 13 infants missed due to COVID-19 regulations

& Infants were discharged before their music therapy period commenced

NIRS monitoring was discontinued as standard of care

Table 1. *Participant characteristics*

	N=20
Gestational age (weeks)	27.4 (26.3-28.6)
Birth weight (grams)	880.0 (775.0-1027.5)
Male sex	11 (55)
Small-for-gestational age	9 (45)
One of a multiple	2 (10)
Postmenstrual age at time of music therapy (days)	30.3 (29.4-31.1)
Postnatal day of music therapy sessions (days)	24.5 (16.5-29.8)
Number of sessions of music therapy (n=44)	
One	20 (46)
Two	13 (30)
Three	7 (16)
Four	3 (7)
Five	1 (3)
Instrument during therapy session (n=43)	
Ocean Disk	24 (56)
Arpeggio Guitar (including Song-of-Kin)	19 (44)
Apgar scores	
1 minute	5.0 (2.0-7.0)
5 minutes	8.0 (7.0-9.0)
10 minutes	8.0 (7.5-9.0)
Respiratory support during music therapy (n=44)	
Heated Humidified High-flow nasal cannula therapy (HHHFNC)	14 (32)
Continuous positive airway pressure	18 (41)
Conventional invasive ventilation	11 (25)
High frequency oscillation	1 (2)
Intraventricular hemorrhages grade 3-4	3 (15)
(Sub)clinical convulsions	1 (0.5)
Gastro-intestinal problems	
Necrotizing enterocolitis#	4 (20)
Spontaneous intestinal perforation	1 (0.5)
Sepsis	11 (55)
Hyperbilirubinemia requiring phototherapy	20 (100)
Red blood cell transfusion during study period	16 (80)
Hemodynamically significant Persistent Ductus Arteriosus^	6 (30)
Circulatory insufficiency	4 (20)

Data are presented as median (interquartile range) or n (%) where appropriate. # Three infants were treated conservatively with nil-per-mouth and antibiotics, one infant was treated surgically. ^ Five infants were treated with ibuprofen, one infant was treated surgically.

Cerebral oxygenation and music therapy

In **Figure 2**, we present the distribution of the mean r_cSO_2 before, during and after neonatal music therapy sessions, which yielded a trend towards a higher r_cSO_2 after therapy compared with during therapy. Upon closer visual inspection of the individual therapy sessions, we identified two distinct reactions to music therapy in the r_cSO_2 values. In one group (n=20 sessions) the r_cSO_2 decreased during therapy compared with before therapy (individual example presented in **Figure 3A**), whereas in the other group (n=24 sessions) the r_cSO_2 increased during therapy compared with before therapy (individual example presented in **Figure 3B**). Within individual infants, reactions to the several sessions differed. In addition, we visually identified several sessions in which the infant was more stable during music therapy compared with before and after therapy, reflected in less desaturations (individual example presented in **Figure 3C**).

Figure 2. *Cerebral oxygenation (r_cSO_2) before, during and after music therapy.* MT: Music Therapy.

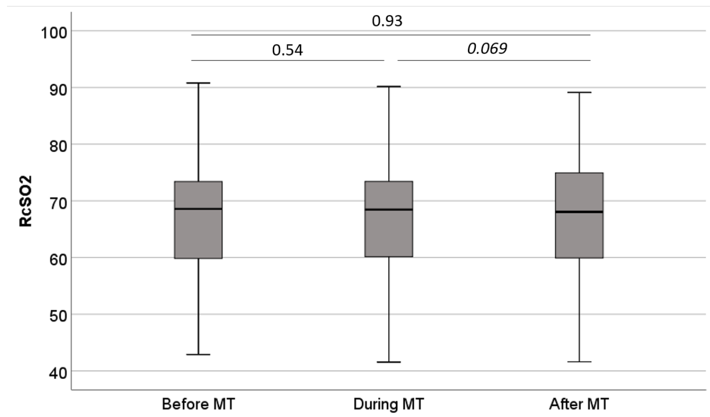
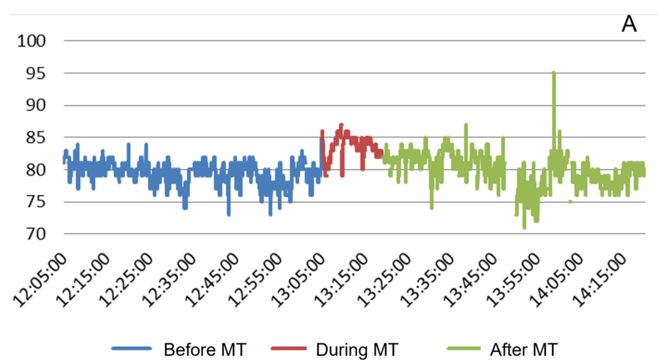
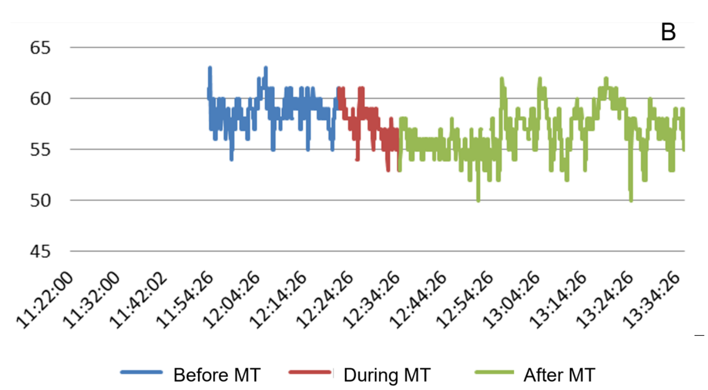


Figure 3. Individual courses of cerebral oxygenation (r_cSO_2) before (blue), during (red) and after (green) music therapy. **A**: example of an individual course where the r_cSO_2 increased during therapy. **B**: example of an individual course where the r_cSO_2 decreased during therapy. **C**: example of an individual course where the infant showed less desaturations during therapy compared with before and after therapy. *MT: Music Therapy.*

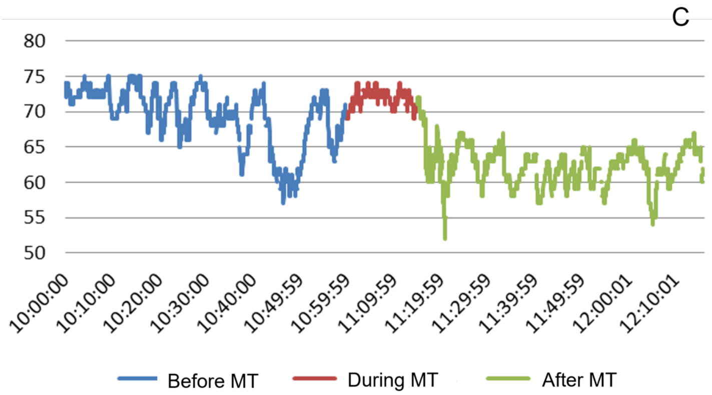
3A



3B



3C



Cerebral fractional tissue oxygen extraction and music therapy

Regarding the $_c$FTOE in these groups, we identified a similar pattern (**Figure 4**). In the group with increased r_cSO_2, the $_c$FTOE decreased during music therapy, and in the group with decreased r_cSO_2, the $_c$FTOE increased during music therapy. We were not able to identify characteristics that could explain the differences in these two distinct reactions (**Table 2**), including the type of instrument used. Moreover, we did not identify any associations with (changes in) heart rate (**Figure 5**) that might explain the differences in these two distinct reactions.

Figure 4. *Cerebral Fractional Tissue Oxygen Extraction ($_c$FTOE) before, during and after music therapy by distinct reactions in r_cSO_2.* MT: Music Therapy

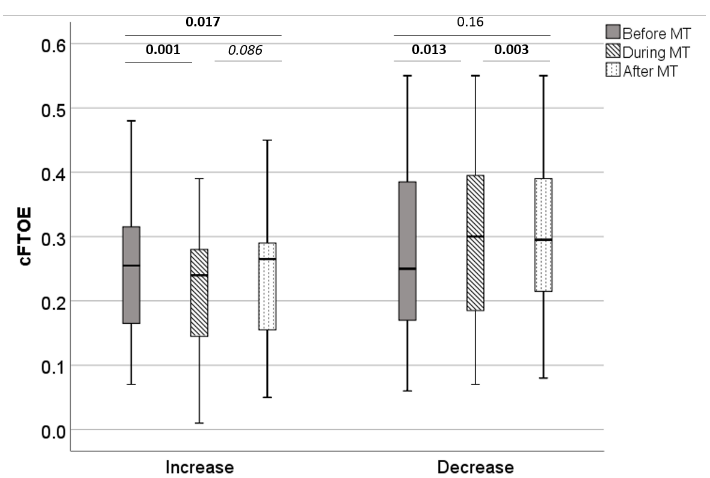
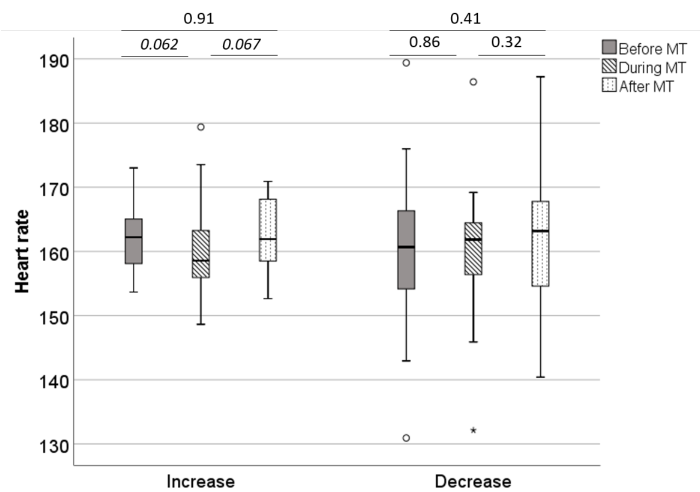


Table 2. Clinical characteristics for the two distinct reactions in r_cSO_2 to music therapy

Characteristic	**Increased r_cSO_2 during therapy (n=20 sessions)**	**Decreased r_cSO_2 during therapy (n=24 sessions)**	**P-value**
Gestational age (weeks)	27.0 (25.6-28.8)	26.6 (25.1-28.0)	0.31
Birth weight (grams)	863 (751-1206)	887 (793-1028)	0.76
Postmenstrual age (weeks)	30.4 (29.5-31.6)	30.3 (29.3-31.0)	0.38
Session number			*0.063*
One	8 (40)	12 (50)	
Two	10 (50)	3 (13)	
Three	0 (0)	7 (29)	
Four	1 (5)	2 (8)	
Five	1 (5)	0 (0)	
Instrument			0.39
Ocean Disc	12 (63)	12 (50)	
Arpeggio Guitar (including Song-of Kin)*	7 (37)	12 (50)	
Respiratory support			0.12
Heated Humidified High-flow nasal cannula therapy (HHHFNC)	7 (35)	7 (29)	
Continuous positive airway pressure	8 (40)	10 (42)	
Conventional ventilation	4 (20)	7 (29)	
High frequency oscillation	1 (5)	0 (0)	
Male sex	11 (55)	12 (50)	0.74
Intraventricular hemorrhages	2 (10)	6 (25)	0.20

*Data are presented as median (interquartile range) or n (%) where appropriate. *For one session, the type of instrument used was not recorded (N=43).*

Figure 5. *Heart rate before, during and after music therapy by distinct reactions in r_cSO_2. MT: Music Therapy*



Discussion

The aim of this study was to further elucidate mechanisms of action of music therapy, by investigating whether live-performed music therapy would affect cerebral oxygenation markers (r_cSO_2 and $_cFTOE$) measured using NIRS in extremely and very preterm infants. We demonstrated that there seem to be two evenly distributed distinct reactions in cerebral oxygenation to music therapy sessions. One group showed an increased r_cSO_2 and decreased $_cFTOE$ during music therapy, whereas the other group showed a decreased r_cSO_2 and increased $_cFTOE$ during music therapy. We did not find any influence of the musical instrument chosen to perform the intervention, nor any other differences in clinical characteristics. We also identified several sessions where music therapy resulted in a physiologically more stable state, reflected in fewer desaturations.

The first type of reaction to music therapy, i.e. where r_cSO_2 increased and $_cFTOE$ decreased, indicates either an increased cerebral blood flow or a decreased metabolism in the brain during music therapy [23]. The few available studies investigating cerebral reactions to music also report an increased r_cSO_2, and conclude that this is due to increased cerebral blood flow [24,25]. Notably, one of these studies did not include $_cFTOE$ as a marker [24], and the other reported no changes in $_cFTOE$ values before, during and after the intervention [25]. In our study, heart rate tended to decrease in this group during music therapy. In preterm infants, this may reflect an increased end-diastolic ventricular filling time, which in turn increases the cardiac output [19,20]. We therefore speculate that this reaction to music therapy partially represents a sedative effect and partially represents an increased cerebral blood flow through increased cardiac output in these infants.

The second type of reaction to music therapy, i.e. where r_cSO_2 decreased and $_cFTOE$ increased, indicates either a decreased cerebral blood flow or an increased metabolism and oxygen consumption in the brain during music therapy [23]. In this group, heart rate slightly increased, albeit non-significantly, which may reflect an attenuated cardiac output, by a decreased end-diastolic ventricular filling time [19,20], in turn resulting in a lowered cerebral blood flow [23]. Potential causes for increased cerebral oxygen consumption have been suggested to be intraventricular hemorrhages, hypoxic circumstances during delivery or seizures [23]. In our analyses, we did not find evidence for these causes, as these characteristics did not differ between the two distinct reacting groups. We therefore hypothesize that these infants react to music therapy partially with increased alertness resulting in increased cerebral oxygen consumption, and partially with a decreased cerebral blood flow through decreased cardiac output.

The interrelation between cerebral oxygenation, heart rate and music therapy may closely interact with the autonomic nervous systems [26]. The autonomic nervous system, which consists of the sympathic and parasympathic branches, is balanced to regulate amongst others the cardiovascular and respiratory systems [27]. Environmental stimuli, such as music therapy intervention, could evoke two reactions, i.e. a sympathic reaction that could lead to tachycardia and a lowered cardiac output, or a parasympathic reaction that could lead to lowered heart rate and increased cardiac output [26]. These two autonomic nervous system branches are known to be different in active sleep (sympathic dominance) versus quiet sleep (parasympathic dominance) [26]. Several studies have attempted to elucidate the maturation of the autonomic nervous branches, which can be measured using heart rate variability, and reported that with increasing gestational age the parasympathic nervous system becomes more influential [28]. In our study, we did not identify a difference in the two reactions by gestational age, postmenstrual age, nor by session number. However, it would be of interest to further investigate the role of the autonomic nervous system in music therapy, using heart rate variability as a marker.

The question remains whether these two distinct reactions are associated with neurological development. Previously, cerebral oxygenation markers (both r_cSO_2 and $_cFTOE$) have been associated with neurodevelopmental outcomes [29]. Brain perfusion and oxygen consumption of the brain may therefore indeed be mechanisms of action underlying the effects of the music therapy intervention. Regarding the two distinct reactions, we speculate that the proposed sedative effect of music (i.e. an increased r_cSO_2, associated decreased $_cFTOE$ and lowered heart rate) may be the result of an attenuated stress response in these infants. This relaxed state could result in better processing of the live-performed music, resulting in enhanced brain development.

Animal studies strengthen this hypothesis, reporting that attenuated stress responses resulted in more synaptogenesis and less neuronal cell death in the prefrontal cortex, hippocampus and amygdala, parts of the brain particularly important for cognitive development and executive functioning [30]. Furthermore, studies in preterm neonates who were offered recorded music report enhanced cognitive brain networks [31,32]. Therefore, we speculate that infants responding to music with a decreased cerebral oxygen consumption may experience more favorable effects of music therapy, which evidently requires further study.

Another finding in this study was the identified sessions in which infants seemed more physiologically stable, reflected in less desaturations. Physiological outcomes (i.e. heart rate, spO_2 or respiratory rate) of music interventions have recently been reviewed by Foroushani *et al.* [33]. The 16 available studies reported mixed results, either positive effects or no effects at all [33]. In addition, only two studies used music *therapy*, whereas all other studies used music [33]. In our study, we could not demonstrate a statistically significant effect on heart rate, although we did find a trend towards lower heart rate during music therapy in the group of children where the r_cSO_2 increased. Considerable variability exists across studies regarding the design and participants, as well as the actual musical intervention, hampering a clear-cut conclusion.

Strengths and limitations

An important strength of this study is the structured live-music therapy intervention that we provided to the included extremely and very preterm infants. We also were able to include several factors that could have influenced the cerebral oxygenation, i.e. intraventricular hemorrhages, hypoxia and seizures, to be able to provide the most plausible explanation for our findings. We also recognize several limitations of this study. First, we included a small number of preterm infants in this study, depending on whether infants received music therapy and NIRS monitoring simultaneously. This could have introduced some selection bias in our study. Second, we lacked information on the exact location (i.e. left or right) of the neonatal SomaSensor. Others have suggested that r_cSO_2 reactions to music therapy differed between left and right hemispheres [25], which we could not consider. Moreover, we did not measure cardiac output nor cerebral perfusion, as this is usually more invasive in nature. We could therefore not discriminate whether the reaction to music therapy is due to changes in cardiac output and cerebral blood flow or to changes in brain metabolism. Finally, we are unable to compare cerebral oxygenation data to behavioral state (i.e. wakefulness or sleep), because we did not measure these. Future studies might incorporate amplitude integrated electroencephalogram measurements to be able to make such a comparison.

Conclusion

In summary, our findings suggest that, in extremely and very preterm infants, there are two distinct reactions in cerebral oxygenation to music therapy. On the one hand, infants may react with a decreased oxygen consumption and a decreased heart rate, possibly reflecting a sedative effect. On the other hand, infants may react with an increased oxygen consumption and increased heart rate, possibly reflecting a hyperalert state. An environmental stimulus such as music therapy may trigger the autonomic nervous system, regulating heart rate and oxygenation of the brain. The clinical significance of these two distinct reactions for music processing and neurological functioning in these infants deserves further investigation.

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References

1. Mooney-Leber SM, Brummelte S. Neonatal pain and reduced maternal care: Early-life stressors interacting to impact brain and behavioral development. *Neuroscience*. 2017;342:21-36
2. Anand KJ. Clinical importance of pain and stress in preterm neonates. *Biol Neonate*. 1998;73:1-9.
3. Valeri BO, Holsti L, Linhares MB. Neonatal pain and developmental outcomes in children born preterm: A systematic review. *Clin J Pain*. 2015;31:355-362.
4. Grunau RE. Neonatal pain in very preterm infants: Long-term effects on brain, neurodevelopment and pain reactivity. *Rambam Maimonides medical journal*. 2013;4:e0025.
5. Bucsea O, Pillai Riddell R. Non-pharmacological pain management in the neonatal intensive care unit: Managing neonatal pain without drugs. *Semin Fetal Neonatal Med*. 2019;24:e101017.
6. American music therapy association. https://www.musictherapy.org/about/quotes/. Accessed 11-1-2021.
7. Anderson DE, Patel AD. Infants born preterm, stress, and neurodevelopment in the neonatal intensive care unit: Might music have an impact? *Dev Med Child Neurol*. 2018;60:256-266.
8. Bieleninik Ł, Ghetti C, Gold C. Music therapy for preterm infants and their parents: A meta-analysis. *Pediatrics*. 2016;138: e20160971
9. Qiu J, Jiang Y, Li F, Tong Q, Rong H, Cheng R. Effect of combined music and touch intervention on pain response and β-endorphin and cortisol concentrations in late preterm infants. *BMC Pediatr*. 2017;17:e38.

10. Lubetzky R, Mimouni FB, Dollberg S, Reifen R, Ashbel G, Mandel D. Effect of music by Mozart on energy expenditure in growing preterm infants. *Pediatrics*. 2010;125:e24.
11. Loewy J, Stewart K, Dassler A, Telsey A, Homel P. The effects of music therapy on vital signs, feeding, and sleep in premature infants. *Pediatrics*. 2013;131:902-918.
12. Standley J. Music therapy research in the NICU: An updated meta-analysis. *Neonatal Netw*. 2012;31:311-316.
13. van Dokkum NH, Jaschke AC, Ravensbergen A, et al. Feasibility of live-performed music therapy for extremely and very preterm infants in a tertiary NICU. *Front Pediatr*. 2020;8:e581372.
14. Loewy J. NICU music therapy: Song of kin as critical lullaby in research and practice. *Ann N Y Acad Sci*. 2015;1337:178-185.
15. Juslin PN. Musical emotions explained: Unlocking the secrets of musical affect. Oxford University Press; 2019.
16. Garvey AA, Dempsey EM. Applications of near infrared spectroscopy in the neonate. *Curr Opin Pediatr*. 2018;30:209-215.
17. Sood BG, McLaughlin K, Cortez J. Near-infrared spectroscopy: Applications in neonates. *Semin Fetal Neonatal Med*. 2015;20:164-172.
18. Kooi EMW, Richter AE. Cerebral autoregulation in sick infants: Current insights. *Clin Perinatol*. 2020;47:449-467.
19. Vrancken SL, van Heijst AF, de Boode WP. Neonatal hemodynamics: From developmental physiology to comprehensive monitoring. *Front Pediatr*. 2018;6:e87.
20. Winberg P, Ergander U. Relationship between heart rate, left ventricular output, and stroke volume in preterm infants during fluctuations in heart rate. *Pediatr Res*. 1992;31:117-120.
21. Javorka K, Lehotska Z, Kozar M, et al. Heart rate variability in newborns. *Physiol Res*. 2017;66:S203-S214.
22. Visser GHA, Eilers PHC, Elferink-Stinkens PM, Merkus, Hans MWM., Wit JM. New Dutch reference curves for birthweight by gestational age. *Early Hum Dev*. 2009;85:737-744.
23. Garvey AA, Kooi EMW, Smith A, Dempsey EM. Interpretation of cerebral oxygenation changes in the preterm infant. *Children (Basel)*. 2018;5:e94.
24. Sakatani K, Chen S, Lichty W, Zuo H, Wang YP. Cerebral blood oxygenation changes induced by auditory stimulation in newborn infants measured by near infrared spectroscopy. *Early Hum Dev*. 1999;55:229-236.
25. Meder U, Tarjanyi E, Kovacs K, et al. Cerebral oxygenation in preterm infants during maternal singing combined with skin-to-skin care. *Pediatr Res*. 2020;Online ahead of print (doi: 10.1038/s41390-020-01235-2).
26. Chiera M, Cerritelli F, Casini A, et al. Heart rate variability in the perinatal period: A critical and conceptual review. *Front Neurosci*. 2020;14:e561186.
27. Fairchild KD, O'Shea TM. Heart rate characteristics: Physiomarkers for detection of late-onset neonatal sepsis. *Clin Perinatol*. 2010;37:581-598.
28. Cardoso S, Silva MJ, Guimarães H. Autonomic nervous system in newborns: A review based on heart rate variability. *Childs Nerv Syst*. 2017;33:1053-1063.
29. Verhagen EA, Van Braeckel KN, Van der Veere CN, et al. Cerebral oxygenation is associated with neurodevelopmental outcome of preterm children at age 2 to 3 years. *Dev Med Child Neurol*. 2015;57:449-455.
30. Haslbeck FB, Bassler D. Music from the very beginning-A neuroscience-based framework for music as therapy for preterm infants and their parents. *Front Behav Neurosci*. 2018;12:e112.
31. Lordier L, Meskaldji D, Grouiller F, et al. Music in premature infants enhances high-level cognitive brain networks. *Proc Natl Acad Sci U S A*. 2019;116:12103-12108.
32. Sa de Almeida J, Lordier L, Zollinger B, et al. Music enhances structural maturation of emotional processing neural pathways in very preterm infants. *Neuroimage*. 2020;207:e116391.
33. Foroushani SM, Herman CA, Wiseman CA, Anthony CM, Drury SS, Howell MP. Evaluating physiologic outcomes of music interventions in the neonatal intensive care unit: A systematic review. *J Perinatol*. 2020;40:1770-1779.

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