FULL PROTOCOL TITLE

Effects of Music Based Intervention (MBI) on Pain Response and Neurodevelopment in Preterm Infants

Principal Investigator

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Supported by:
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1 R61 AT010712-01

Tool Revision History

Version Number: 2.0b

Version Date: November 4, 2019

Summary of Revisions Made:
Page 11 (Bayley's III assessment) - Apologies. The statement about Bayley's III developmental assessment was not supposed to be included in R61 study protocol. It is part of the R33 protocol. I am deleting it from this section.
Page 14 (how will they maintain distance of headphones relative to baby's ears) - In this age group, these preterms have very little motor capability to move much – even while awake. In addition, during each session of intervention, study staff will be monitoring for infant discomfort. Study staff will also monitor to ensure the 1cm distance from the preterms' ears.
Page 15 (hearing loss) - Hearing testing is not typically assessed in preterm babies, but rather at term age. However, hearing loss in preterm babies typically occurs in those who are medically unstable (hyperbilirubinemia, sepsis, or meningitis – approximately 2-4% of the population). That population of preterm infants do not meet eligibility criteria for this trial. Hearing will also be confirmed during ERP testing.
Page 22 (blinded statistician) - Yes. This statistician is only aware of the blinded treatment and is thus blinded also for the study analysis.
Page 27 & 28 (why is PIPP considered the primary outcome but not associated with primary study objective 1) - This R61 is exploratory with little prior research in all aspects, and thus we had challenges regarding sample size/ power considerations. Our main goal is aimed towards meeting Go/NoGo Criteria – each with equal weight in mind. Certainly, the order in which all the Go/NoGo criteria is presented can be switched around with primary outcome PIPP being Primary objective. With minimal prior research, we decided on PIPP to be the primary outcome for statistical calculations. In this exploratory work there is not adequate power to assess hypothesis 1 and 2.

I have put together a version 2.0b study protocol response to these edits with the objectives moved around. Version 2b shows PIPP primary outcome to be associated with primary objective.

Page 28 (eligibility) - Yes. The eligible subjects at the University of Minnesota Neonatal Intensive Care Unit meeting inclusion and exclusion criteria.
Page 29 (missing data) – please see added section 9.6 Missing Data.
Page 29-30 (clarification about sleep duration measure) - Milestone threshold is reached if any 1 or more of the serial EEGs is reached. This threshold was purposely chosen as brain maturation occurs at different stages. This exploratory investigation is important to understand whether there might be critical time periods during prematurity in which the music intervention may have more impact. If the other time points show opposite directions, these may be time periods in which music may be less effective. Because no literature is available to describe this, it is important for us to gather this information.
Page 30-31 (clarification about PIPP/central EEG amplitudes) – similar answers to the above sleep measure regarding importance of reaching thresholds at different time points of prematurity.

Version Number: 2.0c

Version Date: February 7, 2020

Updated inclusion criteria to 30 weeks +/- 2 weeks

Version Number: 3.0

Version Date: June 18, 2020

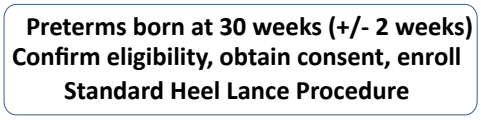
Page 13: PIPP performed during heel lance procedure expanded to include both Minnesota Newborn Screen or required heel lance blood tests

The PIPP is performed when the preterm infant undergoes the heel lance procedure for either the Minnesota Newborn Screen or other required blood tests performed during the same approximate time period within the scheduled protocol timeline. The PIPP should not pose any additional risk to the subject as the heel lance for newborn screening or required blood tests are both standard of care.

The heel lance procedure for the Minnesota Newborn Screen is the same as the heel lance procedure required for other blood tests. Due to new COVID restrictions and limited access to the NICU for research purposes, the opportunity to perform the PIPP has been expanded to include other required blood tests that utilize the same heel lance procedure performed at approximately the same time period within the scheduled protocol timeline. These heel lances are all part of required standard of care and does not significantly change the study protocol.

Page 15 Diagram:

Changed label to Standard Heel Lance Procedure



Instead of Newborn Screen Heel Lance

Page 19: Visit 5/Completion Visit:

1) Added Medical History Review to gather information if there are any ongoing medical issues that might affect ERP results
2) Added music exposure form to gather information on how much music is played at home after NICU discharge

Version Number 4.0

Version Date: August 31, 2020

Page 5, 15, 17, 18, 25: COVID efforts:

Given the need to decrease face to face interactions for COVID precautions, we are slightly decreasing the number of music intervention to an average of 4-5 (instead of 5-6) sessions per week. This is still

frequent enough music intervention to show changes in pain responses and neurodevelopmental findings since the intervention lasts a target of 6 weeks.
Page 5: …average 4-5 intervention or control sessions per week…
Page 15: MBI subjects will receive …intervention averaged at 4-5 sessions per week.
Page 17: …music intervention 4-5 sessions per week...
Page 18: Adherence …as 80% compliance …average of 4-5 intervention or control sessions per week
Page 25: Targeted averaged 4-5 sessions of MBI or Control will be played per week.

Page 16 and 18: Change 6 to 10 playlists:
To randomize songs properly, our biostatistician created 10 playlists, instead of the 6 originally planned.
Page 16: stimulation, habituation, and neurological development, we will use ten playlists.
Page 18: As it is recommended to change song order to increase stimulation, habituation, and neurological development, we will use 10 playlists.

Page 16: “decibel” to “volume”:
The mp3 player/headphone equipment decibel levels will be checked prior to use. Because the mp3 players only display volume levels, this word was changed from “decibel” to “volume”.

Page 16: Daily electronic volume level checks will avoid the need for listening checks of the headphones.

Version Number 5.0

Version Date: March 16, 2021

Pg 14: Clarification that for reliability in scoring, the PIPP will be

done by 2 study team members. The 2nd PIPP can be done in-person or virtually.

Pg 21 and 23 (sec 6.1 and 6.2.2 clarification that HADS is optional for the parent to complete)

Pg 24 (sec 6.2.4): clarification that EEG will have a video component

Pg 25: Hearing status of the child will be confirmed through chart review of the child’s clinical BAER hearing test. This is done clinically prior to discharge (and/or at 1 month clinical care follow-up).

Pg 36 (sec 11.3) clarification the confidentiality of the video EEG

Version Number 6.0

Version Date: August 11, 2021

Updated protocol to reflect that ERPs will be conducted between 43-48 weeks corrected gestational age. See pgs 10,11,15,16, 26, 30. No impact on analysis. This allows for better compliance with allowing for scheduling families with a new born child.

Version Number 7.0

Version Date: February 1, 2022

Pg 20: Updated Section 6.1 Schedule of Evaluations – Screening and Study Visits to remove the 48 hour time period for baseline/visit 1 activities to occur. This change is administrative only as it has no impact on study aims, analysis or safety.

Version Number 8.0

Version Date: March 8, 2022

Pg 20-21: *Updated Section 6.1 Schedule of Evaluations* & Pg 25: *Section 6.2.4 Follow up Visits: Visits 2-5* to move Family Feedback form completion at Visit 4, noting though that this may be completed at any time prior to discharge.

TABLE OF CONTENTS

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STUDY TEAM ROSTER

1) Pediatric Neurology (Sonya Wang) – Sonya Wang is the principal investigator and will oversee the entire project.

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2) Music Therapy (Michael Silverman) - Michael Silverman will be responsible for the creation of the recorded lullaby variations and will ensure safety of headphones, MP3 players with routine quality control checks on music and decibel sound level.

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3) Neonatology (Raghavendra Rao) - Raghavendra Rao is a practicing neonatologist, and core faculty of the Center for Neurobehavioral Development. He will assist in recruitment, data collection, consent, and music based intervention administration.

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4) Biomedical engineering (Theoden Netoff) - Theoden Netoff will supervise EEG data analysis and oversee a biomedical engineering graduate student who will utilize machine learning tools to identify EEG sleep biomarkers for future automatization of EEG interpretation and identification of EEG-sleep wake cycle features.

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5) Statistics (Lynn Eberly, Qi Wang) - Lynn Eberly will provide oversight for biostatistical support. Qi Wang will provide biostatistical analysis support for this project.

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6) Study Coordinators (TBD) – Study coordinators will oversee practical details of the project, including coordinating recruitment, implementation of intervention, organizing details of data collection, data movement, data storage, data security, and data access; data preparation for publications and project reporting.

Contact info TBD

PARTICIPATING STUDY SITES

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PRÉCIS

Study Title

Effects of Music Based Intervention (MBI) on Pain Response and Neurodevelopment in Preterm Infants

Objectives

Primary Objective (1):

Characterize differences in preterm pain responses between MBI and controls

Primary Objective (2):

Identify differences between MBI and controls in preterm brain maturation and early neurodevelopment

Design and Outcomes

Study design: Pilot prospective randomized, double blinded, controlled study to test effect of music based intervention (MBI) on pain response and neurodevelopment in preterm infants.

Aim 1 (R61): Characterize differences in preterm pain responses between MBI and controls.

The objective of this aim is to understand the behavioral processes of MBI on pain in preterm infants by comparing the PIPP and EEG pain responses in the MBI and control cohorts.

Aim 2 (R61): Identify differences between MBI and controls in preterm brain maturation and early neurodevelopment.

The objective of this aim is to explore biological mechanisms of MBI on preterm brain maturation and neurodevelopment using electroencephalography (EEG) and event related potentials (ERPs).

Interventions and Duration

Specific recorded lullabies will be played for 6 weeks (+/- 1 week). Additional follow-up will occur at 43-48 weeks corrected age (~ one month old after the original due date).

Sample Size and Population

Recruit 60 medically stable preterm infants born at approximately 30 weeks to participate in this study. Randomization will be stratified by sex at birth and within each stratum use randomly allocated block sizes of 2 and 4.

1. STUDY OBJECTIVES

1.1 Primary Objective (1)

Aim 1(R61): Characterize differences in preterm pain responses between MBI and controls.

Premature infant pain profiles (PIPP) include physiologic, behavioral, and contextual measures which identifies differences in pain responses between MBI and controls while still in the neonatal intensive care unit (NICU). Central EEG amplitude changes have been time-locked with painful procedures in term infants. We will explore if PIPP scores and central EEG amplitude changes are attenuated with MBI in comparison to controls.

• Hypothesis 1: MBI will show improved pain responses, with lower PIPP

scores and attenuated central EEG amplitude changes during painful procedures, in comparison to the control cohort.

1.2 Primary Objective (2)

Aim 2(R61): Identify differences between MBI and controls in preterm brain maturation and early neurodevelopment.

EEG is a surrogate marker for real time brain function during sleep-wake cycles. Because preterm brain networks develop during sleep, sleep duration is a strong indicator of brain maturation. Serial biweekly EEGs of preterm infants can quantify sleep duration trends and track MBI's influence on sleep. To enhance objectivity, innovative EEG machine-learning tools will be applied to the analyses.

• Hypothesis 2: MBI will enhance preterm EEG brain maturation in comparison to controls.

Due to the natural limitations of evaluating immature neonatal nervous systems, ERPs have been utilized to study early neurodevelopment. ERPs quantify electrical brain potentials changes time-locked with a stimulus. Auditory ERPs performed at 1 month (43-48 weeks) corrected age evaluates attention and discrimination between familiar and novel stimuli - early neurodevelopmental signs of recognition memory function and perceptual learning.

• Hypothesis 3: ERPs at 1 month (43-48 weeks) corrected age will show that MBI has a greater impact on early neurodevelopment when compared to controls.

2. BACKGROUND AND RATIONALE

2.1 Background – Primary Objective (1)

Preterm infants experience up to 14 painful procedures per day during their first 2 weeks of life[26] and neonatal pain experiences prime adult pain responses.[27] In rats, painful events in early life can increase the number or glucocorticoid receptors in the hippocampus which may affect future stress responses.[28] Incision of the rat paw in early life has been associated with a greater magnitude and duration of hyperalgesia in adulthood.[29] Neonatal injury has also been associated with increased intensity, spatial distribution and duration of ionized calcium-binding adaptor molecule-1-positive microglial reactivity in the dorsal horn of the spine (a sensory region of the spine).[29] Anatomical differences in neuronal organization of the sensory active subplate zone have been found in preterms, indicating underlying developmental changes due to painful procedures.[30] Furthermore, greater

neonatal procedural pain has been associated with reduced white matter and subcortical gray matter volumes by term gestation[31] and thinner cortices in the frontal and parietal lobes by age seven.[32]

To control for pain, opioids (including morphine and fentanyl), sedatives (benzodiazepines), and sucrose have been widely used in the NICU.[33,34] However, higher cumulative fentanyl doses in preterm infants correlate with a higher incidence of cerebellar injury and lower cerebellar diameter at term age.[35] Midazolam, a benzodiazepine sedative, exposure in preterm infants correlates with abnormal hippocampal growth and altered neurodevelopmental outcomes in cognitive, language, and motor abilities.[36] Repeated exposure to sucrose for procedural pain in mouse pups reveal smaller white matter volumes in the corpus callosum, stria terminalis, and fimbria, as well as smaller cortical and subcortical grey matter volumes in hippocampus and cerebellum.[37]

Music has been presumed to be effective in treating pain by provoking feelings of familiarity and security.[19] Soothing music redirects attention from pain inducers, allows for release of brain endorphins, and reduces stress hormones.[38] Music has also been shown to relax muscle tone and release body tension.[39] Prior music intervention studies on pain have shown overall positive immediate effects on heart rate, facial expressions, latency to cry, and lower pain scores.[40,41] However, similar to the MBI studies described previously, weaknesses in prior music research on pain in preterm infants consist of inconsistent study approaches with heterogenous study groups, small sample sizes, and varied interventions with mixed outcomes.[20,40,42–48]

2.1a Study Rationale – Primary Objective (1)

Thus, we propose a novel approach to study immediate and lasting effects of MBI on pain responses in the preterm infant utilizing the standardized Premature Infant Pain Profile (PIPP) and EEG (Aim 1). With frequent music intervention throughout the NICU hospitalization (6 weeks +/- 1 week), pain responses are likely to adapt and change over time. PIPP and EEG are objective, quantifiable outcomes measures that when performed serially will provide pain response trends revealing the cumulative effects of MBI on pain responses throughout the NICU stay. Evaluation of MBI in preterm infants will provide a deeper understanding of music's influence on behavioral processes of the pain response and offer a low risk treatment alternative to the current pain treatment regimen of opioids, benzodiazepines, and sucrose that are detrimental to neurodevelopment.

2.2 Background – Primary Objective (2)

In 2018, the World Health Organization reported 15 million (>1 in 10) preterm births with rising annual rates. While over 90% of those born at ~30 weeks gestation survive, approximately 50% of these infants suffer from neurodevelopmental impairments that span motor, behavior and academic

performance.[1] By 18 months, preterm infants have lower language skills (15 points) on IQ tests compared to term infants.[2] The most active period of neurodevelopment occurs from conception to 2 years ("first 1000 days") where the effects of environmental exposures may permanently affect functioning throughout life.[3,4] Healthy neurodevelopment progresses by a complex scaffolding process whereby intricate neural circuits rely on successful completion of previous stages of development.[4] In particular, the 3rd trimester of gestation is a critical period of rapid brain growth.[5–9] Although neurodevelopment continues throughout life, by age 2, the brain has undertaken significant restructuring and many developmental changes cannot occur beyond this sensitive growth period.[4]

Nevertheless, music has the remarkable ability to enhance child neurodevelopment. Auditory listening has been found in 27 week fetuses.[10] Fetal auditory learning discriminates low-pitch sound features such as rhythm of music and prosodic features of speech[11] and fetuses perceive high-pitched sounds in a similar manner that adults recognize speech sounds.[12] Infants exposed to music prenatally have been shown to recognize familiar melodies at 4 months old suggesting that early music exposure can form lasting neural representations in the brain.[13] Preterm music listening has been found to increase immediate sleep duration, enhance physiologic functioning (heart rate and oxygen saturation levels), improve behavioural states and weight gain while decreasing hospital stays and initial weight loss.[14,15] Early music training is associated with enhanced neural speech processing such as pitch changes in speech sounds[16] syllable duration[17] and improved verbal memory skills.[18] Music-based interventions (MBIs) for infants are low-risk[19], minimally-invasive, and low-cost,[20] and thus, an ideal intervention to trial in the neonatal intensive care unit (NICU). The NICU is a necessary but artificial environment created for the survival of preterm infants. It is imperative to carefully select their exposures in this unique environment which overlaps with the timing of the third trimester of pregnancy, a sensitive growth period in which we can have a great impact on immediate and future neurodevelopment.

2.2a Study Rationale – Primary Objective (2)

Thus, we propose a novel approach to study the effects of a longer duration of repeated MBI (6 weeks) by utilizing objective and reproducible measures of electroencephalography (EEG) and event related potentials (ERPs). EEG captures electrical potential oscillations of the brain which yields valuable information about brain function.[25] EEG trends over time reveal brain maturation in preterm infants. Utilizing EEG is a novel approach to studying the biological mechanisms of MBI effects on preterm infants as EEG is objective, captures real-time brain activity, and tracks overall changes in neurodevelopment (Aim 2). Furthermore, ERPs test recognition memory function and cognitive processing of mother vs stranger's voice and offers

another objective measure to further study the early effects of the MBI's biological mechanisms on neurodevelopment of these infants (Aim 2).

Thus, we propose a novel approach to study immediate and lasting effects of MBI on pain responses in the preterm infant utilizing EEG and the standardized Premature Infant Pain Profile (PIPP) (Aim 2). With frequent music intervention throughout the NICU hospitalization (6 weeks), pain responses are likely to adapt and change over time. EEG and PIPP are objective, quantifiable outcomes measures that when performed serially will provide pain response trends revealing the cumulative effects of MBI on pain responses throughout the NICU stay (second clinical primary outcome measure). Evaluation of MBI in preterm infants will provide a deeper understanding of music's influence on behavioral processes of the pain response and offer a low risk treatment alternative to the current pain treatment regimen of opioids, benzodiazepines, and sucrose that are detrimental to neurodevelopment.

Successful completion of studying MBI effects on preterm neurodevelopment using rigorous objective measures of EEG and ERPs provides a deeper understanding of the biological mechanisms underlying music intervention in neurodevelopment and could result in a newly established standard of medical care in the NICU to utilize music intervention in the optimization of preterm neurodevelopment. Evaluation of MBI in preterm pain provides a deeper understanding of music's influence on behavioural processes of pain responses which potentially supports music as a low risk, minimally invasive treatment alternative to the current regimen of opioids, benzodiazepines, and sucrose that have detrimental side effects. Outcomes of this R61 would provide foundational work on music and preterm health which can inform future MBI research in optimizing and tailoring different music based interventions to specific patient populations.

Potential Risks

The planned research procedures include: 1) EEG/ERP, 2) PIPP, and 3) Music based intervention or control. These procedures place participating infants at no more than minimal risk.

EEG/ERP

Risks associated with the EEG and ERP are minimal. The EEG electrode placement via EEG caps could cause minor skin irritation or discomfort. Routine skin care, involving gentle cleaning, will be maintained to prevent these side effects. The ERP data will be gathered using Geodesic Sensor net, which may cause similar mild irritation. The EEG/ERPs will utilize EEG caps and Sensor nets rather than traditional electrodes which further minimizes the risk.

PIPP

The PIPP is an observational tool (pain profile) that doesn't require interaction with the subject. The PIPP is performed when the preterm infant undergoes the heel lance procedure for either the Minnesota Newborn Screen or other required blood tests performed during the same approximate time period within the scheduled protocol timeline. The PIPP should not pose any additional risk to the subject as the heel lance for newborn screening or required blood tests are both standard of care. The PIPP will be performed by two study team members concurrently, with the 2nd person either in-person or virtually through a HIPAA compliant method. This is in order to ensure reliability of data collection. The investigator(s) and study statistician will review any discrepancies for statistical analysis.

Music Based Intervention (MBI)

MBI has been shown to be minimal risk. The MBI in this trial (lullabies) will be played for the infants using headphones placed 1 cm away from the ears to avoid discomfort. During music vs control intervention, a study staff personnel will be actively monitoring the procedure to ensure that cords from the headphones do not interfere with standard of care. The MP3 player has bluetooth capability and should not interfere with routine care. Noise levels are calibrated before every session. Music amplitude will be at no more than 70 dB, which has been shown to be a safe noise level. NICU and study staff will observe for signs of distress during the music therapy, such as crying or finger splay. If these signs continue for more than five minutes, the music therapy will be stopped for that day's session.

Potential risks regarding confidentiality.
There is a potential risk for breach of confidentiality, which will be minimized by the procedures described in the following section.

3. STUDY DESIGN

Pilot randomized, controlled, double blinded music-based intervention (MBI) trial of preterm newborns born at 30 weeks (+/- 2 week) in Neonatal Intensive Care Unit (NICU)

We propose a R61 project to explore the behavioral processes underlying effects of MBI on pain using the premature infant pain profile (PIPP) and EEG. In preterm infants, PIPP scores scale pain responses with painful procedures and central EEG amplitudes change when time-locked to a painful stimulus. The R61 will also explore biological mechanisms of music based intervention (MBI) on preterm brain maturation and neurodevelopment using electroencephalography (EEG) and event related potentials (ERPs). EEG captures electrical potential oscillations of the brain which yields valuable information about brain function. Serial EEGs can track brain maturation in preterm infants. ERPs quantify electrical brain potentials changes time-locked with a stimulus. ERPs at 43-48 corrected gestational

age test recognition memory function and cognitive processing and offers another objective measure to study the early effects of the MBI's on neurodevelopment. Specific recorded lullabies with simple arpeggiated accompaniment will be played for 6 weeks (+/- 1 week) in a small randomized, blinded, controlled study of 60 recruited medically stable 30 week preterms.

Primary Objective - Aim 1: Characterize differences in preterm pain responses between MBI and controls.

Hypothesis/Outcomes:

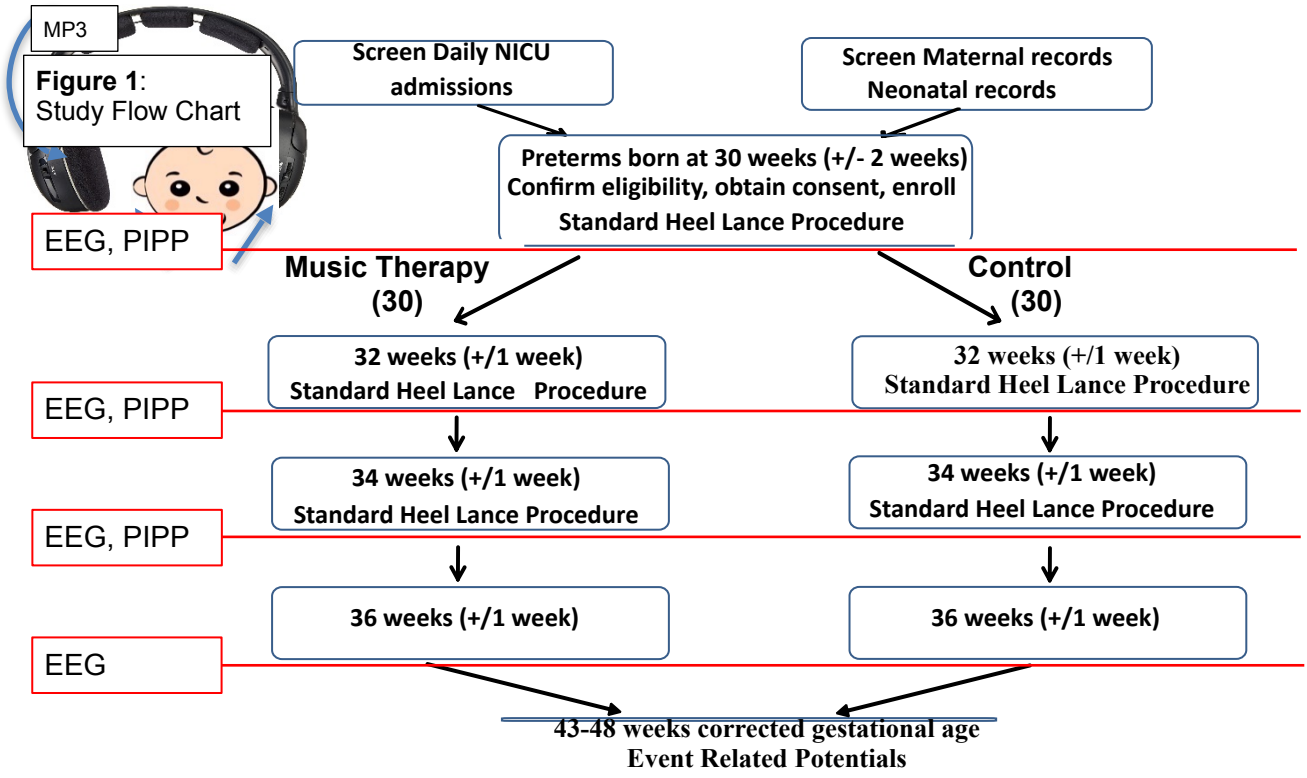
- Hypothesis 1: MBI will show improved pain responses, with lower PIPP scores and attenuated central EEG amplitude changes during painful procedures, in comparison to the control cohort.

Primary Objective – Aim 2: Identify differences between MBI and controls in preterm brain maturation and early neurodevelopment.

Hypothesis/Outcomes:

- Hypothesis 2: MBI cohort will show enhanced preterm EEG maturation when compared to controls.

Hypothesis 3: ERPs at 1 month (43-48 weeks) corrected age will show that MBI has a greater impact on early neurodevelopment when compared to controls.



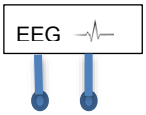
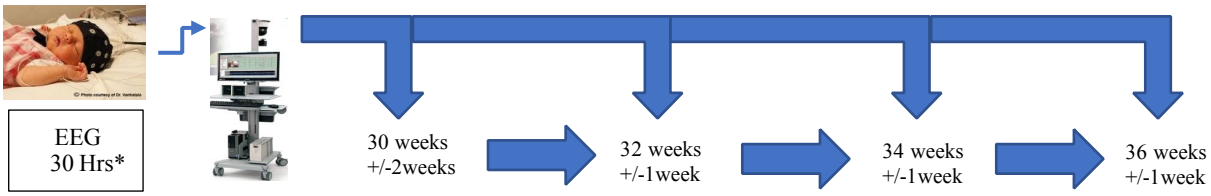


Figure 2: Music Intervention

MBI subjects will receive a total of 1.5 hours of music intervention averaged at 4-5 sessions per week (Figure 2). Music will be alternating: 30 minutes on and 30 minutes off and will be played when the subject is awake to cue pacification and initiate the sleep process. Voice unaccompanied or with single accompanying softly arpeggiated instrument[66] is best for infants. Lullabies meet these criteria and promote language development due to an emphasis on vowels, rising and falling melodic phrases, and the recognition of soothing sounds. Lullabies will all have consistent tempos, with the melody in higher range sung by female or children as infants hear these pitches best.[66] As it is recommended to change song order to increase stimulation, habituation, and neurological development, we will use ten playlists. MP3 players will be loaded in advance with the entire 6 weeks of music. Within each week, order of the playlists will be randomly assigned and prepared in advance by Dr. Eberly. MBI will have minimal interference with routine medical care. Study coordinators will monitor for any distress from music: finger splay, grimace, vital sign disturbance, or persistent crying.[67] If crying lasts longer than 5 minutes, the intervention will be stopped for that day's session. Blinding will be maintained by using identical headphones and MP3 players for all subjects. Headphones will be placed on each side of infant's head such that sound stimuli are received binaurally.[66] Music amplitude will be no more than 70 dB on Scale C.[15,66,68]

Daily electronic volume level checks will avoid the need for listening checks of the headphones. Headphones will be placed 1 cm away from the ears to avoid discomfort from headphone compression. The headphones will either play music or silence for the control group.

Figure 4: Every two weeks, 30 hour EEG* recordings will capture two sessions of background frequencies and changes that occur with Session 1) MBI or Control intervention or Session 2) [MBI + pain] or [Control + pain]



4. SELECTION AND ENROLLMENT OF PARTICIPANTS

4.1 Inclusion Criteria

Participants must meet all of the inclusion criteria to participate in this study:

Preterm infant born at 30 weeks (+/- 2 weeks)

Medically stable

4.2 Exclusion Criteria

All candidates meeting any of the exclusion criteria at baseline will be excluded from study participation:

Treatment for major organ system disease

Significant neurological disorder including, but not limited to, abnormal neurological examination, neonatal abstinence syndrome, intraventricular hemorrhage, seizures, meningitis, or congenital brain malformations

Scalp lesions affecting EEG placement

4.3 Study Enrollment Procedures

Newborns will be recruited from the University of Minnesota Masonic Children's Hospital neonatal intensive care unit (NICU). We will ensure staff in the NICU are aware of the study and post flyers in the NICU to alert neonatology staff to the study's purpose, inclusion criteria, and the pager number needed to contact the study staff regarding potential subjects. The neonatologist will first mention the study to the parents, then ask whether the parents would be willing to further discuss the study with the study investigators. Only parents who express interest in learning about the research study will be approached regarding enrollment of their infant.

The PI and other selected study investigators listed on the IRB will obtain consent. All study investigators who will be obtaining consent will receive training on how to determine eligibility of subjects and how to discuss the study with parents. The study investigator who obtains consent will ensure the parents understand the informed consent process and the requirements of the study. Parents will be informed that they can ask questions about the study at any time and contact information for the study staff will be provided on the consent form.

Once eligibility has been confirmed and consent obtained, subjects will be randomly assigned to the MBI or control treatment cohorts in a 50:50 allocation ratio using randomization tables, stratified by sex and within each stratum use randomly allocated block sizes of 2 and 4, prepared by Dr. Eberly.

5. STUDY INTERVENTIONS

5.1 Interventions, Administration, and Duration

Specific recorded lullabies with simple arpeggiated accompaniment will be played for 6 weeks (+/- 1 week) in a small randomized, blinded, controlled study of 60 recruited medically stable 30 week preterms.

MBI subjects will receive a total of 1.5 hours of music intervention 4-5 sessions per week. Music will be alternating: 30 minutes on and 30 minutes off and will be played when the subject is awake to cue pacification and initiate the sleep process. Voice unaccompanied or with single accompanying

softly arpeggiated instrument is best for infants. Lullabies meet these criteria and promote language development due to an emphasis on vowels, rising and falling melodic phrases, and the recognition of soothing sounds. Lullabies will all have consistent tempos, with the melody in higher range sung by female or children as infants hear these pitches best. As it is recommended to change song order to increase stimulation, habituation, and neurological development, we will use 10 playlists. MP3 players will be loaded in advance with the entire 6 weeks of music. Within each week, order of the playlists will be randomly assigned and prepared in advance by Dr. Eberly. MBI will have minimal interference with routine medical care.

5.2 Handling of Study Interventions

N/A

5.3 Concomitant Interventions

N/A

5.4 Adherence Assessment

Adherence is defined as 80% compliance in this study – average of 4-5 intervention or control sessions per week. If adherence falls below 80%, the Internal QA Reviewer will discuss with the PI methods for improving adherence.

6. STUDY PROCEDURES

6.1 Schedule of Evaluations – Screening and Study Visits

Assessment	Screening	Baseline/ Visit 1	2 weeks (+/- 3 days) Visit 2	4 weeks (+/- 3 days) Visit 3	6 weeks (+/- 3 days) Visit 4	43-48 weeks corrected gestational age Visit 5/ Completion
Informed Consent Form	x					
Randomization	x					
Demographics		x				
Medical History		x				x
Physical Exam		x				
Vital Signs		x	x	x	x	
Baseline Lab Values		x				
Inclusions/Exclusion Criteria		x				
Concomitant Meds		x	x	x	x	x
HADS*		x				
Music Exposure		x				x
Baseline Checklist		x				
On Study Visit Checklist		x	x	x	x	x
EEG		x	x	x	x	
PIPP		x	x	x		
ERP						x
Adverse Events		x	x	x	x	x
SAE		x	x	x	x	x
Family Feedback**					x**	
Study Completion						x

**optional element (parent must provide specific consent to complete this assessment)*

*** Family Feedback may be completed at any point before discharge from the hospital*

6.1a Schedule of Evaluations – Music Based Intervention or Control Sessions

Assessment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
MBI Info Form	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
MBI Info Form	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

6.2 Description of Evaluations

6.2.1 Screening Evaluation

Infants' medical histories will be screened to determine if infant is medically stable (no major organ system illnesses or sepsis) with no abnormal neurological exams, neonatal abstinence syndrome, intraventricular hemorrhage, seizures, meningitis, congenital brain malformations, or scalp lesions affecting the EEG application.

Consenting Procedure

The PI and other selected study investigators listed on the IRB will obtain a single informed consent that describes both the screening and study procedures. All study investigators who will be obtaining consent will receive training on how to determine eligibility of subjects and how to discuss the study with parents. The study investigator who obtains consent will ensure the parents understand the informed consent process and the requirements of the study.

The patient recruitment site will be the University of Minnesota Masonic Children's Hospital neonatal intensive care unit (NICU). Infants born at 30 weeks (+/- 2 weeks) who meet both inclusion and exclusion criteria will be identified by NICU staff. The neonatologist will first mention the study to the parent(s)/guardian(s), then ask whether they would be willing to further discuss the study with the study investigators. Study investigators include: (PI) Sonya Wang, (Music Therapist) Michael Silverman, Raghavendra Rao, and the two study coordinators. The PI, Sonya Wang, will oversee the consent process and make the final determination of the eligibility. Only parents/guardians who consent to hearing about the research study will be approached regarding enrollment of their infant. At the time of recruitment, all procedures, risks, benefits, and the option to withdraw from the study at any time will be explained to the parent(s)/guardian(s) of each participant and written, informed consent will be obtained. The voluntary nature of the study will be emphasized. The consent form will provide a description of the overall purpose of the research, the specific details of the protocol, risks and benefits, costs and payments, confidentiality, contact information for the PI, the posting of information on ClinicalTrials.gov, and the ability to withdraw from participation. At least one parent/guardian will sign the consent form on behalf of their infant. Assent will not be obtained because participants are not capable of providing it given their very young age (preterm newborn infants). A copy of the signed consent form will be kept in the study research files and medical records. The parent(s)/guardian(s) of participants will also be given a copy of their signed consent form. Parents will be informed that they can ask questions about the study at any time and contact information for the study staff will be provided on the consent form.

Process for meeting HHS regulatory requirements for parental permission and child assent

This study meets the requirements for 45 CFR 46.404 – Research not involving greater than minimal risk. Therefore, according to 45 CFR 46.408, the permission of one parent or guardian is sufficient for research to be conducted, pending IRB approval.

Screening

Both male and female potential subjects will be recruited from the patient population admitted to the University of Minnesota Masonic Children's Hospital NICU.

- Gestational age will be determined by mother's last menstrual period, fetal ultrasound measurements, and physical examination characteristics with a variability of +/- 14 days of the targeted 30 week gestational birth age for all subjects.
- Eligible infants must be medically stable (no major organ system illnesses or sepsis) with no abnormal neurological exams, neonatal abstinence syndrome, intraventricular hemorrhage, seizures, meningitis, congenital brain malformations, or scalp lesions affecting the EEG application.

6.2.2 Enrollment, Baseline, and/or Randomization

Enrollment

All infants for whom a parent or legal guardian has signed consent will be considered to be enrolled in the study.

Baseline Assessments/ Visit 1

For participants who have successfully been screened for eligibility and are enrolled into the study, baseline assessments are performed. These include the following:

- Demographics
- Medical History
- Physical Exam
- Vital Signs
- Baseline Lab Values
- Concomitant Meds
- HADS (Hospital Anxiety and Depression Scale) for Moms
    - **optional element (mom must provide specific consent to complete this assessment)*
- Music Exposure during Pregnancy

- EEG
- PIPP
- Adverse Events
- SAE

Randomization

Once eligibility has been confirmed and consent obtained, subjects will be randomly assigned to the MBI or control treatment cohorts in a 50:50 allocation ratio using randomization tables, stratified by sex, prepared by Dr. Eberly. Randomization will occur immediately after screening.

6.2.3 Blinding

Blinding will be maintained by using identical headphones and MP3 players for all subjects. The PI, any personnel involved in collecting the clinical outcomes (e.g., PIPP) or collecting or quantifying EEG outcomes, and the statistician will be blinded. The statistician will be aware of blinded treatment group, so that e.g. safety summaries by blinded treatment group can be prepared for the Data Safety and Monitoring Board (DSMB) closed sessions if it so requests. Should the DSMB request unblinded comparisons, the study team will contract with the University of Minnesota CTSI's Biostatistical Design and Analysis Center (BDAC) for an independent statistician to work with the DSMB using unblinded data.

The unblinded staff includes Michael Silverman (music therapist) and Erin Osterholm (Director of the NICU). Silverman will prepare the music players. Both will be unblinded to handle randomization, delivery of interventions, and preparations of unblinded reports to the independent study monitors. They will not be involved with data monitoring, analyses, or outcome assessments.

The study coordinators who are assessing the PIPP will wear earplugs and noise cancelling headphones, which will ensure that s/he cannot hear what is emanating from the infant's headphones. In addition, family members and NICU staff will be reminded frequently to not disclose what they think they hear coming from the infant's headphones.

6.2.4 Followup Visits: Visits 2-5 (See above table for timing of visits)

EEG: provides real time information about cerebral function by measuring cerebral electrical activity recorded from electrodes placed on the scalp. This EEG includes a video component that will be recording the child during the EEG, in order to assess if any movement was occurring during the EEG.
PIPP: The Premature Infant Pain Profile is a 7-item multidimensional measure of pain that includes 3 behavioral (brow bulge, eye squeeze, nasolabial furrow), 2 physiological (heart rate, oxygen saturation), and 2 contextual

(gestational age and behavioral state) measurements. The measurements are scaled on a 4 point scale (0,1,2,3) with increasing change of each variable from baseline. The 7 items are summed for a total pain intensity score.

Visit 2 and 3:

- Vital Signs
- Concomitant Meds
- On Study Visit Checklist
- EEG
- PIPP
- Adverse Events
- SAE

Visit 4:

- Vital Signs
- Concomitant Meds
- On Study Visit Checklist
- EEG
- Adverse Events
- SAE
- Family Feedback** may be completed at visit 4 or any time before discharge from the hospital

Prior to discharge the study team will review the child's medical chart to collect the clinical BAER score. The BAER stands for the *Brainstem **auditory** evoked response (**BAER**) test* which evaluates a child's hearing and is done as part of clinical care. If the child does not pass while hospitalized, the clinical care team may conduct a follow-up BAER test at their clinical care follow-up appointment. For a child that has a follow-up BAER test, the study team will review the medical chart and collect this information to ensure that hearing is confirmed prior to the child's study ERP, as part of Visit 5/Completion Visit. If the child does not pass this 2nd hearing test, the PI may withdraw the family from the ERP portion of the study visit.

Visit 5/Completion Visit:

- Concomitant Meds
- Medical History
- Music Exposure
- On Study Visit Checklist
- Adverse Events
- SAE
- Study Completion
- ERP: Event related potentials quantify changes in electrical brain potentials timelocked in milliseconds with a stimulus and they are widely used to study cognitive abilities such as discrimination, attention, and memory.

Event related potential (ERP) testing will occur on follow-up at 43-48 weeks corrected gestational age in all subjects to evaluate early neurodevelopment.

6.2.5 Music Based Intervention or Control Sessions

Targeted averaged 4-5 sessions of MBI or Control will be played per week. With each session of intervention, evaluations of vital signs and MBI information will be obtained. Target 6 weeks of MBI or control (+/- 1 week).

- Vital Signs
- MBI Information

7. SAFETY ASSESSMENTS

7.1 Specification of Safety Parameters

Study coordinators will monitor for any distress from music: finger splay, grimace, vital sign disturbance, or persistent crying. If crying lasts longer than 5 minutes, the intervention will be stopped for that session.

7.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

NA

7.3 Adverse Events and Serious Adverse Events

An **adverse event (AE)** is generally defined as any unfavorable and unintended diagnosis, symptom, sign (including an abnormal laboratory finding), syndrome or disease which either occurs during the study, having been absent at baseline, or if present at baseline, appears to worsen. Adverse events are to be recording regardless of their relationship to the

study intervention.

A **serious adverse event (SAE)** is generally defined as any untoward medical occurrence that results in death, is life threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly.

The main adverse effects that may occur during the study are:

Distress resulting from music based intervention and mild skin irritation or discomfort from EEG/ERP electrodes. NICU and research staff will be instructed to observe for signs of distress lasting more than five minutes during music based intervention that do not appear to be associated with other co-occurring interventions or conditions. Signs of distress will include finger splay, grimace, vital sign disturbance, or persistent crying. If these signs of distress last longer than 5 minutes, music based intervention will be stopped for that session and this event will be reported to the PI. Routine skin monitoring and cares will be performed by NICU staff and research coordinators while EEG leads are in place. Care will be taken when placing the brain net for EEG or 128-channel Geodesic Sensor net for ERP to avoid discomfort.

7.4 Reporting Procedures

A core group from the research team consisting of the PI, co-investigators, and statisticians will be responsible for ongoing monitoring of the trial. They will also be responsible for reporting any issues regarding the safety of the study or threats to data integrity to the IRB.

All adverse events will be reported to the PI immediately or within 24 hours. The PI will report adverse events that meet University of Minnesota IRB reporting requirements to the IRB per IRB guidelines. We do not foresee any serious adverse events from participation in this minimal-risk music-based intervention study. For each adverse event (serious or non-serious), the investigator will provide the onset, duration, intensity, treatment, action taken, and outcome. The investigator will determine the relationship of the adverse event to the study-related procedures. Study coordinators will enter safety monitoring and adverse event data into the electronic database. Eligibility verification and data completion will be monitored by the research coordinator and PI.

7.5 Follow-up for Adverse Events

The PI will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each EEG/ERP study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

7.6 Safety Monitoring

This research study involves no greater than minimal risk to participants. All participants will be monitored by the Principal Investigator (PI) and study coordinators.

A Data Safety Monitoring Board (DSMB) has been convened for this study. It is an independent group of experts charged with reviewing study data for subject safety, study conduct and progress, and providing formal recommendations regarding study continuation, modification, and termination.

The University of Minnesota CTSI Independent Monitor(s) will review study materials (documents, records, drug/device accountability, Case Report Forms, etc.) to assure that the study is conducted, recorded, and reported in compliance with FDA Good Clinical Practice.

8. INTERVENTION DISCONTINUATION

Safety findings that would prompt temporary suspension of enrollment and/or study interventions until a complete safety review is convened (either routine or ad hoc) include the following – lasting longer than 5 minutes:

1) Finger splay
2) Grimacing
3) Vital Sign disturbances associated directly with intervention
4) Crying

Should these findings last longer than 5 minutes in duration during intervention, the intervention will be stopped for that intervention session. If that preterm displays these findings >50% of their sessions within any one week (i.e., in 4, 5, or 6 sessions in that week), the intervention will be halted for 3 days and then re-initiated. During the 3 days of intervention halting for that infant, the NICU staff will be consulted on whether they recommend protocol modification for this preterm upon re-initiation. If this happens again (i.e., findings at 4, 5, or 6 sessions within one week) for this preterm after re-initiation, the intervention will be discontinued for that preterm but outcome measures will still be collected for that infant's complete follow-up.

After the first 3 preterms have been enrolled, if 2 or 3 of the 3 enrolled preterms have this temporary halting of the intervention, the DSMB/ CTSI Independent Monitor(s) will be consulted regarding whether the protocol should be modified. After at least 10 infants have been enrolled, if >40% of infants have this temporary halting of the intervention, the DSMB/ CTSI Independent Monitor(s) will be consulted regarding whether the protocol should continue as is, proceed with enhanced monitoring, be further investigated, be discontinued, or be modified and then proceed. Suspension of enrollment (for a particular group, a particular study site or for the entire

study) is a potential outcome of a DSMB/ CTSI Independent Monitor(s) safety review.

Subsequent review of serious, unexpected, and related AEs by the DSMB/ CTSI Independent Monitor(s), IRB, and NCCIH or relevant local regulatory authorities may also result in suspension of further study interventions/ administration of study product at a site. NCCIH retain the authority to suspend additional enrollment and study interventions/administration of study product for the entire study, as applicable.

9. STATISTICAL CONSIDERATIONS

9.1 General Design Issues

The R61 phase of this clinical trial - Effects of Music Based Intervention (MBI) on Pain Response and Neurodevelopment in Preterm Infants – is exploratory and designed to inform planning of the R33 phase.

Primary Objective (1): Characterize differences in preterm pain responses between MBI and controls. Premature infant pain profiles (PIPP) include physiologic, behavioral, and contextual measures which identifies differences in pain responses between MBI and controls while still in the neonatal intensive care unit (NICU). Central EEG amplitude changes have been time-locked with painful procedures in term infants. We will explore if PIPP scores and central EEG amplitudes change with MBI compared to controls.

This primary objective(1) addresses the first hypothesis and the milestone measures (3 and 4) described in Appendix A.

- Hypothesis 1: MBI will show improved pain responses, with lower PIPP scores and attenuated central EEG amplitude changes during painful procedures, in comparison to the control cohort.

  Measure 3) PIPP scores comparing MBI to controls during a painful procedure.

  Measure 4) Central EEG amplitude changes comparing MBI to controls during a painful procedure.

Primary Objective (2): Identify differences between MBI and controls in preterm brain maturation and early neurodevelopment.

EEG is a surrogate marker for real time brain function during sleep-wake cycles. Because preterm brain networks develop during sleep, sleep duration is a strong indicator of brain maturation. Serial biweekly EEGs of preterm infants can quantify sleep duration trends and track MBI's influence on sleep.

Due to the natural limitations of evaluating immature neonatal nervous systems, ERPs have been utilized to study early neurodevelopment. ERPs quantify electrical brain potentials changes time-locked with a stimulus.

Auditory ERPs performed at 43-48 weeks corrected age evaluates attention and discrimination between familiar and novel stimuli - early neurodevelopmental signs of recognition memory function and perceptual learning.

This primary objective(2) addresses the below two hypotheses and the milestone measures (1 and 2) described in Appendix A.

- Hypothesis 2: MBI will enhance preterm EEG brain maturation in comparison to controls.

  Measure 1) Average sleep (REM + non-REM) duration difference between MBI and controls using EEG.

- Hypothesis 3: ERPs at 1 month (43-48 weeks) corrected age will show that MBI has a greater impact on early neurodevelopment when compared to controls.

  Measure 2) Late Slow Wave amplitude difference in auditory ERPs comparing MBI to controls.

9.2 Sample Size and Randomization

The R61 phase is exploratory. Based on the number of eligible infants and assuming 60% of eligible subjects enrollment, we can feasibly enroll 60 infants in 1.5 years.. This sample size is similar to or larger than other MBI studies in infants.[21,23,68,107]

The clinical measure Premature Infant Pain Profile (PIPP) at 4 weeks is the primary outcome for the R61-phase pilot clinical trial. With expected recruitment of at least 60 preterms, expected 50 completed PIPPs at 4 weeks represents a loss to follow-up of (60-50)/60 = ~17%. Such a loss rate is slightly more conservative than previous studies completed in the University of Minnesota NICU (which has had ~15% loss rates). This sample size is similar to or larger than other previous MBI studies in infants.

With 50 completed PIPPs at 4 weeks, for comparing MBI to control (25 per group), there is 80% power to detect a group difference in PIPP of 2.9 and 85% power to detect a group difference in PIPP of 3.1. These calculations assume a between-infant standard deviation of 3.6, estimated from the placebo group of a recent study of sucrose as an analgesic for pain from a standard-of-care heel lance. These calculations were carried out in SAS with the following code:

```
proc power;
twosamplemeans test=diff
      meandiff = .
      stddev = 3.6
      npergroup = 25
```

```
        power = 0.80 0.85
        alpha = 0.05
    ;
    run;
```

The R61 exploratory phase data from primary outcome clinical measure PIPP at 4 weeks will be utilized to assist in planning of the R33 phase. The associations of baseline characteristics (infant, e.g. birthweight, and maternal pregnancy history, etc) may inform the design of the R33 phase. For example, these secondary analyses might indicate that we should stratify randomization in the R33 phase by a dichotomized infant birthweight. These analyses, using linear models or non-parametric alternatives if needed (e.g., measures are highly skewed), will use both intervention groups and we will examine the magnitudes of associations in each group and, if appropriate, in the pooled groups.

Treatment Assignment Procedures

Once eligibility has been confirmed and consent obtained*,* **Randomization** will be accomplished through the use of randomization tables, stratified by sex and within each stratum use randomly allocated block sizes of 2 and 4. These will be prepared by the study statistician (Dr. Eberly).

Blinding will be maintained by using identical headphones and MP3 players for all subjects. The PI, any personnel involved in collecting the clinical outcomes (e.g., PIPP) or collecting or quantifying EEG outcomes, and the statistician will be blinded. The statistician will be aware of blinded treatment group, so that e.g. safety summaries by blinded treatment group can be prepared for the Data Safety and Monitoring Board (DSMB) closed sessions if it so requests. Should the DSMB request unblinded comparisons, the study team will contract with the University of Minnesota CTSI's Biostatistical Design and Analysis Center (BDAC) for an independent statistician to work with the DSMB using unblinded data.

The unblinded staff includes Michael Silverman (music therapist) and Erin Osterholm (Director of the NICU). Silverman will prepare the music players. Both will be unblinded to handle randomization, delivery of interventions, and preparations of unblinded reports to the independent study monitors. They will not be involved with data monitoring, analyses, or outcome assessments.

The study coordinators who are assessing the PIPP will wear earplugs and noise cancelling headphones, which will ensure that s/he cannot hear what is emanating from the infant's headphones. In addition, family members and NICU staff will be reminded frequently to not disclose what they think they hear coming from the infant's headphones.

9.3 Definition of Populations

N/A

9.4 Interim Analyses and Stopping Rules

N/A

9.5 Outcomes: Milestone Measures and Data Analyses

Measure 1: Average sleep (REM+nonREM) duration difference between MBI and controls using EEG. Normal preterm brain development is dependent on cycling between wakefulness and sleep with sleep durations gradually increasing by about 5% increments every two weeks until term age. EEG will be used to measure the average duration of sleep (REM + non-REM) between MBI and controls. Average sleep duration measurements will determine if MBI promotes/enhances brain maturation.

Statistical Analysis: Four quality sleep durations from within each EEG session will be averaged per-infant per-timepoint (one EEG session for each of the 4 age timepoints, 30 weeks, 32 weeks, 34 weeks, 36 weeks). At each timepoint, average sleep duration will be compared between MBI infants and controls. Milestone threshold will be reached if >5% longer average durations of sleep are found in the MBI cohort compared to controls in any 1 or more of the serial EEGs.

Measure 2: Late Slow Wave amplitude difference in auditory ERPs comparing MBI to controls. Due to immature nervous systems, early infant neurodevelopment studies have centered on attentional capture paradigms using ERPs. ERPs quantify changes in electrical brain potentials time-locked to a stimulus and are widely used to study cognitive abilities such as learning, discrimination, attention, and memory. At one month age, the ERP Late Slow Wave of a mom/stranger stimulus is considered a reflection of perceptual learning and memory. ERP Late Slow amplitude measurements will determine if MBI enhances neurodevelopment.

Statistical Analysis: ERP Late Slow Wave amplitudes from the 100 trials of the auditory recognition task will be averaged. Within-infant average Late Slow Wave amplitudes across mother's voice trials minus the same average across stranger's voice trials will be computed and averaged. Mother-vs-stranger Late Slow Wave average difference between MBI and controls will be compared. Milestone threshold will be reached if ≥1 μV greater amplitude difference in ERPs of the MBI cohort compared to controls is found at one month adjusted age.

Measure 3: PIPP scores comparing MBI to controls during a painful procedure. The premature infant pain profile (PIPP) is well established and has undergone extensive psychometric testing. Music creates a sense of

familiarity and security in preterm infants and redirects their attention away from the painful stimulus; thus, PIPP scores will be used to measure if MBI has an effect on pain response in comparison to controls.

Statistical Analysis: The PIPP will be scored at 3 age timepoints (30 weeks, 32 weeks, 34 weeks). At each timepoint, average PIPP among MBI infants will be compared to average PIPP among control infants. Milestone threshold will be reached if MBI cohort PIPP scores are ≥1 point lower than controls at any 1 or more of the scoring points.

Measure 4: Central EEG amplitude changes comparing MBI to controls during a painful procedure. The heel lance painful procedure has been shown to evoke central EEG activity with significantly higher amplitudes, ≥10 μV in infants. EEG will be used to measure amplitude changes in central EEG activity to determine if MBI alters responses to a painful procedure.

Statistical Analysis: Three quantifiable changes in central EEG amplitudes will be collected - one for each age timepoint at which a heel lance is done (at 30, 32, and 34 weeks). Within-infant central EEG amplitude differences between heel sticks and resting states will be computed. Average central EEG amplitude change (heel-stick minus resting-state) among MBI infants will be compared to controls. Milestone threshold is reached if ≥10 μV attenuation in central EEG amplitudes occurs with a painful procedure in the MBI cohort compared to controls in any of the serial EEGs.

9.6 Missing Data

With regards to missing data, the built-in loss rate of 17% includes loss of subjects and also loss due to missing data. Thus, with our recruitment of 60 subjects, we should still be able to reach our 50 subject target completing PIPP data at 4 weeks of MBI.

This pilot clinical trial in the R61 phase is sufficiently small in sample size that imputation methods (e.g., missing week 4 PIPP value or sleep duration) are likely to perform poorly, specifically are likely to add substantial noise to the quantification of the Go/NoGo outcomes. The Go/NoGo quantifications will be computed without imputation and compared to the declared thresholds. Detailed infant and maternal characteristics from baseline assessments will be able to well describe the characteristics of those infants with missing data compared to those with complete data.

10. DATA COLLECTION AND QUALITY ASSURANCE

10.1 Data Collection Forms

NICU-based study activities. PI, Sonya Wang, with the assistance of Co-Investigators, Raghavendra Rao and Lynn Eberly, will be responsible for overseeing study implementation and all NICU-based data collection. Specifically, they will oversee the research coordinators who provide day-to-day supervision of participant informed consent and enrollment, MBI vs control intervention, and outcome assessments including EEGs and PIPPs.

Neurodevelopmental testing. Tracking of study participants after NICU discharge and coordination of follow-up visits will be overseen by PI, Sonya Wang with the assistance of study coordinators. ERP testing will be conducted by Neely Miller at the Center for Neurobehavioral Development. Bayley's III Neurodevelopmental testing will be conducted by the psychometrician at the Center for Neurobehavioral Development.

Data coordination and management will involve design of data collection forms in REDCap for NICU and follow-up data. These will be managed by the study coordinators with PI, Sonya Wang, and Co-Investigator, Lynn Eberly, oversight. REDCap data analysis will be conducted by PI, Sonya Wang, Co-Investigators, Lynn Eberly, and Biostatistician, Qi Wang. EEG data will be stored in password protected encrypted hard drives by study coordinators. EEG data will be managed by study coordinators and PI, Sonya Wang. EEG data analysis will be conducted by PI, Sonya Wang, and Co-Investigators, Theoden Netoff, Lynn Eberly, and the biomedical engineering graduate student.

Data collected specifically for this study will be for clinical trial research purposes only and will include clinical data regarding the subject's medical history and hospital course including clinical characteristics such as age, daily weight including birthweight, race and any underlying neonatal, perinatal and maternal health conditions. Most clinical data will be obtained from subjects' medical records. In addition, EEG, PIPP, ERP, and neurodevelopmental assessment data will be collected. Study staff will have access to individually identifiable information about the infants enrolled in the study. Data will be collected by the study coordinator and recorded onto case report forms (CRFs). Data from the CRFs will subsequently be entered into the research database located on the encrypted study laptops. The CRFs and the electronic database will only have de- identified information to preserve confidentiality of patients' protected health information. Files that link the study identification number for each subject to the subject's personal information (such as name, medical record number, parents' names) will filed separately and only be accessible to the PI and study coordinators.

10.2 Data Management

See section 10.1 above.

10.3 Quality Assurance

10.3.1 Training

The PI will ensure all staff are qualified for their study roles and trained on the protocol.

10.3.2 Quality Control Committee

This study will also be monitored semi- annually via independent monitoring services from the Clinical and Translational Science Institute (CTSI) at the University of Minnesota.

CTSI Independent Monitor(s) review study materials (documents, records, Case Report Forms, etc) to assure that the study is conducted, recorded, and reported in compliance with FDA Good Clinical Practice.

10.3.3 Metrics

CTSI Independent Monitor(s) will also ensure that the study is conducted in accordance with the protocol and inclusion/exclusion criteria as approved by the IRB. The goal is to promote and facilitate compliance with Good Clinical Practice through:

- Regular monitoring visits
- Quality assurance
- Data query resolution
- Review of study regulatory files
- Adverse Event/Serious Adverse Event (AE/SAE) reviews
- Compliance consultation services
- Typical review of subject specific documents includes but is not limited to:
  - Signed informed consent/HIPAA documents
  - Case Report Forms (CRFs)
  - Medical records (for AE/SAE)
  - Regulatory binders
  - Communications with FDA/IRB

10.3.4 Protocol Deviations

Protocol deviations will be documented in the study file and those that meet IRB reporting criteria will be reported accordingly.

10.3.5 Monitoring

This research study involves no greater than minimal risk to participants. See above 10.3.2 for above details of monitoring.

11. PARTICIPANT RIGHTS AND CONFIDENTIALITY

11.1 Institutional Review Board (IRB) Review

The IRB at the University of Minnesota is a fully authorized Institutional Review Board that provides oversight to research conducted at the university. It functions in compliance with the congressional statutes governing Assurance of Compliance with Health and Human Services (HHS) Regulations for Protection of Human Research Subjects. This board will be providing oversight to the current study.

11.2 Informed Consent Forms

The PI and other selected study investigators listed on the IRB will obtain consent. All study investigators who will be obtaining consent will receive training on how to determine eligibility of subjects and how to discuss the study with parents. The study investigator who obtains consent will ensure the parents understand the informed consent process and the requirements of the study. Parents will be informed that they can ask questions about the study at any time and contact information for the study staff will be provided on the consent form.

11.3 Participant Confidentiality

Every effort will be made during this study to ensure confidentiality. Only authorized study personnel and others authorized for regulatory purposes as described in the consent form will have access to this information. Study data will be de-identified and entered by the study coordinators into a password protected, encrypted electronic database for storage, retrieval, and analysis. EEG files will be de-identified and recorded onto a password protected, encrypted computer workstation. Each EEG file will be downloaded onto an encrypted hard drive with one extra backup copy. The EEG files will include video of the child and those who may be caring for the child during the EEG, such as a parent or staff member. There is also sound captured during the EEG but this is not listened to by the investigator. All confidentiality processes, as outlined above, will be followed for all video recordings. In all records, presentations, and manuscripts to be made public, the videos taken during the EEG will be de-identified so not to show the infant's face.

Families will be approached by research staff in private settings e.g. the mother's or infant's private hospital room. All information and data collected for the sole purpose of this study will be kept confidential. Only authorized study personnel and others authorized for regulatory purposes as described in the consent form will have access to this information. Medical information collected during this study will become part of the subject's hospital record, if the information is determined to be pertinent to the subject's medical care or is a usual part of the subject's care (e.g., results of tests ordered by treating clinicians that are also recorded for research purposes). Medical records are available to other health care professionals at the hospital and may be

reviewed by hospital staff in their course of carrying out their responsibilities. However, they are required to maintain confidentiality in accordance with applicable laws and hospital policies. Study information that is not included in the medical record and could be personally identifiable will be under restricted access and viewable only by members of the research team (PI and study coordinators). These research records will not be made available to anyone not on the research team except upon a parent's request or as required by law. Deidentified EEG data will be recorded onto a password protected, encrypted computer workstation. Each EEG file will be downloaded onto an encrypted hard drive with one extra backup copy. Data from various domains will be entered by the study coordinators into an electronic database for storage, retrieval, and analysis. Additional measures needed to ensure restricted access and confidentiality will be considered and implemented as deemed necessary. In all records, presentations, and manuscripts to be made public, information or data will not be personally identifiable. In the case that data or references to specific study patients are used, the patients will only be identified by code in order to maintain confidentiality.

11.4 Study Discontinuation

The study may be discontinued at any time by the IRB, the NCCIH, the OHRP, the FDA, or other government agencies as part of their duties to ensure that research participants are protected.

12. COMMITTEES

N/A.

13. PUBLICATION OF RESEARCH FINDINGS

We will ensure that this trial "Effects of Music Based Intervention (MBI) on Neurodevelopment and Pain Response in Preterm Infants**"** is appropriately registered and results information is submitted to ClinicalTrials.gov, as outlined in the NIH policy and according to the specific timelines stated in the policy. Any presentation, abstract, or manuscript will be made available for review by the sponsor and the NCCIH prior to submission.

14. REFERENCES

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15. SUPPLEMENTS/APPENDICES