Recorded Maternal Song to Reduce Crying Duration in Neonatal Abstinence Syndrome

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RECORDED MATERNAL SONG TO REDUCE CRYING DURATION IN NEONATAL ABSTINENCE SYNDROME

A Thesis Presented to
The Faculty of the School of Medicine
Yale University

In Candidacy for the degree of
Master of Medical Science

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ABSTRACT

Neonatal abstinence syndrome is a constellation of symptoms that include high pitched crying and irritability resulting from abrupt postnatal cessation of opioid exposure. Maternal recorded song reduces crying duration in preterm and full-term infants. However, the effect of maternal recorded song on crying duration in affected infants is unknown. In this prospective single-blind randomized control trial, we compare the effect of maternal recorded song and ambient noise in reducing crying duration in infants with neonatal abstinence syndrome. We hypothesize that exposure to maternal recorded song will result in a significant difference in mean duration of crying compared to ambient noise in neonates with neonatal abstinence syndrome. The use of maternal recorded song may reduce neonatal crying among all infants. Ultimately, this study will contribute to non-pharmacological management of infants with neonatal abstinence syndrome.
CHAPTER ONE: INTRODUCTION

1.1 BACKGROUND

1.1.1 The Burden of Neonatal Abstinence Syndrome

Maternal substance use during pregnancy can result in infant withdrawal after birth. Neonatal opioid withdrawal syndrome refers to the constellation of symptoms resulting from abrupt postnatal cessation of opioid use. More broadly, these signs are classified as neonatal abstinence syndrome (NAS) which includes substance exposure to stimulants, benzodiazepines and antidepressant medications in utero. Perinatally, affected infants face short term consequences of withdrawal including irritability and inconsolability which challenge parents and increase their long-term risk of abuse.

The incidence of NAS has risen in parallel with opioid use such that in the United States, it is estimated that every 15 minutes an infant is born with NAS. In particular, between 2000 and 2012 the incidence of NAS rose five-fold with the most recent reported incidence to be 6.7 per 1000 hospital births. Over the same period, aggregate hospital charges for treatment of NAS rose from $190 million to $1.5 billion largely due to an increase in pharmacotherapy use leading to average treatment charges of $44,720. Additionally, infants with NAS experience long hospital stays ranging between 6 and 79 days compared to the typical 11 day stay of unaffected infants. In addition, infants with NAS are twice as likely to be readmitted compared to healthy neonates. Ultimately, the cost of pharmacotherapy, extended hospital stays, and rising incidence explain the extraordinary aggregate charges of NAS.

Nonetheless, the burden of NAS is not equal among populations and geographic regions. American Indians and Alaskan populations have the highest racial incidence of
15.9 per 1000 hospital births followed by non-Hispanic whites with an incidence of 10.5 per 1000 hospital births\(^5\). Incidence also varies by geographic region with the highest incidence reported in regions with the highest rates of opioid prescription. In particular, Kentucky, Tennessee, Mississippi and Alabama, report the highest national incidence of NAS of 16.2 per 1000 hospital births with New England reporting the second highest incidence of 13.7 per 1000 births. Across all regions, the incidence of NAS is highest among those in the lowest quartile of income\(^4,5\) with 89% of infants with NAS utilizing public insurance\(^6\).

**1.1.2 Clinical Presentation**

Infants with NAS show signs of neurologic excitability and gastrointestinal dysfunction which reflect a high concentration of mu opioid receptors within the central nervous system and gastrointestinal tract respectively. Characteristic signs of neurologic excitability in NAS include tremor, irritability, increased muscle tone, seizure and disrupted sleep\(^1\). However, the hallmark sign of NAS is high-pitched continuous crying which can be classified as excessive and inconsolable in turn overwhelming parents and caregivers\(^1\). Gastrointestinal dysfunction presents with poor digestion, feeding and weight gain whereas increased autonomic activity includes sweating, temperature instability and increased respiratory rate\(^1,11\). Severity of infant withdrawal and clinical presentation vary depending upon the specific opioid of exposure, dose of opioid or opioid replacement therapy, polysubstance abuse, tobacco exposure, prematurity and breastfeeding\(^12,13\).

The clinical onset of signs of NAS vary in timing in accordance with the substance of exposure. Signs of withdrawal most commonly present within 24 to 72 hours of birth for short acting opioids like methadone\(^12,14\). However, signs can develop
within the first 24 hours after heroin exposure\textsuperscript{1} or later between 40 and 70 hours in the case of buprenorphine exposure\textsuperscript{15}. Recent studies suggest that infants exposed to buprenorphine may experience a less severe withdrawal compared to methadone exposed infants evident through a shorter treatment duration and reduction in pharmacotherapy use\textsuperscript{16-18}. Given the variability in timing of presentation, the American Academy of Pediatrics (AAP) recommends observing an infant for signs of withdrawal for at least 3 days in the case of exposure to opioids with a short half-life and for 5 to 7 days for opioids with a long half-life\textsuperscript{1}.

\textit{1.1.3 Screening and Diagnosis}

Identification of infants exposed to substance use in utero can be difficult and involves a combination of maternal interview and toxicology screening\textsuperscript{19}. Most hospitals utilize a risk-based screening approach\textsuperscript{20,21} which relies on patient interviews to identify infants at risk of NAS requiring confirmation by biologic toxicology screening. Findings warranting further screening include suspicion of drug use, insufficient prenatal care, placental abruption, admission from a justice center and maternal sexually transmitted diseases including HIV, hepatitis B and hepatitis C\textsuperscript{22}. These criteria are based on evidence that pregnant women with substance use disorder seek prenatal care later in pregnancy, frequently miss appointments and have poor weight gain in pregnancy\textsuperscript{23}. Maternal interview also identifies pregnant women with substance use disorder requiring referral to methadone or buprenorphine treatment during pregnancy\textsuperscript{23}. Nonetheless, maternal self-reporting alone is unreliable because mothers face guilt and shame as well as fear of the child welfare system and the legal system leading to under reporting\textsuperscript{23-25}. Contributing to this fear are racial disparities in screening and reporting as black infants
are four times more likely than white infants to be reported to child protective services\textsuperscript{26}. Thus, universal toxicology screening identifies infants at risk of NAS where maternal reporting fails\textsuperscript{22}.

Most commonly, neonatal urine is tested because it is accessible and offers the quickest result time\textsuperscript{1,25}. However, this method is limited to detecting maternal substance use within 24 to 72 hours prior to delivery resulting in false negative results with antenatal abstinence. Alternatively, meconium is considered the specimen of choice in detecting substance exposure in utero due to its high sensitivity and window of detection beginning from twelve weeks of gestation. Thus, meconium testing is helpful when there is high clinical suspicion despite a negative urine screen\textsuperscript{11,27}, however infants are often discharged prior to receiving the results of analysis due to a longer testing process\textsuperscript{25,28,29}. Further, meconium collection is challenging as it must be collected prior to the infant’s first feeding, it is contaminated by urine, and it is not collectable if passed in utero\textsuperscript{25,27}. Alternative screening samples include neonatal hair\textsuperscript{30} and umbilical cord tissue\textsuperscript{31} but are used less frequently\textsuperscript{25}.

1.1.4 Assessment and Criteria for Initiation of Pharmacotherapy

A variety of scoring tools can be used to assess severity of infant withdrawal and guide treatment of NAS. Current recommendations from the AAP include utilizing a standardized scoring system to assess neonatal withdrawal, however, the guidelines do not establish a single assessment tool as superior\textsuperscript{11}. Infants with NAS are most commonly assessed using the Finnegan Neonatal Abstinence Scoring System (FNASS)\textsuperscript{21,32}, however other available assessment tools include the Lipsitz tool\textsuperscript{33}, the Neonatal Withdrawal Inventory\textsuperscript{34} and the MOTHER NAS scale\textsuperscript{16}. The FNASS assesses for the presence of
twenty-one signs of withdrawal and guides initiation and weaning of pharmacotherapy treatment according to withdrawal severity\(^1\). FNASS scores are recorded every two to six hours and pharmacologic therapy is initiated after three consecutive scores greater than eight or two consecutive scores greater than twelve. Notably, this scoring system leads to delays in pharmacotherapy while documenting the required FNASS scores. This system also utilizes a long weaning process resulting in an average length of stay of three weeks for infants assessed by FNASS\(^2,35\). Finally, Finnegan scoring includes disturbing the infant to assess for the Moro reflex and tremors which directly contradicts non-pharmacologic care for these infants\(^35\). Recently, an alternative scoring tool entitled Eat, Sleep, Console (ESC) was developed to guide management of NAS. An infant is assessed on its ability to eat at least one ounce per feed or breastfeed well, to sleep undisturbed for at least one hour and to be consoled within ten minutes from crying onset\(^36\). If an infant fails to meet the pillars, then non-pharmacological interventions are optimized as primary therapy which includes offering a non-stimulatory environment with soothing techniques and ensuring adequate feeding. If primary therapy fails, single doses of pharmacotherapy are initiated on an as needed basis. This approach has shown a reduction in frequency and dose of pharmacotherapy\(^36-38\) as well as a reduction in length of stay by ten days\(^36\).

1.1.5 Non-Pharmacologic Interventions

Non-pharmacologic therapy is the cornerstone in treatment of NAS and is essential to promote adequate sleep, weight gain and social integration\(^1\). However, current literature surrounding non-pharmacologic interventions is limited and despite optimization of studied interventions, infants with NAS continue to cry inconsolably. Broadly, non-pharmacologic care can be classified into domains including soothing
techniques, social integration, environmental control and feeding methods. Soothing techniques reduce overstimulation which exacerbates signs of NAS. These include swaddling and holding, opportunities for non-nutritive sucking and skin-to-skin contact. Specifically, swaddling prolongs sleep which decreases arousal, distress and crying in infants. Social integration of the mother-infant dyad through rooming-in strengthens maternal-infant bonding through breastfeeding, cuddling and skin to skin contact while reducing length of stay and cost of hospitalization. Rooming-in may also strengthen maternal confidence in managing difficult behaviors specific to infants with NAS. In situations when the mother is absent, hospital volunteer cuddlers may also console and soothe infants with similar benefits.

Environmental control includes minimizing auditory and visual environmental stimuli in a quiet, dim lit environment. Infants with NAS are cared for in multiple settings with varying levels of stimulation including neonatal intensive care units (NICU), pediatric in-patient units and post-natal wards. Thus, it is imperative to carefully consider the environment in which an infant is cared for in order to provide an appropriately low stimulation environment. Most commonly, infants are cared for in the highly stimulating NICU which is associated with more frequent pharmacotherapy use compared to floor management. Alternatively, postnatal ward and pediatric in-patient unit management may reduce length of stay and treatment costs compared to NICU care. Since pediatric units offer rooming-in and low stimulation environments, they may be a more appropriate location of care for infants with NAS.

Non-pharmacologic feeding methods involve optimizing feeding and nutrition given the rapid metabolism of infants with NAS. Specifically, small volume frequent
feedings minimize stress and dysregulation and prevent weight loss associated with regurgitation and loose stools\(^1,47\). Additionally, hypercaloric formula may support weight gain despite gastrointestinal dysfunction\(^1\). Current guidelines support breast feeding among mothers undergoing methadone treatment\(^48\) as breastfeeding may reduce severity of withdrawal\(^49,50\). Finally, alternative studied non-pharmacologic interventions for infants with NAS include aromatherapy\(^51\), laser acupuncture\(^52\), acupressure\(^53\) and reiki therapy\(^54\).

Despite the described non-pharmacologic interventions, infants with NAS may continue to cry inconsolably highlighting the need for additional alternative measures to comfort affected infants potentially through maternal voice and song. Importantly, non-pharmacologic care utilized in other infant populations is not well studied in infants with NAS. Thus, sound interventions such as white noise, maternal voice and maternal recorded song may have a role in soothing and caring for affected infants.

### 1.1.6 Pharmacologic Interventions

Despite optimization of non-pharmacologic therapy, many infants still require pharmacotherapy to ameliorate signs of severe withdrawal\(^1\) and prevent complications including poor sleep, seizures, dehydration and weight loss\(^11,55\). Unfortunately, pharmacotherapy requires a gradual weaning process which often extends an infant’s length of stay\(^1\). Opioids are recommended as the first line medication for NAS which include morphine, methadone and buprenorphine. Although the AAP does not recommend any opioid as superior, morphine is most commonly used\(^7,11,18\). Some studies suggest that the course of withdrawal may vary depending upon management with buprenorphine, methadone or morphine\(^56,57\). If opioid treatment alone fails, sedative
medications can be utilized\textsuperscript{1, 58}; however phenobarbital carries a risk of neurotoxicity and developmental delays\textsuperscript{11, 32, 59}. Regardless of the pharmacotherapeutic agent used, a stringent protocol-based weaning reduces the duration of opioid treatment and hospital stay compared to non-protocol-based weaning\textsuperscript{60}.

1.1.7 Long Term Consequences and Care

Studies examining the long-term outcomes of affected infants are limited by loss to follow up due to high rates of stress and poverty, dysfunctional or inconsistent caregiving and family instability common among infants with NAS\textsuperscript{1, 35, 61}. Limited research on physical outcomes reveals a risk of reduced visual acuity\textsuperscript{62}, delayed motor skills and attention deficits including memory, hyperactivity and impulsivity\textsuperscript{63}. Notably, by 12 to 18 months, affected infants exhibit deficits in mental and psychomotor development compared with their non-substance exposed, matched counterparts\textsuperscript{64}. By age three, infants with NAS exhibit impaired and delayed language and cognition\textsuperscript{65} which may account for the higher utilization of special education services\textsuperscript{66}. In high school, substance-exposed infants score worse in reading, writing, grammar, spelling and numeracy than non-substance exposed peers, worsening school performance overall\textsuperscript{67}.

Maternal substance abuse also creates a challenging environment for infant development postnatally. In particular, mothers who used buprenorphine score higher on scales of abuse and belief in corporal punishment while scoring lower on empathy and appropriate parenting skills\textsuperscript{68}. Parental substance abuse increases an infant’s risk of abuse twofold compared to non-substance exposed infants\textsuperscript{69, 70}. In addition, substance using mothers often have a personal history of abuse thus increasing the likelihood of abusing their own infant\textsuperscript{71}. Evidently, treatment of infants with NAS should also include
supporting maternal mental health through referral to mental health providers\cite{1,68}. In addition, clinicians should educate parents on challenging symptoms of withdrawal including inconsolable crying which can be overwhelming\cite{11}. Mothers should also learn strategies to console infants thus increasing maternal confidence and preparation to safely care for her infant at home\cite{11}. Importantly, parental perception of excessive crying durations places an infant at risk of abuse and non-accidental trauma\cite{72}. Thus, interventions aimed at soothing infants and reducing crying durations may reduce maternal stress and abuse in infants with NAS.

1.1.8 Maternal Recorded Song

Inconsolable crying is a hallmark of NAS which has deleterious effects on infants and their caregivers. While soothing techniques, environmental control and optimized feeding help to alleviate the burden of withdrawal, non-pharmacologic care may fail to adequately console affected infants demanding utilization of alternative non-pharmacologic measures. Maternal recorded song has been used in other infant populations to reduce daily crying duration both in the hospital and throughout infancy in premature and full-term infants\cite{73-76}. In premature infants, maternal song promotes physiologic stability\cite{77,78}, strengthens mother-infant bonding\cite{79} and reduces maternal stress\cite{80}. Despite the widespread use of lullaby to quiet infants, literature surrounding recorded maternal song in infants with NAS is limited. One study reported that maternal recorded song delivered through pacifier may reduce severity of NAS withdrawal\cite{76}. Another study of recorded music reported a trend toward reduced crying in infants with NAS\cite{75}. These studies suggest that maternal recorded song may be an inexpensive and readily available non-pharmacologic adjunct to console excessively crying infants with
NAS both immediately in the hospital and long term at home. Understanding recent literature surrounding music in other infant populations, we propose that recorded maternal song will reduce crying duration in infants with NAS.

1.2. STATEMENT OF THE PROBLEM

Initial treatment for NAS includes non-pharmacologic supportive measures focused on minimizing environmental stimuli and maximizing maternal comforting techniques including swaying, swaddling and skin to skin contact\textsuperscript{1,11}. Still, many mothers report feelings of guilt, shame and judgement which limit how frequently they visit their infant in the NICU\textsuperscript{81}. Thus, the stigma of NAS can limit maternal care of her potentially irritable, hyper arousable and inconsolably crying infant in turn threatening mother-infant bonding and increasing healthcare resource utilization\textsuperscript{11}. Evidently, additional means to soothe affected infants are needed to limit infant crying and reduce the burden of care on healthcare staff and mothers alike. Maternal recorded song may be a feasible and accessible method to soothe crying infants during periods of maternal absence.

Literature regarding non-pharmacologic therapy for NAS is predominantly comprised of quality improvement studies using the Eat, Sleep, Console approach, rooming in and on-demand small volume feedings\textsuperscript{1,36,42,43}. There is a paucity of randomized controlled trials studying non-pharmacologic care in infants with NAS and given the variability in NAS care between institutions\textsuperscript{20}, the generalizability of existing literature is unknown. Further, only two studies have evaluated the use of maternal recorded song in infants with NAS\textsuperscript{75,76} highlighting a lack robust evidence supporting music intervention. Further studies examining the role of maternal recorded song in infants with NAS are needed in order to offer an inexpensive and widely available adjunct to non-pharmacologic care to
soothe and console infants with NAS while preventing long term consequences of crying such as abuse.

1.3 GOALS AND OBJECTIVES

In this randomized controlled trial we aim to investigate the use of recorded maternal song as a non-pharmacologic intervention to treat infants with NAS. We will accomplish this by comparing crying durations over seventy-two hours between infants exposed to recorded maternal song and ambient hospital noise. Our objective is to demonstrate a significant reduction in crying duration as a result of recorded maternal song in affected infants. Ultimately, our goal is to determine if there is a role for maternal recorded song as an adjunct in non-pharmacologic therapy of infants with NAS.

All infants with NAS meeting eligibility criteria with maternal consent will be enrolled in the study. Participants will be randomly assigned to either ambient hospital noise or recorded maternal song intervention during hospitalization. The primary objective is to determine differences in crying duration, scored from voice activated recordings, between the two groups. Secondary outcomes include total longest crying duration, number of crying episodes, average length of crying episode and diurnal distribution of crying. Additional non-crying secondary outcomes include heart rate, respiratory rate and oxygen saturation measured by pulse oximetry as well as pharmacotherapy use, frequency of pharmacotherapy, average calories consumed daily and weight gain which serve as proxies of withdrawal severity.

1.4 HYPOTHESIS

Neonates greater than 35 weeks and 2000 grams diagnosed with neonatal abstinence syndrome without medical conditions requiring admission to the neonatal intensive care
unit who are exposed to recorded maternal song for thirty minutes after feeding daily will have a statistically significant difference in seventy-two hour crying duration scored from voice activated audio recording compared to infants exposed to ambient hospital noise after feeding.

1.5 REFERENCES


CHAPTER TWO: REVIEW OF THE LITERATURE

2.1 INTRODUCTION

A search of relevant medical literature was conducted between June 2020 and June 2021 using OVID Medline, Cochrane, Scopus, Pubmed and CINAHL. Primary systematic searches were conducted using combinations of MeSH terms including “neonatal abstinence syndrome”, “substance exposed”, “mothers”, “singing”, “music” and “music therapy”. Additional search terms included neonatal opioid withdrawal syndrome, maternal song, lullaby, maternal recorded song, crying, breast feeding, breast fed, swaddle, swaddling, kangaroo care, skin to skin contact, crying duration and crying amount. The search was limited to articles studying infant human subjects published in English. Article titles, abstracts and reference lists of primary articles were reviewed for topic relevance to our study. Clinical trials, systematic reviews and meta-analyses were prioritized while observational case studies were excluded. Experimental trials evaluating crying duration after a procedure were excluded. The literature search and review demonstrates diversity of types of music interventions among infants. Still, the efficacy of music to calm infants with NAS remains largely unstudied.

2.2 REVIEW OF EMPIRICAL STUDIES

2.2.1 Novel Non-Pharmacologic Therapy for Infants with NAS

Since non-pharmacologic care is considered the preferred treatment of NAS, recent literature has examined how to optimize these interventions to best care for affected infants. Rooming-in brings together mothers and infants immediately after birth which increases opportunities for maternal care and on-demand breast feeding. A retrospective cohort study in British Columbia evaluated the severity of NAS among
infants who roomed-in compared to both a historical cohort at the same hospital and a concurrent cohort managed at a nearby community hospital without rooming-in\(^1\). In this study, rooming-in reduced NAS treatment compared to both the historical cohort (95% CI 0.20-0.78) and the concurrent cohort (95% CI 0.20-0.75). Further, rooming-in was associated with a shorter hospital stay (p=0.01 historical cohort, p=0.0001 concurrent cohort) and a higher incidence of breast feeding (62.5%) compared to the historical and concurrent cohorts (7.9% and 11.1% respectively). Notably, cohorts varied in race, familial support and maternal smoking habits at baseline thus limiting validity of results. Still, this study suggests that rooming-in may be associated with less severe withdrawal and higher breast-feeding rates in infants\(^1\). Another retrospective rooming-in study of infants with NAS demonstrated a reduction in morphine use among breast fed infants compared to formula fed infants (OR 0.21 ± 1.44, p < 0.001)\(^2\). Evidently, rooming-in may be associated with decreased withdrawal severity, increased breast feeding and reduced utilization of pharmacotherapy\(^1,2\).

Similarly, a quality improvement study implementing rooming-in, on demand feeding and skin to skin care among infants with NAS demonstrated a reduction in pharmacotherapy use, average hospital charge and length of stay (16.9 days to 12.3 days) among infants that roomed-in. Notably, there were no reported adverse events or changes in readmission rates (p = 0.46). Still, the study population had a high incidence of buprenorphine exposure which may limit generalizability of results to all substance exposed infants. Additionally, the study failed to report values of statistical significance thus limiting validity\(^3\). Another quality improvement study at Yale New Haven Children’s Hospital (YNHCH) emphasized low stimulation environments, parent
engagement through rooming-in and breastfeeding among infants managed according to ESC assessment. In this study, rooming-in reduced the length of stay by 74% (p < 0.001), morphine use (p < 0.001), hospital charges (p < 0.001) and increased breastfeeding rates 25% (p = 0.01). Again, there was no increase in 30-day readmission rates, ICU transfers or seizures. A limitation of this study is the lack of recorded maternal time with infants thus inhibiting a determination of a dose response of rooming-in. Other retrospective analyses have demonstrated a reduction in duration of therapy (IQR 24-38.5 days with rooming-in, IQR 25-54.4 days without rooming-in) and length of stay among infants who roomed-in (IQR 28-48 days with rooming-in, IQR 30.3-54.5 days without rooming-in). Yet another rooming-in study demonstrated reduced pharmacotherapy use (p < 0.001) and average length of stay (p < 0.001). Notably, maternal surveys reveal a preference for rooming-in as well as more frequent breastfeeding.

Another key non-pharmacologic intervention among infants with NAS is breastfeeding. A retrospective cohort study demonstrated a reduction in Finnegan scores (p < 0.05) and pharmacotherapy use (p < 0.001) as well as a delayed onset of withdrawal (p < 0.01) in breast-fed infants with NAS compared to formula fed infants. In this study, formula fed infants faced more challenges as they were more commonly exposed to polydrug use (p = 0.001), received less prenatal care and were more often premature compared to breast fed infants. Similarly, another retrospective study of methadone exposed infants revealed that breast fed infants had fewer FNAS scores (p=0.001) and lower mean FNAS scores (p = 0.0001) compared to both combination and formula fed infants suggesting that breastfeeding may be associated with a less severe withdrawal. This study was limited as it did not control for cigarette smoking, a known confounder of
NAS. Together, these studies provide compelling evidence to support breastfeeding as an essential non-pharmacological intervention that should be utilized in conjunction with rooming-in among infants with NAS.

2.2.2 Effects of Music Therapy on Infants

Studied in a variety of infant populations, music therapy may be an important non-pharmacologic intervention for affected infants. Music therapy entrains music to an infant’s physiological and behavioral state which can be used to alter infant behaviors and promote stability. The Rhythm, Breath Lullaby (RBL) intervention employs three musical interventions including the ocean disc instrument which mimics sounds of intrauterine fluid to support respiration and sleep in infants. The Gato Box instrument mimics a mother’s heartbeat, thus soothing infants and slowing heart rates. Finally, the Song of Kin is a parent selected, culturally important lullaby that cues sleep. Music therapy can be delivered either by a certified music therapist or by a trained parent.

Recently, two studies have examined the effects of music therapy among infants with NAS. One randomized controlled trial (RCT) examined the effects of recorded sedative music or lullaby (RSM), multimodal stimulation which included live parental signing (MMS) or combination RSM and MMS on infants with NAS. Although the study demonstrated no significant effects on FNASS score, it is novel as it is the first study to compare the effects of live and recorded music on infants with NAS and included both preterm and full-term infants. Still, the study is limited by a small sample size and disproportionate lengths of withdrawal between groups suggesting that future studies should either stratify infants according to substance of exposure or ensure successful randomization. The study reported a trend toward reduced crying in RSM and
RSM/MMS groups, increased sleep in the MMS group and reduced FNAS in the central nervous system component, which includes crying, among all treatment groups. The study fails to report any statistical analysis thus limiting the validity of reported trends.

A second quality improvement study examined the use of Pacifier Activated Lullaby (PAL) among 20 full term infants with NAS admitted to the NICU for treatment. The PAL intervention plays music when infants demonstrate strong non-nutritive suck which reinforces sucking and feeding development in infants. The study demonstrated a reduction in FNAS score of infants using PAL compared to infants using a standard pacifier (p = 0.05) suggesting that PAL may reduce severity of NAS withdrawal. This study is limited in its design as a quality improvement project and small sample size. Further, the study did not account for the length of time an infant was held by family and infants were not stratified according to substance of exposure which can affect the length of withdrawal. Overall, these two studies support a soothing role of music therapy in infants with NAS.

Although evidence surrounding music therapy for infants with NAS is limited, there is robust evidence to support its beneficial effects among other infant populations. A crossover RCT studied the effects of weekly RBL intervention among premature infants admitted to the NICU. The ocean disc reduced heart rate (p = 0.001) and improved sleep patterns (p <0.001) while showing a trend toward reduced respiratory rate (p = 0.07). The Gato box intervention reduced heart rate (p = 0.04) and showed trends toward improved sleep patterns (p= 0.08) and reduced respiratory rate (p = 0.71). Finally, the song of kin intervention demonstrated decreased heart rate (p < 0.0001) and increased feeding (p = 0.02). Notably, music decreased self-reported parental stress (p < 0.001).
Strengths of this study include a large sample size and randomized cross over design; however, its generalizability is limited to premature infants diagnosed with sepsis, respiratory distress or small for gestation age requiring NICU care. Still, this study suggests that music therapy may promote physiologic stability among infants.

Additionally, several meta-analyses have demonstrated beneficial effects of music therapy in premature infants. The first meta-analysis demonstrated music therapy to have a significant effect (95% CI 0.68 to 0.97) on behavioral state, physiologic parameters, weight gain and length of hospital stay. This study suggests that 20-to-30-minute recorded lullaby music interventions may pacify infants. Another meta-analysis of music therapy in preterm infants admitted to the NICU demonstrated a positive effect of music among infants 28 to 31 weeks gestation (p = 0.01) and 32 to 35 weeks (p < 0.01), however music therapy did not impact studied outcomes in infants greater than 35 weeks gestation (p=0.84). Given the intended population of premature infants, there was a small sample size of infants greater than 35 weeks gestation suggesting that results in full term infants may be skewed and lack validity. Another meta-analysis compared music therapy to standard of care and demonstrated reduced infant respiratory rates (95% CI -7.8 to -0.03) and maternal anxiety (95% CI -2.42 to -1.22). Finally, a meta-analysis of music therapy in infants less than 37 weeks gestation demonstrated reductions in heart rate (p = 0.06), respiratory rate (p = 0.0002), maternal anxiety (p < 0.00001) and maternal stress level (p < 0.00001). The study reported significant heterogeneity of study design between trials highlighting the need for well-designed RCTs studying music therapy. Considered together, these studies suggest music therapy may promote stability among premature infants and full term infants including those with NAS.
2.2.3 Effects of Lullabies on Infants

Several studies have examined the effects of live and recorded lullabies on preterm neonates. One prospective controlled trial compared the effects of live music therapy, recorded music therapy and ambient noise on physiological and behavioral parameters of preterm infants in the NICU\textsuperscript{17}. Infants exposed to live music showed a reduction in heart rate (p < 0.01) and in behavioral state (p < 0.001) 25 minutes after exposure without changes in oxygen saturation (p=0.08) suggesting that live music may calm premature infants. However, infants exposed to recorded music therapy showed reductions only in heart rate (p <0.01) without changes in behavioral state (p > 0.93)\textsuperscript{17}. The study is generalizable to preterm infants less than 32 weeks gestation admitted to the NICU and informs only short-term outcomes of music therapy. Similarly, another study of premature infants demonstrated reduced heart rates among infants listening to live music (p< 0.001) and recorded music (p <0.001) compared to ambient noise\textsuperscript{18}. Live-music exposed infants also showed calmer behavioral states (p = 0.003) than infants listening to ambient noise whereas recorded-music exposed infants showed no change in behavioral state (p=0.93). Further, live lullabies were associated with calmer states compared to recorded lullabies (p = 0.02)\textsuperscript{18}. Both of these studies demonstrate that music promotes physiologic stability among preterm infants\textsuperscript{17,18}. Conversely, a third larger RCT of premature infants delivered music through headphones and reported no difference in heart rate, oxygen saturation or behavioral state 10 minutes after intervention (p= 0.81, p = 0.31, p=0.34 respectively)\textsuperscript{19}. However, the follow up period of 10 minutes was shorter than the previous two studies which found significant differences 15 minutes after exposure. Further, the introduction of headphones may have agitated infants thus limiting
effects on studied outcomes\textsuperscript{19}. Notably, the study excluded all infants exposed to sedating medications and infants born to mothers with a history of drug use or alcohol use thus limiting its generalizability to infants with NAS.

Other studies have examined only recorded lullaby intervention in premature infants. A study of low-birth-weight premature infants examined the effect of recorded lullaby on physiologic state over 8 sessions, a longer intervention than the aforementioned studies. The study reported higher respiratory rates (p=0.01) and more stable oxygen saturations (p=0.001) without changes in heart rate (p=0.24) in infants listening to recorded lullabies compared to those listening to ambient noise\textsuperscript{20}. Finally, a fifth controlled trial of music intervention in inconsolable premature infants reported a reduction in heart rate (p <0.001), respiratory rate (p < 0.001) and crying outcomes (p<0.0001) with an increase in oxygen saturation (p < 0.01) in response to music intervention\textsuperscript{21}. Notably, this study excluded all infants in pain or experiencing withdrawal again limiting its generalizability to infants with NAS\textsuperscript{21}. Nonetheless, these five studies provide strong evidence that recorded song can improve physiologic state and stability in premature infants thus warranting further study in full term infants and those with NAS.

\textbf{2.2.4 Effects of Maternal Song on Infants}

Maternal song has demonstrated benefits on physiologic parameters, behavioral state, feeding and attachment in premature and full-term infants. One single cohort crossover study compared the effect of recorded maternal lullaby to ambient noise on the physiologic parameters of preterm infants\textsuperscript{22}. Maternal recorded lullaby increased oxygen saturation (p=0.039) and decreased heart rate (p=0.03) compared to ambient noise exposure without affecting respiratory rate (p=0.070) suggesting that maternal lullabies
positively affect physiological parameters of infants\textsuperscript{22}. Limitations include a single group design and a lack of blinding to music intervention. Another study compared the effects of maternal speech, maternal signing and ambient noise on physiological outcomes of stable preterm infants\textsuperscript{23}. Compared to control infants, both maternal voice groups experienced increased heart rate (p=0.049) and oxygen saturations (p=0.033) without a change in the number of critical events (p<0.0001) suggesting that maternal voice promotes infant stability without causing adverse events. There were no differences in physiologic parameters during exposure to maternal speech compared to maternal singing suggesting that maternal voice positively effects the studied outcomes\textsuperscript{23}. Importantly, there is variation between studies regarding heart rate in response to music as some studies report increases in heart rate while others report reductions. This may be due to infant age as older infants are stimulated and captivated by maternal voice whereas younger infants are soothed as they would be in utero. A third RCT utilized maternal recorded lullaby played through pacifier activated music (PAM) compared to routine pacifier in preterm infants\textsuperscript{24}. Infants using PAM during non-nutritive sucking had increased feeding volumes (p=0.001) and number of oral feeds per day (p<0.001) compared to infants using standard pacifiers\textsuperscript{24}. Thus, recorded maternal song may support physiologic stability and oral feeding in preterm infants.

Additionally, three studies have demonstrated beneficial effects of maternal song on full term infants. One RCT compared the effects of classical music and maternal lullaby played during routine nursing care on physiologic and stress parameters of full-term infants. Both classical and lullaby music improved oxygen saturation (p = 0.036) and reduced stress symptoms (p=0.001) compared to ambient noise. However, music did
not affect heart rate or blood pressure in either group (p > 0.05)\textsuperscript{25}. The study did not blind participants or nursing staff, nor did it assess long-term outcomes of the studied parameters. Another concurrent cohort study examined the effects of maternal song on maternal-infant bonding and newborn crying. The singing cohort participated in song directed antenatal classes, whereas the control cohort participated in speech directed antenatal classes. Maternal singing strengthened self-reported mother-infant bonding (p < 0.001) and decreased maternal stress due to nighttime awakening (p < 0.05). Additionally, infants in the singing cohort cried less often (p < 0.00001) and experienced less colic (p=0.003) at one month of age compared to the concurrent cohort\textsuperscript{26}. Finally, a RCT analyzed the effect of contingent maternal lullaby on full-term infants over the first 6 weeks of life\textsuperscript{27}. Contingent lullaby consisted of maternal singing when the infant was quiet and alert thus reinforcing quiet behaviors. Infants receiving contingent lullaby showed reductions in mean daily crying time over six weeks (p < 0.01) compared to control infants\textsuperscript{27}. Limitations of this study include a lack of recording of baseline maternal signing practices which may have affected frequency and persistent use of contingent lullaby to reduce crying. As a whole, these three studies demonstrate that maternal recorded song may reduce stress states, crying duration and crying frequency among full term infants.

\subsection*{2.2.5 Effects of Music on Infant Crying}

There are a limited number of studies that examine the relationship between music and crying patterns. As previously mentioned, Persico et al’s concurrent cohort study demonstrated a reduction in crying frequency (p < 0.00001 at 1 month, p < 0.00001 at 2 months) and colic (p=0.003 at 1 month, p=0.02 at 2 months) in the singing cohort.
compared to the speech directed cohort\textsuperscript{26}. This study not blind mothers to allocation and was ethically unable to prohibit maternal signing in the concurrent cohort where eight percent of mothers sang to their infants. Nonetheless, this study suggests that maternal song may reduce crying measures. Another single blind RCT examined the effect of music intervention during kangaroo care or skin to skin contact on crying duration of preterm infants. Music exposed infants had fewer crying episodes ($p < 0.05$) compared to infants listening to ambient noise. However, results may be limited by infrequent sampling of 10-minute intervals thus restricting the ability to detect changes in the infants’ physiologic state\textsuperscript{28}.

Another aforementioned study demonstrated that music listening during crying episodes improved crying measures among inconsolable premature infants. Music listening reduced frequency of crying episodes from 7.21 to 4.29 episodes per day ($p < 0.001$) and duration of crying episodes from 23.14 minutes to 5.53 minutes ($p < 0.001$) compared to ambient noise\textsuperscript{21}. Finally, Robertson et al. demonstrated a reduction in mean daily crying time in full term infants exposed to contingent lullaby compared to routine care ($p < 0.01$). In the first week of life, infants receiving contingent lullaby cried $23.47 \pm 24.83$ minutes compared to $31.25 \pm 36.35$ minutes among control infants\textsuperscript{27}. Although the literature is limited, current evidence suggests that music interventions may reduce crying frequency and duration in infants.

\textbf{2.2.6 Interventions to Reduce Daily Crying Duration}

A literature review offers multiple interventions to reduce intermittent neonatal crying episodes, however, this review will focus only on interventions aiming to reduce daily crying duration. Two studies have evaluated the effect of skin to skin contact (SSC)
on crying duration. A quasi-experimental study of premature infants in the NICU demonstrated that infants exposed to SSC cried less often than infants exposed to routine neonatal care across the first 5 days of life ($p=0.002$ day 1, $p=0.005$ day 2, $p < 0.001$ day 3, $p=0.007$ day 4, $p=0.010$ day 5). The study demonstrated significant selection bias as allocation to SSC was by maternal choice\textsuperscript{29}. Similarly, a case-controlled study demonstrated reduced crying times among colicky infants exposed to kangaroo mother care (KMC). Fussing and crying time declined from $2.21 \pm 1.54$ hours per day to $1.16 \pm 1.13$ hours per day after KMC compared to control infants ($p=0.001$). The study reported that KMC is more effective among female infants ($p=0.001$) suggesting that sex may play a role in crying duration\textsuperscript{30}. Considered together, these studies demonstrate that skin to skin contact may reduce crying in premature and colicky infants.

Swaddling has also been studied as an intervention to reduce crying duration. A RCT evaluated the effect of swaddling in excessively crying infants. At baseline, the mean crying duration for both groups was 5.76 hours per day (SD 3.31). Over the first week, there was a 42\% reduction in crying time (62.1 minutes per day) and 49.5\% reduction in the second week (70.7 minutes per day). Overall, there was no difference in crying time between groups over 12 weeks, however, infants 1-7 weeks of age at randomization benefited from being swaddled compared to the infants 8-13 weeks of age (95\% CI 0.023 – 0.107). The randomization and RCT design were of high quality; however, the study does not report all statistical analyses required to support reported findings thus limiting validity of results. Nonetheless, swaddling may reduce daily crying duration among excessively crying infants in the first seven weeks of life\textsuperscript{31}. Overall,
despite limited evidence, skin to skin contact and swaddling may affect crying outcomes in infants.

2.3 REVIEW OF RELEVANT METHODOLOGY

2.3.1 Study Design

The proposed study will be a single blind two arm RCT examining the benefits of recorded maternal song on crying duration among infants with NAS. As discussed, several RCTs have examined the effects of recorded maternal song on premature infants\(^{22,24,25}\). However, these studies lack generalizability to all neonates given the location of care and physiologic differences of premature infants. A review of the literature provides several RCTs studying pharmacotherapy among infants with NAS\(^{32-37}\), however most studies of nonpharmacological care for NAS are either quality improvement studies or of retrospective cohort design\(^{1,3,4,7}\). To our knowledge, there are only two studies examining non-pharmacological music interventions in affected infants\(^{10,11}\). Nonetheless, several RCTs have demonstrated positive effects of music intervention among premature and full-term infants\(^{12,17,20,27}\). The proposed study will parallel the RCT design of music studies in other infant populations to limit bias introduced by known confounding variables of NAS.

The proposed study will recruit all infants at risk of NAS from three labor and delivery units and multiple clinics affiliated with YNHHS. The large number of recruitment sites aims to account for the incidence of NAS in Connecticut and to achieve the desired sample size. Given the diversity of patients treated by YNHHS, these authors believe that the proposed study will be widely generalizable. Recruitment in this study adopts convenience sampling utilized by another study in which all infants with NAS
meeting inclusion criteria within the hospital were recruited. Through this recruitment approach, we hope to enroll a large and diverse population to maximize the generalizability of our results.

2.3.2 Selection Criteria

Selection criteria were chosen based upon previous studies of similar populations. Eligible mother-infant dyads include infants diagnosed with NAS within 24 hours of delivery who are greater than 35 weeks gestational age and weigh more than 2000 grams at birth. Unlike other studies of infants with NAS that included only methadone exposed infants, we will include all substance exposed infants with a diagnosis of NAS. We will exclude infants with medical conditions requiring treatment in the NICU as well as any major congenital malformations, hearing impairment or need for surgical procedures with anesthesia. Infants with neurological problems will be excluded in accordance with other neonatal music studies. The proposed exclusion criteria parallels other studies of infants with NAS which barred all infants who were not otherwise well newborns eliminating any infants with sepsis, prematurity or need for either surgical or respiratory support. Exclusion of these medical conditions will reduce the risk of results being attributed to these conditions rather than to music intervention and increase the internal validity of the study. Unlike prior studies of music interventions among preterm neonates, we will not exclude infants receiving phenobarbital as it is a sedative medication used in the treatment of NAS.

2.3.3 Potential Confounding Variables

Confounding variables for this study were selected from other studies centered on infants with NAS. We identify confounding variables of NAS to be gender,
gestational age, polysubstance use, tobacco use, breastfeeding and substance of exposure which we will control for through randomization. Gestational age introduces the opportunity for bias as premature infants can experience milder withdrawal compared to full term infants\(^40\), thus our study excludes all infants less than 35 weeks gestation at birth. Conversely, the role of gender in withdrawal severity is conflicting. One study reported higher NAS scores and pharmacotherapy use in males compared to females\(^41\), however, another study demonstrated no sex related differences in NAS development, severity, duration or pharmacotherapy\(^42\). Thus, we will randomize infants to limit the impact of all known potential confounding variables to strengthen study validity.

Substance of exposure and polysubstance use may also affect severity of NAS\(^39\). One study comparing NAS withdrawal in methadone exposed, and buprenorphine exposed infants demonstrated that buprenorphine may be associated with a less severe withdrawal compared to methadone\(^36\). Further, despite primary substance of exposure, polysubstance use can be associated with more severe NAS symptoms\(^43\). Similarly, cigarette use within 30 days of delivery may be associated with more severe withdrawal supported by longer hospital stays and treatment duration\(^44\). The quantity of daily cigarette use may also be associated with severity and timing of NAS in methadone exposed infants\(^45\), but not in buprenorphine exposed infants\(^43\). In the proposed study, randomization controls for differences in substance of exposure and cigarette use in order to limit bias introduced from known confounding variables. Notably, the proposed study relies upon maternal reported substance use instead of biological sample testing.

Maternal breastfeeding is another confounding variable among infants with NAS. Breast feeding may be associated with NAS severity, decreased need for
pharmacotherapy and delayed onset of NAS\textsuperscript{7}. Evidence suggests that breast milk may be associated with shorter hospital stay compared to formula\textsuperscript{46}. Further, another study reported that infants receiving breast milk recorded lower Finnegan scores than formula fed infants suggesting that breastfeeding may mitigate withdrawal\textsuperscript{7}. Proposed explanations include delivery of opiates through the breast milk which may reduce withdrawal, or an increase in skin-to-skin contact which soothes infants\textsuperscript{47}. The proposed study will not limit breast feeding due to its reported benefits. We will encourage all able mothers to breastfeed, and nurses will document the feeding method used.

\textbf{2.3.4 Randomization and Blinding Technique}

The proposed method of randomization is based upon a RCT which used an online number generator to allocate infants to music intervention or routine care\textsuperscript{11}. Random allocation will control for baseline characteristics to reduce bias introduced by known confounding variables and strengthen the likelihood that reported results are due to music intervention alone. Several music studies utilized a cross over design with random order generators to randomly assign infants to a specific order of music intervention over several days\textsuperscript{27}; however, the proposed study is not of crossover design. In the proposed parallel RCT study, a random number generator will randomize participants to either recorded maternal song or ambient noise intervention.

This will be a single blind study in which outcome assessors and research staff will be blinded to allocation. Given the nature of the music intervention, it is impossible to blind infants, mothers and nursing staff to allocation. Still, we believe the single blinded nature of this study will minimize reporting bias and information bias to provide the strongest validity possible. This design stems from a previous study comparing live
music, recorded music and no music among premature neonates in which investigators were blinded to allocation and the primary outcome of physiological parameters during data analysis\textsuperscript{17}. Our proposed study will similarly blind research staff to allocation at baseline and at all assessment points to create a single blind trial.

\textbf{2.3.5 Intervention}

The maternal recorded song intervention will parallel music studies of premature infants\textsuperscript{17-19,28} while maintaining a noise level below the 50 decibel recommendation from the AAP to protect the auditory systems of studied infants\textsuperscript{48}. During observation periods and intervention, all infants will be supine in an open crib 30 centimeters from the music source. Studies vary in the distance of music source to the infant ranging from 30 centimeters to 2 meters\textsuperscript{17,18}, however, since sound level will be monitored and maintained according to the AAP, the proposed study will use a 30 centimeter distance which has demonstrated significant effects in premature populations\textsuperscript{18}.

There is significant diversity in frequency, duration and length of music interventions used in other music studies. One study of live and recorded music offered thirty minutes of daily music one hour after feeding for three consecutive days\textsuperscript{17}. Two other studies similarly offered twenty minutes of daily music thirty minutes after feeding for three consecutive days\textsuperscript{18,19}. Despite the brevity of these interventions and short duration of treatment, these music interventions showed beneficial short-term effects on premature infants\textsuperscript{17-19,28}. While other studies demonstrated effects of music on crying over the period of weeks\textsuperscript{27}, infants with NAS do not experience hospital stays long enough to accommodate a similar timeline for intervention. Thus, the proposed study will implement a music intervention for thirty minutes over three consecutive days one hour
after eating which limits loss to follow up as the intervention will be completed within the hospital stay. Further, the frequency of once daily intervention limits the burden placed on nurses delivering the music intervention and on mothers prohibited from holding their infant around the time of intervention as holding and skin to skin contact are known confounding variables of NAS. Importantly, the frequency, dose and length of the proposed intervention will not impede infant feeding periods or routine nursing care as the timing of intervention will be implemented at nursing discretion after appropriate training.

Music interventions vary widely in delivery as live or recorded music as well as the selected song as either a song of kin, classical music or traditional lullaby\textsuperscript{12,17,25}. Classical music has been demonstrated to maintain body temperature and oxygen saturation as well as reducing stress symptoms in full term neonates\textsuperscript{25}. A study that compared parent selected song of kin intervention to the folk song “Twinkle Twinkle Little Star” showed higher oxygen saturation among infants listening to “Twinkle” compared to the song of kin. However, the song of kin was associated with feeding benefits\textsuperscript{49}. Studies consistently choose “Twinkle” as the lullaby intervention\textsuperscript{12,49,50} as this song follows a repetitive patterns thus easing delivery in non-musicians and offers a melody well known across cultures\textsuperscript{12}. Specifically, both “Ba Ba Black Sheep” and the “ABC’s” follow the same melody which further increases its familiarity among parents and will limit cultural bias from song of kin intervention.

\textbf{2.3.6 Primary and Secondary Outcome Measures}

The primary outcome of the proposed study is seventy-two hour crying duration as excessive crying is a hallmark characteristic of NAS withdrawal and increases risk of
Thus, interventions which reduce crying in affected infants may represent a less severe withdrawal, reduce risk of abuse and improve long term outcomes. In the proposed study, crying duration will be measured via continuous audio recording of infant vocalization which will be scored by research staff using automated segmentation of recorded crying. In the proposed study, a member of the research team will sum annotated inspiratory and expiratory bouts of crying vocalizations to determine crying duration and crying episodes. These calculations will then be used to determine seventy-two-hour crying duration. Recording of infant vocalizations is based upon a previous study that compared crying durations recorded by subjective maternal crying logs to objective audio recordings obtained through a wireless microphone placed on an infant’s cloth belt for 24 hours. The receiver was connected to a voice activated recorder which marked the start of vocalization indicating the start of crying. In this study, crying was defined as continuous, sustained expiratory vocalization longer than 5 seconds and a crying episode was defined from the start of crying until the last vocalization prior to 5 minutes of silence. All audio recordings were played back for scoring of crying episodes via stopwatch thus requiring research staff to listen to all infant vocalizations. In the proposed study, we will use the same recording method to detect infant vocalizations, however, analysis of crying duration will be done through automatic segmentation. Naithani and his colleagues describe a cry analysis system using hidden Markov models which identifies parts of the cry signal and labels inspiratory and expiratory phases of crying. The program is 89.2% accurate for crying annotation and has capability for training and learning of new crying sounds. Importantly, one study analyzing cry signals reported no difference in the acoustic cry signals of infants with infant abuse. Thus, interventions which reduce crying in affected infants may represent a less severe withdrawal, reduce risk of abuse and improve long term outcomes. In the proposed study, crying duration will be measured via continuous audio recording of infant vocalization which will be scored by research staff using automated segmentation of recorded crying. In the proposed study, a member of the research team will sum annotated inspiratory and expiratory bouts of crying vocalizations to determine crying duration and crying episodes. These calculations will then be used to determine seventy-two-hour crying duration. Recording of infant vocalizations is based upon a previous study that compared crying durations recorded by subjective maternal crying logs to objective audio recordings obtained through a wireless microphone placed on an infant’s cloth belt for 24 hours. The receiver was connected to a voice activated recorder which marked the start of vocalization indicating the start of crying. In this study, crying was defined as continuous, sustained expiratory vocalization longer than 5 seconds and a crying episode was defined from the start of crying until the last vocalization prior to 5 minutes of silence. All audio recordings were played back for scoring of crying episodes via stopwatch thus requiring research staff to listen to all infant vocalizations. In the proposed study, we will use the same recording method to detect infant vocalizations, however, analysis of crying duration will be done through automatic segmentation. Naithani and his colleagues describe a cry analysis system using hidden Markov models which identifies parts of the cry signal and labels inspiratory and expiratory phases of crying. The program is 89.2% accurate for crying annotation and has capability for training and learning of new crying sounds. Importantly, one study analyzing cry signals reported no difference in the acoustic cry signals of infants with infant abuse.
NAS compared to healthy neonates\textsuperscript{55}. An alternative method to determine crying duration is 24-hour maternal crying diaries, however this tool requires continuous maternal presence and is limited by reporting bias\textsuperscript{53}. In one study comparing voice activated recording and maternal reported crying diaries, mothers reported less total crying (p<0.02) and fewer crying episodes (p <0.01) compared to digital recordings of crying. The study demonstrated that mothers failed to report 24% of total crying duration thus emphasizing the limitations of crying diaries\textsuperscript{53}. The proposed study will instead determine all crying outcomes through the objective measures described.

Secondary outcomes of the proposed study also include crying measures such as longest crying duration, number of crying episodes and average length of crying episode. These measures will be calculated from the segmented crying increments. Further, the study will examine the diurnal distribution of crying episodes. These outcomes were selected based upon a study which measured frequency of crying, mean duration of crying episodes and diurnal distribution with reported crying occurring most frequently between 3:00 pm and 9:00 pm\textsuperscript{53}. Our study will examine diurnal variation in 6-hour increments beginning at midnight following another study in which the primary outcome was diurnal crying distribution\textsuperscript{56}.

Additional secondary outcomes including physiological parameters of heart rate, respiratory rate and oxygen saturation were selected based upon several studies which examined the effect of music intervention on physiologic parameters of premature infants\textsuperscript{12,17-20,25}. The proposed study models the recording method described by Alipour et al. in which oxygen saturation and heart rate were measured via pulse oximetry attached to the infant’s foot and respiratory rate was counted by an investigator observing chest
rise and fall. Physiologic parameters were recorded in 5-minute epochs prior to and after the music intervention\textsuperscript{19}. Similarly, we propose to use pulse oximetry to record heart rate, respiratory rate and oxygen saturation which will later be extracted from telemetry monitors by research staff in 5-minute epochs. Since some studies did not observe significant physiologic differences until twenty minutes after intervention\textsuperscript{17,18}, we plan to monitor physiologic parameters for thirty minutes after intervention.

Several additional secondary outcomes proposed include the use of pharmacological medication and frequency of pharmacotherapy use. Other studies of infants with NAS rely upon mean Finnegan scores\textsuperscript{11}, however, the proposed study site at YNHCH does not utilize Finnegan scores to monitor NAS withdrawal. Instead, pharmacotherapy measures, which indicate severe or unmanaged withdrawal, will serve as proxies to assess severity of NAS. Additionally, the average number of calories consumed per day and weight gain are important makers of NAS severity as affected infants experience gastrointestinal disturbances.

\textbf{2.3.7 Sample Size and Statistical Significance}

The sample size for the proposed study is based upon another study that aimed to reduce crying duration among infants with colic\textsuperscript{57}. To our knowledge, there are no prior studies that quantify mean daily crying duration among infants with NAS. Similarly, there are no studies that use music to reduce mean crying duration among infants with colic. A recent meta-analysis of mean infant crying duration revealed that in the first six weeks of life, healthy neonates fuss or cry for 117 to 133 minutes per day\textsuperscript{58}. Conversely, a small cohort study demonstrated a mean daily crying duration of 25 minutes measured
by digital voice activated recording\textsuperscript{59}. This study separated crying and fussing time which may account for the marked difference in daily crying durations.

Considering the irritability of infants with NAS attributable to withdrawal and the characteristic high pitched, excessive crying of these infants, we believe that healthy neonatal crying durations will underestimate the true crying duration of infants with NAS. Thus our sample size calculation is based upon a study aimed to reduce crying in infants with colic\textsuperscript{57}. This study found that a mean change of 52.7 minutes was significant in reducing daily crying duration among infants with colic. Given the results of this study, we aim to detect the same effect size of 52.7 minutes of daily crying with a standard deviation of 110 minutes.

A review of prior clinical trials using non-pharmacological care in infants with NAS demonstrated dropout rates between 1.2 and 6.6\%\textsuperscript{60,61}. Our study conservatively estimates a dropout rate of 5\% given that a three-day intervention is within the recommended observation period for infants with NAS\textsuperscript{39}. We propose a study with a power of 80\% and alpha of 0.05 and a dropout rate of 5\%. Thus a sample size of 75 infants per group or 150 total infants must be recruited for the proposed study.

2.4 CONCLUSION

This literature review details studies demonstrating beneficial effects of music intervention among various infant populations including preterm and full-term infants as well as infants with NAS. There is evidence that recorded lullabies, music therapy and maternal song positively impact infant physiologic and behavioral states while reducing crying duration. Two studies have utilized recorded maternal song among infants with NAS, however, neither have elucidated the effect of music on crying duration which may
indicate severe withdrawal and increase risk of infant abuse. Further, these studies were limited by sample size and design. Thus, we propose a single blinded RCT comparing the effect of maternal recorded song to ambient hospital noise in reducing crying duration among infants with NAS. This study will address the stated gap in the literature and establish the role of recorded maternal song among infants with NAS. This study will offer both medical providers and mothers alike an inexpensive and effective non-pharmacologic intervention to comfort inconsolably crying infants with NAS.

2.5 REFERENCES


CHAPTER 3: STUDY METHODOLOGY

3.1 STUDY DESIGN

We propose a two arm, single blind randomized control trial (RCT) analyzing the effects of maternal recorded song and ambient hospital noise on crying duration in neonates with NAS. A baseline characteristics enrollment survey will be used to assess demographic data and audio recordings will quantify infant crying over three days. Student t-tests will assess for a difference in total crying duration between maternal recorded song and ambient noise.

3.2 STUDY POPULATION AND SAMPLING

The source population is women delivering infants with in-utero opioid exposure within the Yale New Haven Health System (YNHHS). Given the incidence of NAS, participants will be sampled in a non-random, consecutive basis via convenience sampling to maximize enrollment. Infants meeting inclusion criteria will be recruited from mothers delivering at multiple clinics and hospitals within YNHHS. Barring consent, all eligible infants with NAS will be included in the study.

Table 1. Inclusion and Exclusion Criteria

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<tr>
<td>Infant born with a diagnosis of neonatal abstinence syndrome within 24 hours of delivery</td>
<td>Medical conditions necessitating admission to the neonatal intensive care unit</td>
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<tr>
<td>Gestational age greater than 35 weeks at birth</td>
<td>Major congenital malformations</td>
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<tr>
<td>Weight greater than 2000 grams at birth</td>
<td>Major surgical procedures with anesthesia</td>
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<td>Mother willing to record lullabies prior to enrollment</td>
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3.3 RECRUITMENT

The primary recruitment process will occur within Obstetrics and Gynecology clinics and the Prenatal Neonatal Abstinence Syndrome Clinic associated with YNHHS. Recruitment will also occur in the labor and delivery units of Yale New Haven Hospital York Street Campus, Lawrence and Memorial Hospital and Bridgeport Hospital. Research staff will be responsible for assessing the eligibility and interest of all mother-infant dyads at the above sites as well as educating the dyads on the risks and benefits of participation. If willing to enroll, mothers will complete the enrollment survey and record themselves singing a lullaby with a research investigator. Recruitment will occur in an ongoing basis over twenty-two months.

3.4 SUBJECT PROTECTION AND CONFIDENTIALITY

This study will be conducted after ethical approval by the institutional review board granted by the Yale Human Investigations Committee (HIC) at the Yale School of Medicine and YNHHS. Prior to the study all research staff will complete Health Insurance Portability and Accessibility Act (HIPAA) training. All protected health information will be accessed only by HIPAA-trained research staff as necessitated in the study. Written informed parental consent will be obtained via the Authorization and Consent Form (Appendix A) prior to enrollment after all questions are answered by research staff. Consent must be given by the mother with legal custody of each neonate in accordance with the Connecticut Department of Children and Families.

3.5 STUDY VARIABLES AND MEASURES

Recorded Maternal Song Intervention: Neonates will be exposed to maternal recorded song after feeding for thirty minutes over three consecutive days. The music intervention
will be played from a MP3 player through two speakers placed thirty centimeters from the head of the neonate. Nursing staff will initiate the self-terminating recording thirty minutes after feeding is completed when the neonate is quiet and settled. Recorded song will not be played if feedings are less than two hours apart.

All mothers will be recorded singing “Twinkle, Twinkle, Little Star” in a recording session with a research assistant prior to randomization to prevent drop-out if randomized to ambient noise intervention\(^1\). The noise level will be measured with a Cirrus (Cr274) sound level meter and adjusted not to exceed 50 decibels\(^2\).

**Ambient Noise Control:** Ambient hospital noise will serve as the control exposure in this study. Outcomes will be assessed thirty minutes after feeding when the settled infant is exposed only to the ambient noise of the pediatric in-patient unit at hospitals within YNHHS. Ambient noise will be measured in decibels with a Cirrus (Cr274) sound level meter to assess baseline noise levels in the pediatric in-patient unit.

**All Infants:** Regardless of allocation, all groups will receive the standard of care within the Eat, Sleep, Console approach to NAS in accordance with the guidelines set forth by YNHCH. Mothers will not be restricted in daily singing to their infants, holding their infants or being present at the bedside; however, the occurrence of these soothing factors will be self-reported by mothers and recorded by nursing staff via documentation in the electronic health record. Mothers will not be allowed to hold their infant for 90 minutes surrounding noise intervention which includes 30 minutes prior to intervention to ensure the infant is settled in the crib.

**Primary Outcome:** The primary outcome measure is total crying duration over seventy-two hours which will be measured by audio recording of infant vocalization and scored
by research staff using automated segmentation of recorded crying. Crying duration is defined as the summation of each crying period throughout the day measured in minutes per day expressed as a mean with standard deviations.

The audio recording system is a wireless microphone and receiver sitting in a terry cloth belt on the infant. The receiver connects to a voice activated recorder (VLR-8CT, Omnicron Electronics) which records 8 hours of crying onto a 60-minute tape. The voice activated recordings will be analyzed by a hidden Markov model which segments recorded crying into inspiratory, expiratory and residual phases. In this study, total crying duration is defined as the sum of inspiratory and expiratory phases as annotated by the Audacity program. Any crying vocalization lasting more than 5 seconds will be counted as crying for the appropriate minute. A crying increment will terminate after 5 consecutive minutes of silence. Appendix B details the recording and quantification of crying duration for all studied outcomes.

**Secondary Outcomes:** Secondary outcomes of the study include physiologic parameters of heart rate, respiratory rate and oxygen saturation as measured by pulse oximetry placed on the left foot of each neonate. Additional secondary outcomes include pharmacotherapy use (yes or no), frequency of pharmacotherapy (total number of doses), average number of calories consumed per day and weight gain (grams per day) which will be extracted from the electronic health record by a research assistant at discharge.

We are also interested in additional measures of crying recorded in minutes including 24 hour crying duration, longest crying duration, number of crying episodes and average length of crying episode. We will examine the diurnal distribution of crying
durations in various time blocks defined as night (12am – 6am), morning (6am – 12pm), afternoon (12pm – 6pm) and evening (6pm – 12am).

**Baseline Variables:** Baseline demographic data as well as postnatal infant care will be collected at enrollment, during the study and at discharge via extraction from the electronic health record by a research assistant blinded to allocation (Table 2). Maternal characteristics of interest include age, opioid used, polysubstance use, cigarette smoking, alcohol use, insurance status and delivery by cesarean section. Neonatal data of interest includes gender, race, birth weight, gestational age, APGAR score at 5 minutes, head circumference and insurance status. Postnatal infant care characteristics will include feeding method (formula, breast milk or both), pharmacotherapy use (yes or no), average calories consumed per day, frequency of pharmacotherapy use, kangaroo care time, maternal live singing at bedside, holding time per day and family time at bedside. Family presence at the bedside will be recorded in nursing documentation. Singing at bedside, kangaroo care time and holding time will be self-reported by the mother (Appendix C) and documented by nurses in the electronic health record for extraction at discharge.

**3.6 ADDITIONAL METHODOLOGY CONSIDERATIONS**

To our knowledge no studies have quantified daily crying duration in infants with NAS. Since these infants may be more irritable than the general population of neonates, we believe crying durations of healthy neonates will underestimate those of infants with NAS. In this study we propose an effect size and sample size based upon crying durations of infants with colic as we believe it is reasonable to connect the agitation and irritability of infants with NAS to that of infants with colic. We expect that both groups experience extended crying durations compared to those of healthy infants.
## TABLE 2. CHARACTERISTICS OF THE NEONATE, THE MOTHER AND POSTNATAL CARE

<table>
<thead>
<tr>
<th>Characteristics of the Neonate</th>
<th>Maternal Recorded Song N = 71</th>
<th>Ambient Noise N = 71</th>
<th>P Value (0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, number (%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Race, number (%)</td>
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<td></td>
<td></td>
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<tr>
<td>Caucasian</td>
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<td></td>
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<tr>
<td>African American</td>
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<td></td>
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<tr>
<td>Hispanic</td>
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<td></td>
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<tr>
<td>Other</td>
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<td></td>
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<tr>
<td>Gestational age (weeks)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Birthweight (kg)</td>
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<td></td>
<td></td>
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<tr>
<td>Apgar score at 5 minutes</td>
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<td></td>
<td></td>
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<tr>
<td>Head circumference (cm)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Public insurance, number (%)</td>
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</tbody>
</table>

### Characteristics of the Mother

<table>
<thead>
<tr>
<th>Characteristics of the Mother</th>
<th>Maternal Recorded Song N = 71</th>
<th>Ambient Noise N = 71</th>
<th>P Value (0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother’s age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prenatal Opioid Used, number (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methadone</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Buprenorphine</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polysubstance use, number (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cigarette smoking, number (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol use, number (%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Public insurance, number (%)</td>
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<tr>
<td>Delivery by cesarean section, number (%)</td>
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</table>

### Characteristics of Postnatal Infant Care

<table>
<thead>
<tr>
<th>Characteristics of Postnatal Infant Care</th>
<th>Maternal Recorded Song N = 71</th>
<th>Ambient Noise N = 71</th>
<th>P Value (0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding method</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brest Feeding*</td>
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<td></td>
<td></td>
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<tr>
<td>Formula</td>
<td></td>
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<tr>
<td>Average calories consumed per day (calories)</td>
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<td></td>
</tr>
<tr>
<td>Pharmacotherapy use, number (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pharmacotherapy frequency, number of doses</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family member at bedside, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Other Family</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family time at bedside (minutes per day)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Holding time (minutes per day)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Kangaroo care (minutes per day)</td>
<td></td>
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<td></td>
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<tr>
<td>Maternal live singing at bedside (minutes per day)</td>
<td></td>
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<td></td>
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</tbody>
</table>

*Considered to be breast feeding if given breast milk > 50% of feedings†
3.7 BLINDING OF INTERVENTION AND OUTCOME

**Blinding of Intervention:** Inherent to the intervention, it is not possible to blind participants, nursing staff or parents in any of the groups. Nonetheless, participants, parents and nursing staff will be blinded to the hypothesis and will be instructed not to discuss assigned intervention until trial completion.

**Blinding of Outcome:** The single blinded approach of the study will include blinding of investigators and research assistants to allocation at baseline and intervention during data analysis. All research staff will be blinded to allocation during data extraction from pulse oximetry and electronic health records as well as data analysis. Research assistants recording total crying durations from audio playback will not be blinded as they will hear the intervention during the recording.

3.8 ASSIGNMENT TO INTERVENTION

**Assignment to Intervention:** All participants will complete the baseline characteristics survey. Then a computerized random number generator will randomize participants in a 1:1 ratio to maternal recorded song or ambient noise. Investigators will be blind to allocation by means of sequentially numbered, opaque sealed envelopes. No demographic data will be accessible to research staff prior to allocation. All demographic data retrieved from the electronic health record will be done by a research assistant blind to allocation.

**Adherence to Intervention:** Adherence to each intervention will be ensured by the non-blinded nursing staff who will record the onset and termination of noise intervention within the electronic health record. A research assistant will conduct random observation on 20% of sessions to assure appropriate protocol adherence.
3.9 MONITORING FOR ADVERSE EVENTS

No adverse events are expected. If any intervention increases frequency of apnea, desaturation or bradycardia in a neonate, data collection from the neonate will cease and data will be excluded. All infants with a change in medical condition requiring admission to the NICU or limiting completion of the trial will also be excluded.

3.10 DATA COLLECTION

Data collection will begin at 24 hours of life and continue for three days, terminating on day four of life. For three consecutive days voice activated audio recorders in the neonate’s open crib will record all vocalizations. There is no long term follow up period in this study. Two non-blinded research assistants will extract crying durations from audio playback to calculate the sum of crying periods and to determine total crying duration in minutes over seventy-two-hours. Both research assistants will randomly score 20% of recordings to ensure agreement in scoring of crying with goal reliability of 80% between scorers.

Physiologic data including heart rate, respiratory rate and oxygen saturation will be extracted from pulse oximetry recording in 5-minute epochs by a research assistant beginning 10 minutes prior to noise exposure, throughout the 30-minute intervention and the 30-minute follow up period. Other secondary outcomes including use of pharmacotherapy, pharmacotherapy frequency, average calories consumed per day and weight gain will extracted from the electronic health record by a research assistant at discharge.

3.11 SAMPLE SIZE CALCULATION
A simple t-test calculator Power and Precision version 4.0 (Biostat Inc.) was used to look for a difference in means for an anticipated effect size of 52.7 minutes of crying with standard deviation of 110 minutes. This effect size showed statistical significance in mean crying duration among inconsolably crying infants with colic\textsuperscript{5}. A sample size of 71 participants per arm is needed to provide 80% power with alpha of 0.05. The study anticipates a dropout rate of 5% and thus a total of 75 neonates will be randomized to each arm creating a total study sample size of 150 participants. We expect a low dropout rate given the duration of study is included within the average five-day length of stay of infants with NAS at YNHCH.

3.12 STATISTICAL ANALYSIS

Descriptive statistics of baseline calculations will be run to assess effectiveness of randomization. Categorical variables will be assessed with relative frequencies using a chi-squared test. Categorical variables pertinent to the mother include opioid used, polypharmacy, cigarette smoking, alcohol use and insurance status. Categorical variables pertinent to the neonate include gender, race, delivery by cesarean section and neonatal insurance status. Categorical postnatal care characteristics include feeding method and pharmacotherapy use.

Continuous variables including maternal age, neonatal birth weight, gestational age, neonatal average calories consumed daily, neonatal pharmacotherapy frequency, APGAR score at 5 minutes and neonatal head circumference will be assessed with means and standard deviations using a student t-test at baseline. Continuous postnatal care characteristics include average calories consumed per day, pharmacotherapy frequency
and duration of daily kangaroo care, maternal live singing, family presence at bedside and daily holding time which will also be assessed using a student t-test.

All outcome data will be analyzed using an intention to treat analysis. The primary outcome of crying duration over seventy-two hours with standard deviation measured in minutes will be operationalized as a continuous variable. A student t-test will assess for statistical significance in total mean crying duration between recorded maternal song and ambient noise after three days of intervention.

Secondary outcomes related to crying are continuous variables assessed as means with standard deviation measured in minutes including longest crying duration, total number of crying episodes and average duration of crying. These outcomes will be assessed for significance using student t-tests. Likewise secondary outcomes of heart rate, respiratory rate, oxygen saturation, frequency of pharmacotherapy, average calories consumed per day and weight gain will be operationalized as continuous variables and compared with means and standard deviations using a student t-test. Crying time of day and pharmacotherapy use will be analyzed as a categorical variable with a chi-square test.

3.13 TIMELINE AND RESOURCES

The proposed study will be completed within two years. One week will be dedicated to training research staff and nurses in the pediatric unit of each hospital on study protocols and equipment set up. Over the next twenty-two months enrollment will occur on a rolling basis. Data collection of primary and secondary outcomes will begin at 24 hours of life. For three days the infant will be exposed to either maternal recorded song or ambient noise and crying duration will be recorded. All data collection will be completed during the hospital stay, thus there is no follow up assessment after discharge.
Data analysis and manuscript preparation will be completed within six weeks of study completion.

The principal investigator (PI) of this study will be Doctor Matthew Grossman M.D. and the co-principal investigator (co-PI) will be Alecia Cunniff, PA-SII. Research assistants and a statistician will be recruited from Yale University and will be trained in recording of maternal song and scoring of crying on audio-recording. Two full time nurses from each of the three hospitals will be trained on speaker and MP3 set up for noise intervention, 24-hour audio tape recording and documentation of intervention and secondary outcomes in the electronic health record. Throughout the study the PI and co-PI will approach mother-infant dyads meeting inclusion criteria for recruitment in the trial. As compensation, all mother-infant dyads will be offered an electronic copy of their recorded lullaby upon discharge.

3.14 REFERENCES
CHAPTER 4: CONCLUSION

4.1 ADVANTAGES AND DISADVANTAGES

The proposed study has several strengths rooted in its novelty, study design and diversity of outcomes. Current literature surrounding maternal song is sparse and focuses predominantly on premature infants. Thus, this study is novel in its population of full-term infants, especially in infants with NAS. Further, the strength of this study is underscored by its design as a RCT which limits the impact of known confounding variables of NAS. Careful review of existing literature also ensures the study is accurately powered to detect a difference in crying duration and that specific crying measures and methodology are well defined. Specifically, this study reports participant demographic information, electronic models for recording and detailed methods of analysis to ensure replicability of results which are lacking in other crying studies.1 Another strength of this study is its commitment to a diverse study population achieved through utilizing the YNHHS to optimize the generalizability of reported results. Finally, the diversity of crying, physiologic and pharmacologic outcomes of this study will provide a robust contribution to the literature regarding the effect of maternal song on infant crying among infants with NAS. If maternal recorded song reduces crying in affected infants, it may dampen the burden of care placed on healthcare workers and mothers while also improving maternal stress. Ultimately, reducing crying may reduce risk of abuse and improve mother-infant bonding.

Still, the proposed study has several logistical limitations including its single blind nature, short duration of intervention and standardization of recorded song. As music intervention eliminates the opportunity for a double-blind study, we believe that a single
blind study will offer the strongest internal validity possible. The study is also limited by a short three-day intervention; however, this length was intentionally selected to limit loss to follow up as the study falls within the AAP’s recommended length of hospital observation for infants with NAS.\textsuperscript{2} Still, we cannot exclude the possibility that other significant effects would be made clear in a longer study. Moreover, the design of this trial and short intervention limits the ability to study other outcomes specific to NAS including length of hospital stay or cost of treatment. Additionally, this study does not limit maternal singing or skin to skin contact which are confounding variables known to soothe infants.\textsuperscript{3,4} While we understand that maternal self-reporting of these variables introduces the possibility of reporting bias, limiting these interventions would be unethical. We also acknowledge that standardizing maternal song eliminates cultural lullabies\textsuperscript{5} and that recorded song may be inferior to live singing\textsuperscript{6,7}. However, we believe that standardizing music choice and utilizing recorded song offers a consistent intervention and improves study feasibility. Finally, the largest limitation of this study is the constant voice activated recording which may impede enrollment by distrusting mothers who already face large barriers to care.\textsuperscript{8} Further, the study enrolls many at risk groups including pregnant women, infants and those with substance use disorder. Nonetheless, we believe the proposed study will provide objective data to support the use of maternal recorded song among infants with NAS and we hope that clinician-based education and discussion will reduce maternal hesitancy to enroll their infant in the proposed study.

Overall, this study is generalizable to full term infants with NAS who room-in with their mothers on pediatric units. This intervention could be readily implemented by
nursing staff at YNHCH and other institutions using ESC. Maternal recorded song could also be widely implemented at all institutions optimizing non-pharmacologic care of affected infants; however, replication studies may be needed to assess effectiveness in infants managed on post-natal units, in NICUs or with Finnegan scoring. This intervention is likely safe given the absence of reported adverse effects in previous music studies. Further, future studies should include studying maternal song in healthy, full term infants and in infants requiring NICU admission for medical conditions apart from prematurity and NAS. Ultimately, these studies could lead to widespread use of recorded maternal song to promote stability among all neonates.

4.2 CLINICAL AND PUBLIC HEALTH SIGNIFICANCE

Infants diagnosed with NAS experience postnatal withdrawal symptoms including excessive crying and disrupted sleep which adversely affect infants and their caregivers. As the incidence of NAS continues to rise, the cost of NAS and demand on caregivers has increased dramatically. Soothing environments marked by maternal care and low stimulation are hallmarks of non-pharmacologic care for infants with NAS that can ease withdrawal symptoms. If the findings of this study demonstrate that recorded maternal song decreases crying duration among affected infants, providers will have an inexpensive and accessible adjunct to non-pharmacologic care to ease withdrawal and irritability among infants with NAS. Ultimately, recorded maternal song may offer a tool to soothe infants both in hospital and at home ultimately reducing the burden of care on both healthcare workers and mothers alike.

4.3 REFERENCES


APPENDICIES

APPENDIX A: PARENTAL CONSENT FORM

COMPOUND AUTHORIZATION AND CONSENT FOR PARTICIPATION IN A RESEARCH PROJECT

YALE UNIVERSITY SCHOOL OF MEDICINE
YALE NEW HAVEN HOSPITAL
BRIDGEPORT HOSPITAL
LAWRENCE AND MEMORIAL HOSPITAL

Study Title: RECORDED MATERNAL SONG TO REDUCE CRYING DURATION IN NEONATAL ABSTINENCE SYNDROME

Principal Investigator Matthew R. Grossman, MD
Co-Investigator: Alecia Cunniff, PA-SII
Affiliation: Yale University School of Medicine and Yale New Haven Health System

Research Study Summary
- We are asking you to join a research study.
- The purpose of this research study is to examine the effect of recorded maternal song on crying duration among infants with neonatal abstinence syndrome (NAS).
- Study procedures will include exposure to maternal recorded song or ambient noise, voice activated recording of infant crying continuously for 72 hours, pulse oximetry recording of heart rate, respiratory rate and oxygen saturation, logging of pharmacotherapy use and frequency, calories consumed per day and weight gain.
- No additional visits are needed after discharge from the hospital.
- There are some risks and inconveniences of participating in the study including decreased heart rate, decreased rate of breathing and decreased oxygen saturation; however these vital signs will be monitored throughout your infant’s hospital stay. Inconveniences include restriction on infant holding during daily noise intervention.
- This study may not have benefits for your infant. This study may or may not reduce crying and irritability in in your infant. This study has the potential to guide non-pharmacologic treatment of infants with NAS and strengthen the mother-infant bond.
- There are other choices available to you outside of this research. If you choose not to participate in this study, your child will still receive high quality care to manage their NAS and you will have the opportunity to sing for them at the bedside if you desire.
- Participating in this study is your choice. You can choose to take part, or you can choose not to take part in this study. You can change your mind at any time. Regardless of your choice, you will not lose access to your medical care or give up any legal rights or benefits.
At the end of the study, we will provide you with an electronic copy of your recorded song for use at home if you desire. After your infant is discharged, we recommend relying upon your preferences and child’s pediatrician for guidance on continued singing to your infant. We will not collect any information about your infant after discharge.

If you are interested in learning more about the study, please continue reading, or have someone read to you, the rest of this document. Take as much time as needed to make your decision. Please ask study staff for clarifications and questions you have. Once you understand the study, we will ask you if you wish to participate. If so, you will sign this form.

Why is this study being offered to me and my infant?
We are asking you and your infant to take part in a research study because you are pregnant or recently post-partum and expressed use of methadone, buprenorphine or opioids during a period of or throughout your pregnancy. Your baby is likely to experience some symptoms associated with neonatal abstinence syndrome (NAS). This study may help to regulate their crying and irritability in the first four days of life. We are looking for approximately 150 participants to be part of this research study.

Who is paying for this study?
The Yale Physician Associate Program at the Yale School of Medicine will be paying for this study.

What is this study about?
The purpose of this study is to evaluate the effect of recorded maternal song compared to ambient hospital noise on mean daily crying duration in infants born with NAS.

What are you asking me to do and how long will it take?
If you agree to participate in this study, this is what will occur:

Participation in this study will focus on the effect of maternal recorded song on your infant. You will be asked to record “Twinkle, Twinkle Little Star” with the help of a research assistant for playback to your infant. Each day one hour after feeding, your infant will be exposed to either the recorded song or ambient hospital noise. You will not be allowed to speak to or hold your infant for 30 minutes after the feeding during the 30-minute noise exposure or for 30 minutes after while the infant is monitored. Nursing staff will assist you in completing a form daily indicating the amount of time spent singing to your infant, holding your infant or performing skin to skin contact with your infant, however, aside from time surrounding the noise intervention, singing, holding and skin to skin contact will not be restricted.

What are the risks and discomforts of participating?
Participation in the study involves randomization to maternal recorded song or ambient hospital noise. This decision will be assigned through randomization. There is not currently a standard of care for the role of recorded maternal song for infants with NAS. Maternal song is used at maternal discretion in affected infants. You will still be allowed
to sing to your infant regardless of treatment group. Though uncommon, there may be potential risks to increase arousal of your infant or reduce vital signs with exposure to recorded song depending upon your infant’s gestational age. The study team will monitor your infant throughout the study and will stop the study in the unlikely case of an adverse event. The study team will guide you through education on how to console and comfort your infant.

**How will I know about new risks or important information about the study?**
We will tell you if we learn any information that could change your mind about taking part in this study.

**How can the study possibly benefit me?**
This study may result in decreased crying time of your infant and a stronger mother-infant bond. You will be provided a copy of your recorded song for home use.

**How can the study possibly benefit other people?**
This study will benefit science by developing an understanding of the use of recorded maternal song as a non-pharmacologic adjunct to care for infants born with NAS. This will inform non-pharmacologic management and consoling of affected infants.

**Are there any costs to participation?**
If you wish to participate in this study, you will not incur any additional cost outside of routine hospital care. You or your health insurance must pay for services, supplies, procedures and care that is part of routine medical care for infants with NAS. You will be responsible for any co-payments required by your insurance.

**Will I be paid for participation?**
You will not be paid for taking part in this study.

**What are my choices if I decide not to take part in this study?**
Instead of participating in this study you have other choices. You could
- Get treatment without being in the study. Your infant will still be treated for any irritability and crying related to NAS according to standard hospital protocol.
- Take part in another study.

**How will you keep my data safe and private?**
We will keep information we collect about you and your infant confidential. With your consent, we will share your data if you agree to it or if mandated by U.S. or State law. For example, we will tell somebody if we learn that you are hurting a child.

When we publish the results of the research or present it in conferences, we will not use your infants name, date of birth or any identifying information. All personal health identifiers and confidential information will be stored on an encrypted, password-protected laptop in a secure Box. All data collected from you, or your infant will be de-
identified and connected to a random series of numbers that cannot be traced back to you. All HIPAA identifiers will be deleted upon completion of the study.

Identifiers will be separated from identifiable private information. After removal, information may be used for future research or distributed to another investigator for future research studies without additional informed consent of the subject.

**What information will you collect about me in this study?**
The information we are asking you to share is called “Protected Health Information”. It is protected by a federal law called the Privacy Rule of the Health Insurance Portability and Accountability Act (HIPAA). We cannot use or share your health information for research without your permission. If desired, we can provide more information about the Privacy Rule. If you have additional questions about your rights, you can speak to a Yale Privacy Officer at 203-432-5919.

We will collect information about you and your health as well as that of your child. Information we will collect, use and share includes:
- Research study records including phone calls and study visits
- Medical and laboratory records of only services provided in connection to this study
- The entire research record and related medical records for the mother and infant held by Yale New Haven Hospital

**How will you use and share my information?**
We will use your information to conduct the study outlined in this consent form. Information collected during the study will be used to determine best practices for recorded maternal song intervention in infants with NAS. If shared, your information will be shared only in aggregate form without personal identifying information.

We will use your information to conduct the study described in this consent form. We may share your information with:
- The U.S. Department of Health and Human Services (DHHS) agencies
- The U.S. Food and Drug Administration (FDA)
- Representatives from Yale University, the Yale Human Research Protection Program and the Institutional Review Board (a committee that reviews, approves and monitors research on human participants), who together are responsible for ensuring research compliance. These groups are required to keep all information confidential
- Government agencies to whom certain diseases (reportable diseases) must be reported
- Health care providers who provide services to you in connection with this study
- Laboratories and other individuals and organizations that analyze your health information in connection with this study, according to study plan
- Principal investigator of the study
- Co-Investigators and other investigators
- Study coordinator and members of the research team
Independent data consultant and others authorized to monitor the conduct of the study

We will do our best to ensure your information stays private. But, if we share information with individuals who do not have to follow the Privacy Rule, your information will no longer be protected by the Privacy Rule. Please ask any questions you have. To best protect your health information, agreements are in place with individuals and companies that require them to keep your information confidential.

**Why must I sign this document?**
By signing this form, you will allow researchers to use and disclose your information described above for this research study. This is to ensure that the information related to this research is available to all parties who may need it for research purposes. You always have the right to review and copy your health information in your medical record. You will not be allowed to look at or copy information related to the study until after research is complete.

**What if I change my mind?**
The authorization to use and disclose your health information collected during your participation in this study will never expire. However, you may withdraw or take away your permission at any time. You may withdraw your permission by telling the study staff or writing to the principal investigator **Matthew Grossman, MD at Pediatric Critical Care Medicine PO Box 208064** at Yale University, New Haven, CT 06520-8046.

If you withdraw your permission, you will not be able to stay in the study, however, the care you receive from your provider outside of the study will not change. No new health information will be gathered after the date of your withdrawal. Information already collected may still be used and given to others until the end of the research study to ensure integrity of the study.

**Who will pay for treatment if I am injured or ill due to participation in the study?**
If you or your infant are injured during the study, seek treatment and contact the study doctor as soon as you are able. Yale School of Medicine and Yale New Haven Hospital do not provide funds for the treatment of research related injury. If you or your infant are injured as a result of participation in this study, treatment will be provided. You or your insurance carrier will be expected to pay the costs of this treatment. No additional financial compensation for injury or lost wages will be available. By signing this form you do not give up any of your legal rights.

**What if I want to refuse or end participation before the study is over?**
Participating in this study is your choice. You can choose to take part, or you can choose not to take part in the study. You can change your mind at any time. No matter what choice you make, you will not lose access to your medical care or give up any legal rights or benefits.
We will still treat your infant with standard therapy, or if you request, refer you and your infant to a clinic or doctor who can provide such treatment. Not participating or choosing to withdraw will not damage your relationship with your doctors or with this institution.

To withdraw from the study, you can call a member of the research team at any time and inform them that you no longer wish to participate in the study.

The researchers may withdraw you from participating in the research if necessary. Your infant will be withdrawn from the study if your infant:
- Experiences decreased heart rates
- Experiences periods without breathing
- Experiences decreases in oxygen saturation

**What will happen with my data if I stop participating?**
If you choose to stop participating in the study, we will use the data (crying duration and reported adverse events) obtained up until the day of withdrawal. No further data will be collected. Also, if the study is stopped early by choice or because of an adverse event above, no further data will be collected. The recorded song will be stored electronically with a deidentified number.

**Who should I contact if I have questions?**
Please feel free to ask about anything you do not understand.

If you have further questions about this project or if you have a research-related problem, you may contact the Principal Investigator Dr. Matthew Grossman at 203-785-465.

If after you have signed this form, you have any questions about your privacy rights, please contact the Yale Privacy Officer at 203-432-5919.

If you would like to talk with someone other than the researchers to discuss problems, concerns and questions you may have concerning this research, or to discuss your rights as a research subject, you may contact the Yale Human Investigation Committee at (203) 785-4688.

**Authorization and Permission**
I have read (or someone has read to me) this form, and I have decided to allow both my child and me to participate in the project described above. Its general purpose, the involvement of my child and potential hazards and inconveniences have been explained to me in full. My signatures also indicates that I have received a copy of this permission form.

By signing this form, I give permission to the researchers to use and collect information about my child for the purposes outlined in this form. By refusing to give permission, I understand that my child will not be included in this research.
<table>
<thead>
<tr>
<th>Participant Name (Mother)</th>
<th>Participant Signature (Mother)</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participant Name (Infant)</td>
<td>Parent Signature</td>
<td>Date</td>
</tr>
<tr>
<td>Person Obtaining Consent Printed Name</td>
<td>Person Obtaining Consent Signature</td>
<td>Date</td>
</tr>
</tbody>
</table>
APPENDIX B: CRYING DURATION EXTRACTION FROM AUDIO RECORDING

RECORDED MATERNAL SONG TO REDUCE CRYING DURATION IN NEONATAL ABSTINENCE SYNDROME
PI Dr. Mathew Grossman, Co-PI Alecia Cunniff PA-SII

Complete the crying increment form for the twenty-four-hour voice activated recording as trained and calculate the specified measures below.

**TABLE 1: CRYING INCREMENTS**

<table>
<thead>
<tr>
<th>Increment</th>
<th>Start Time</th>
<th>Stop Time</th>
<th>Duration</th>
<th>Time of Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>15</td>
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</tr>
</tbody>
</table>

**Total crying duration:**

**Longest crying duration:**

**Total number of crying episodes:**

**Average duration of crying:**

**Number of crying episodes 12am – 6 pm (night):**

**Number of crying episodes 6am – 12 pm (morning):**

**Number of crying episodes 12pm – 6pm (afternoon):**

**Number of crying episodes 6pm – 12 am (evening):**

**Total crying duration = sum of all durations in Table 1**

**Longest crying increment = longest recorded duration**

**Average duration of crying = Sum of all crying durations divided by the number of crying increments**
APPENDIX C: MATERNAL BESIDE INFANT CARE

RECORDED MATERNAL SONG TO REDUCE CRYING DURATION IN NEONATAL ABSTINENCE SYNDROME
PI Dr. Mathew Grossman, Co-PI Alecia Cunniff PA-SII

| Mother’s Name               | ______________________ |
| Infant’s Name               | ______________________ |
| Date of Recording           | ______________________ |
| Nurse Receiving Form        | ______________________ |

INSTRUCTIONS
Please record the time spent holding your infant during your visit today as well as the time spent singing to your infant. You are not required to either sing to or hold your infant during your visit but please record all singing and holding times below.

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Start Time</th>
<th>Stop Time</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singing to Infant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Holding Infant</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**APPENDIX D: SAMPLE SIZE CALCULATION**

<table>
<thead>
<tr>
<th>Group</th>
<th>Population Mean</th>
<th>Standard Deviation</th>
<th>N Per Group</th>
<th>Standard Error</th>
<th>95% Lower</th>
<th>95% Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population 1</td>
<td>222.100</td>
<td>110.700</td>
<td>71</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Population 2</td>
<td>169.400</td>
<td>110.700</td>
<td>71</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Mean Difference**

| Mean Difference | 52.700      | 110.700    | 142         | 18.579         | 16.055    | 89.345    |

Alpha = 0.050, Tails = 2

Power = 80%


