Study Protocol

Title: Randomized Controlled Trial to Improve Multisensory Neural Processing, Language & Motor Outcomes in Preterm Infants

NCT Number: NCT03232931

Protocol date: December 19, 2022

PROTOCOL TITLE: **Trial to Improve Multisensory Neural Processing, Language & Motor Outcomes in Preterm Infants**

NCT03232931

PRINCIPAL INVESTIGATOR: Nathalie Maitre, MD, PhD
Department of Pediatrics/Division of Neonatology
Nathalie.linda.maitre@emory.edu

VERSION: **3.0 Emory version**

National Institutes of Health (NIH)
Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)

Table of Contents

Study Summary

Project Title	**Trial to Improve Multisensory Neural Processing, Language & Motor Outcomes in Preterm Infants**
Project Design	Randomized Controlled Trial
Primary Objective	To demonstrate that preterm infants receiving a standardized, parent-supported, auditory-tactile intervention in addition to standard of care in the NICU will have more typical cortical multisensory processing at discharge to home and better sensory adaptation and motor and language outcomes than infants receiving only standard of care.
Secondary Objectives	To test the role of multisensory responses at discharge in mediating intervention effects on later sensory adaptation, and motor and language outcomes. To explore the role of m responses in mediating intervention effects on later motor and language outcomes.
Research Interventions/Interactions	Listed in Table 1 on page 4
Study Population	Preterm infants 32-36 weeks postmenstrual age(PMA)
Sample Size	230
Study Duration for individual participants	NICU intervention between 32-36 week PMA/Follow up at 3 months to 2 years of age
Study Specific Abbreviations/ Definitions	Bayley III – Bayley Scales of Infant Development 3rd edition BCQ – Baby Care Questionnaire HNNE – Hammersmith Neonatal Neurological Examination ITSP – Infant Toddler Sensory Profile MSP – Multisensory Procedures PLS5 – Preschool Language Scales 5th edition

	PSDQ – Parenting Styles and Dimensions Questionnaire STS – Skin to Skin
Funding Source	National Institute of Child Health and Development

Table 1

Construct	Procedure(s)	Timing	Variable derived	Variable type
Multisensory response (Auditory-Tactile processing)	ERP to simultaneous puff + speech sound	Pre and post intervention (near discharge)	Pre-Post change in amplitude at frontal scalp locations in 176-377 ms time window	continuous
			IMP: Percentage of time spent in near-typical neural activation	continuous
Sensory reactivity and adaptation to environment	ITSP	9-12 months	Behavioral neurological threshold score (typical or atypical)	nominal
			Raw score in sensory processing domain	continuous
Language	Bayley III	22-26 months	Composite standard score Scaled standard scores for expressive and receptive components	continuous
Motor	Bayley III	22-26 months	Composite standard score Scaled standard scores for fine and gross components	continuous
	Neurological Exam	9-12 months 22-26 months		
Tactile processing	ERP to calibrated air puff	Baseline/ After intervention	Amplitude of response in 171-240 time window at central and frontal scalp locations	continuous
Functional tactile connectivity	ERP to calibrated air puff	Baseline/ After intervention	Absolute clustering coefficient of alpha band coherence network	continuous
Speech Sound differentiation	ERP to 6 speech sounds	Baseline/ After intervention	Difference in absolute amplitude between /du/ and /gu/ in frontal scalp locations in 250-350 ms time window	continuous

Introduction

Background and rationale

Every year, half a million infants are born prematurely in the United States and 15 million worldwide. The vast majority of preterm infants will have only moderate to mild impairments or delays in early childhood, with intellectual and behavioral consequences of prematurity only apparent at school age and beyond. These infants contribute most of the societal and economic burden of preterm birth, yet have often been overlooked in the face of other devastating and immediately visible consequences of extreme prematurity. Almost all preterm infants suffer from atypical brain maturation and its developmental consequences resulting from interactions between brain immaturity and premature extra-uterine sensory experience.

Brain development in the neonatal period is experience-dependent, yet the neonatal intensive care experience is largely comprised of atypical sensory stimuli. The critical importance of establishing functional sensory systems in infancy as the basis for all higher order processes (cognition, communication, behavioral adaptation) has been demonstrated in both animal models and humans. Preterm infants at discharge to home often have altered sensory reactivity and modulation in response to their environment, which are associated with negative neurodevelopmental outcomes in childhood. For the auditory system, preterm infants with deficient exposure to infant-directed speech sounds have poorer brain microstructure at discharge and worse neurodevelopmental outcomes at two years. For the tactile system, preterm infants with higher numbers of painful experiences have worse processing of light touch at discharge, decreased thalamic organization, decreased behavioral reactivity to tactile stimuli in the home environment, and less typical multisensory processing. Finally, brain responses to multisensory stimuli are supra-additive (greater than the sum of brain responses to unisensory stimuli).

Parents are critical in scaffolding early learning and development, especially with regards to early sensory exposures and responses. In particular, parental linguistic input is a key concept in learning language. Importantly, this input is more effective when it is contingent and immediate (i.e., language is presented immediately after infant action). This precept holds true even in early pre-linguistic phases, when infants differentiating among speech sounds, which is necessary for later development of higher-order language milestones.

Another type of scaffolding provided by parents is the multisensory support of skin-to-skin (STS), which helps maintain quiet and alert states in immature infants who have frequent autonomic instability. Unfortunately, providing STS is often challenging for parents who often have to travel long distances to see their infant, while balancing responsibilities of other children and jobs, in addition to potential unreliable transportation and/or lack of social support.

Multisensory processes (MSPs) are rarely studied in neonates, yet in children and adults MSPs are essential to building a coherent and unified perception of the world, a foundation for learning and social interactions. In order to improve multisensory systems function of hospitalized preterm infants, further research is needed to design rigorous, standardized and mechanistically-based multisensory interventions using feasibly modifiable sensory exposures. There are currently no interventions that address rehabilitation of sensory function in the neonatal period, when brain-plasticity is at its greatest and when improvements can have an exponential downstream effect on later neurodevelopment.

The few associative studies, while critical, have not examined causal effects of interventions on neural processing changes to mediate neurodevelopmental outcomes. Fortunately, more infant-directed speech is associated with better language outcomes in the first 18 months and increased STS care with improved autonomic system stability and muscle tone in the hospital, and improved behavioral and motor outcomes in infancy. However, ours is the first study to use brain-based measures to test predictions regarding how the brain changes in response to multisensory treatment, which in turn affects functional outcomes.

Our study is in direct response to the NIH announcement calling for applications that "elucidate the mechanisms and/or behavioral outcomes of multisensory processing, the integration or processing of at least two distinct types of sensory input" (PA-15-347). In addition, our study meets two of the top new research priorities of the National Center for Medical Rehabilitation Research, to "Explore multimodal approaches that promote plasticity and sensorimotor function" and to "Develop objective measures to monitor functional progress."

To accomplish these goals, our team has leveraged expertise in high-density functional EEG in the NICU, showing that we can rapidly and non-invasively acquire large datasets of brain-based sensory measures and analyze them with innovative spatiotemporal computational tools.

We have utilized these methodologies in pilot studies demonstrating that speech sound differentiation of preterm infants improves in response to a parent-supported standardized

language intervention and that preterm infants exposed to STS care have increased multisensory processing efficiency compared to those who do not.
We have now designed a prospective, interventional RCT in preterm infants aimed at restoring more typical multisensory, rather than the unisensory, processing measures that were the outcomes of our pilot studies.

Our multisensory intervention using parents' voice and nurturing touch can be administered regularly in the NICU during sensitive periods of sensory development, even when parents cannot always be present. Our test of this multisensory intervention will involve 23 sessions of standardized, therapist-administered, auditory-tactile stimulation. This treatment will combine contingent presentation of recorded mother's voice delivered using a suck-activated system during holding with supportive tactile containment against the therapist's chest. This treatment will be tested in an internally-valid RCT. In addition to testing the efficacy of the treatment on gold-standard measures of language and motor functioning, we will also test whether the treatment effect is due to intermediate treatment effects on multisensory and unisensory processing. Understanding the mechanism by which the treatment works is important for laying the foundations of future improvements and potential recommendations for treatment that might be useful for wide-spread use even in lower resource settings with the ultimate goal of improving neurodevelopmental outcomes for these premature infants.

Methods and Analysis

Study aims

Our aims for this study are first, to demonstrate that preterm infants receiving a standardized, parent-supported, auditory-tactile intervention in addition to standard of care in the NICU will have more typical cortical multisensory processing at discharge to home and better sensory adaptation and motor and language outcomes than infants receiving only standard of care. Second, to test the role of multisensory responses at discharge in mediating intervention effects on later sensory adaptation, and motor and language outcomes. Finally, to explore the role of m responses in mediating intervention effects on later motor and language outcomes.

Study design and setting

In order to test these aims, we are conducting an RCT of a multisensory (MS) intervention with 230 hospitalized preterm infants. Infants starting at 31 weeks will be randomized to either the control or intervention group. Both the control and intervention groups will receive standard care. Infants assigned to the MS group will also receive the MS intervention over two weeks,

starting at 32 0/7 weeks postmenstrual age (PMA). ERP testing will be performed prior to intervention and after intervention is complete., which occurs at 36 weeks PMA on average. ERP testing takes approximately 30 to 40 minutes. All infants will be seen at the NICU Follow-Up Program clinics at 3-4months PMA, 9-12 months PMA (Year 1) and 22-24 months PMA (Year 2), where neurodevelopmental outcomes will be assessed using standardized methods. If for any reason these standardized assessments are not able to be performed at the NICU Follow Up Clinic (e.g., parent request, infant not eligible to be seen in the NICU Follow Up Clinic), then the follow up visits will be conducted in the BBOP lab space with trained study personnel. Home visits may be provided on occasion, at the discretion of the Principal Investigator. If an in person visit is not able to be performed, we may do assessments virtually (e.g., zoom).

Study procedures

For infants who meet inclusion criteria at 31 weeks, consent is obtained and parent's voice is recorded and parent-scented T-shirt may be collected when possible. Study staff may approach parents of eligible infants about the study and obtain consent slightly before the infant is 31 weeks, however, other study procedures will not start until the infant reaches 31 weeks. Infants are then randomized to the control or intervention group. If a patient is randomized to the control group, parents will be given the opportunity to consent to the secondary part of the study if they are interested. This part of the study is observational only and does not influence randomization. ERP testing is performed prior to the intervention. Infants assigned to the MS group receive the standardized MS intervention over 2 weeks, starting at 32 0/7 weeks PMA or at enrollment prior to 36 weeks. For both control and intervention groups, standard care includes skin-to-skin care on parent, when present, and playing of parents' voice non-contingent on infant suck. Parents may be asked to complete the Baby Care Questionnaire (BCQ) during infant's stay in the NICU or as soon as possible after discharge. Prior to the infant's discharge, a brief neurological examination (HNNE) and motor assessment (GMA) may be completed. ERP is repeated after intervention is completed, which occurs at 36 weeks PMA on average.

All infants are seen at the NICU Follow-Up Program clinics at 3-4, 9-12 (Year 1) and 22-26 months (Year 2). At the 3-4 month visit, study staff may obtain the Baby Care Questionnaire (BCQ), a brief neurological exam (HINE), and a brief motor assessment (GMA). At the Year 1 visit, study staff may obtain the BCQ, Infant Toddler Sensory Profile (ITSP), HINE, GMA, Bayley III, and the Parenting Styles and Dimensions Questionnaire (PSDQ). At Year 2, study staff may obtain the the Bayley III, HINE, BCQ, CBCL, PSDQ, and PLS-5.

If for any reason these standardized assessments are not able to be performed at the NICU Follow Up Clinic (e.g., parent request, infant not eligible to be seen in the NICU Follow Up Clinic), then the follow up visits will be conducted in the BBOP lab space with trained study personnel. Home visits may be provided on occasion, at the discretion of the Principal Investigator. If an in person visit is not able to be performed, we may do assessments virtually (e.g., zoom). Individual assessment results may be shared with the families at their request and after the scoring has been completed. In addition, if a participant is seen outside of the NICU Follow Up clinic for their study follow up assessments, we may also share results with a participant's primary care provider or the NICU Follow up Clinic (with the parent's permission) with any concerns that were identified.

Inclusion and Exclusion Criteria

The study population was originally comprised of 200 preterm infants. Due to Covid 19 losses to follow up, this number has been revised to 230 preterm infants. We anticipate the distribution to be 45% male and 55% female, as females have a small survival advantage after extreme prematurity. All races and ethnicities will be included, with an expected skew towards slightly more black children due to disproportionate effects of prematurity on race.

Inclusion criteria includes PMA 32 0/7 weeks gestation-36 0/7 weeks gestation. Exclusion criteria will be ventilation using an endotracheal tube, major congenital malformations, family history of genetic hearing loss, and use of sedatives or seizure medications. These medications may mask sensory processing as assessed by ERPs.

Randomization

Random assignment to groups will be carried out using unified reproducible methods (i.e. saved random number seeds) provided by our Biostatistics core. We will try to conceal allocation from all study personnel who could influence scores. Except for parent reports, examiners will be blind to treatment assignment. We will obtain recorded parent's voice from both groups with the intent of masking parents to assignment. Although we cannot fully mask parents to group allocation, as bedside nurses communicate regularly with them, we will address the potential effect on the parent questionnaire at one-year by deemphasizing the sensory nature of the outcomes to both groups, and instead focusing on the proven positive links between parental involvement and improved intersubjectivity.

In addition to randomization, we have addressed the problems of RCT design as follows:
Adherence to treatment protocol: A highly manualized treatment for the MS treatment while monitored control treatments will be used by hospital staff during NICU hospitalization. These measures should help ensure uniform, high-quality implementation of the MS treatment. Sufficiently and randomly sampled fidelity of treatment (FOT) measures will be collected. Two of the first twelve MS sessions will be video recorded for study team review to ensure FOT. Treatment is provided in the NICU by experienced staff therapists.

Total attrition: Total attrition is expected to be less than 10% We have experienced <5% data loss due to non-compliance with electrode placement in our prior studies. Should motion artifact occur, we will retest patients with insufficient data within 24 hours. For the Bayley, should children prove unable to complete follow-up testing during scheduled visits, they will be rescheduled within one week of the scheduled visit. Testers are trained to perform the Bayley III in the home environment, if necessary, The ITSP can be performed over the phone or through the mail if necessary and will be performed within one week of a missed visit.

The BCQ and PSDQ may be completed by the parent in writing or via phone. Analysis will follow an intent-to-treat protocol. As such, all randomized participants will be analyzed and missing data will be handled using multiple imputation.

Differential attrition: Differential attrition is unlikely because children must be in the NICU during the treatment phase due to health concerns. There are no non-NICU treatments during the treatment phase. Thus, compensatory responses due to parental displeasure of group assignment is unlikely.

Covariates: Biological variables are factored into our research design as potential covariates. Several pretreatment covariates will be statistically controlled if needed. These variables will be quantified at the pretreatment period, as past research or theory suggests they may be associated with neurodevelopmental outcomes or sensory processing. If preliminary tests of between-group differences of these pretreatment variables and the pretreatment variables showing between-group differences are associated with putative mediators or neurodevelopmental outcomes, these will be statistical controlled. Their statistical interaction with treatment group must be tested as part of the process of determining whether they should be statistically controlled. Thus, the possibility that treatment varies as a function of these biological variables will also be explored. These include: gestational age at birth, sex, presence of severe white neural injury on neuroimaging (intraventricular hemorrhage grade III or IV, periventricular leukomalacia, cerebellar hemorrhage, ischemic or thrombotic injury), presence of systemic inflammatory conditions (history of necrotizing enterocolitis Bells Stage

IIA or above, culture documented sepsis or meningitis, moderate or severe bronchopulmonary dysplasia per modified Shennan definition), cumulative caffeine exposure post intervention, total parental skin-to-skin time during the study period and pretreatment status on the General Movements Assessment (GMA).

Intervention

Standard Care

The standard care of infant currently follows two medical protocols, one for STS holding and one for exposure to parent's voice. The protocols will be enhanced and monitored as follows:

Exposure to recorded parent's voice:

Preterm infants in the NICU currently receive noncontingent recorded parents' voice during two 20 minute sessions per day. Recordings are standardized. Recordings are then played through a sterilizable device (DINO-egg).

To enhance standard care, we will ensure that all parents have a voice recording before study start when possible so that the majority of infants are exposed to parent's voice. A standard therapist recording may be used when it is not possible to obtain parent's voice. Monitoring of standard care will be accomplished through bedside logs, designed to be filled out by nursing staff and family when DINO-egg is played.

Skin-to-skin holding: Per standard care, STS care will be carried out by parents in both groups. Parents in the NICU currently use either their hands or positioners to facilitate prolonged STS. Infants are placed in a prone position with head positioned over the sternum, allowing transmission of breath and heart sounds to the developing ear. Deeper pressure is applied to offer support and feedback to the child's bottom. Session length is set to a minimum of 45 minutes per unit protocols. When primary caregivers are not comfortable with direct skin-to-skin contact (e.g. are not a direct relation to the child, or parent or child skin problem), a thin single-use hospital gown that is not previously imprinted with the parent's scent is used to facilitate the experience without hindering sound transmission. In order to ensure safety during STS holding, vital signs including heart rate, breathing patterns and rate, oxygen saturation and temperature are continuously and automatically monitored with preset alarms per unit protocol. If any negative deviation from the infant's daily vital patterns occurs, the nurses examine the infant and decide whether to stop the STS.

To monitor STS, we will review the medical record for daily start time, duration and caregiver during STS. We may also leave a STS log at bedside for parents who provide frequent STS to

facilitate greater recording accuracy. We will also record any deviations from autonomic stability during STS (tachy/bradycardic events tachypnea or apnea).

Multisensory Intervention

Rational: Studies have shown that infant acquisition of early speech sound differentiation in term and preterm infants is essential to the construction of more complex cortical functions such as receptive language development. Furthermore, learning of language in infants is improved when language exposure is presented contingent on infant action and promptly after the action. Therefore, we will use preterm infants' active non-nutritive sucking as the behavior that activates an audio recording of mother's voice within a one second after the suck. The mother's voice provides auditory input, which purportedly contributes to the infants' representation of initial speech sound categories.

The mother's voice is a preferred stimulus and it's frequency and patterns are distinguished early in life from other sounds.

The infant must repeat the action every ten seconds to continue to receive the positive reinforcement of mother's voice. This approach enables contingent linguistic input by the infant's mother's voice without parent having to be present. Because infant uptake of linguistic input may be enhanced during periods of calm attention to the auditory stimulus, the contingent linguistic input will be provided while the infant is in a state of autonomic stability. Thus, we will present the suck-contingent playing of parents' voice while skin-to-skin (STS) holding occurs. STS holding is a form of MS stimulation combining tactile, vestibular, olfactory and auditory input that has been demonstrated to improve autonomic nervous system stability. Although STS can induce deep sleep, the contingent presentation of parent's voice will be provided at the beginning of STS containment, when past work indicates that infants are still awake.

MS intervention protocol: The MS intervention will be carried out in addition to standard care and will include two components: holding and light pressure containment of the infant against the cotton T-shirt covered chest of the therapist for tactile and non-specific auditory stimulation **simultaneous with** playing of mother's voice contingent on infant pacifier sucking for during a study session of no more than 15 minutes duration. No more than one study session will be completed per care period for each subject. Additionally, when possible, a 100% cotton T-shirt square scented with parent's skin may be placed within 2 cm of infant's face on the therapists' chest, to provide olfactory stimulation without risk of suffocation. The 12-23 intervention sessions will be dispersed across 2-3 weeks.

On the rare occasion that the parent is present, parental STS will always take precedence over the intervention and the intervention session will be separated from previous parental STS by a minimum of 1 nursing care interval (3 hours) in one 24-hour period. Infant autonomic stability during the MS intervention with the therapist will be recorded as above for parent STS

Contingent parent's voice exposure: We will use the Pacifier Activated Lullaby® (PAL®) device, a 510k FDA approved digital music delivery system that integrates a sensor, a pacifier routinely used in the NICU, and a receiver). It delivers a predetermined 10 seconds of recorded parent's voice singing lullabies upon detection of a suck that meets a preset pressure threshold. The original systems were modified for research use by decreasing the lower limit of activation thresholds for delivering the recording. Minimal effort is required to trigger the device.

However, the settings ensure that regular attempts are needed to continue to receive continual presentation of the recording of mother's voice by requiring another suck after 10 seconds.

The auditory stimulation with PAL will be provided when the infants are still awake (i.e., at the beginning of the session).

STS holding: The therapist will wear a clean 100% cotton T-shirt with a clean hospital gown on top of the shirt (dependent on hospital guidelines) and wrap the "kangaroo" positioner securely over the gown. The positioner will allow containment with one-hand and PAL operations with the other. Should assistance be required, personnel will use the unit standard personal voice-activated call system to request a team member and minimize disruptions to the infant. The parent-scented T-shirt squares may be placed next to the infant's nose will be obtained by having the parent wear the T-shirt for a minimum of one hour and storing it in a sealed bag when possible. Once collected, the shirt will be cut into squares, and when possible, the first square will be used within 3 hours of cutting for the multisensory intervention. After each session the cloth will be stored in its own sealed bag and a new square will be used every 3 days. Each square will be replaced if contaminated with infant's bodily fluids.

Relaxation training of the MS therapy team: Because effects of STS holding are thought to be partly mediated by the holder's heart and respiratory rate, it is essential to maintain calm throughout the PAL administration in the way that parents would while holding their infant without additional activities. To promote this, therapists will be trained on mindfulness techniques and practice this prior to start of the intervention.

Ensuring high intervention fidelity*:*

The intervention will be implemented using a standardized training procedure implemented in the PI's lab for fidelity of parent training in a previous clinical trial. A script for the therapy session detailing the essential steps and sequence of the procedure is produced in a video. All

study therapists and an independent observer in the laboratory study the videos prior to beginning implementation. During a common training phase, the observer scores the therapists on all aspects of the treatment, as indicated by the aforementioned script sequence. The therapists also score themselves on the fidelity rating scale in order to immediately compare their self-assessments with those of the observer. The training phase is concluded when there is 90% adherence to the protocol and concordance between therapists and observer scoring. This allows for development of a gold-standard non-therapist observer and back-up observers that will be used to monitor possible drift during the treatment phases.

A random sample of 10% of all MS treatment sessions will be video recorded and assessed for fidelity with the fidelity rating scale by the trained observer.

Twenty percent of the sessions coded for MS treatment fidelity will be randomly selected and independently recoded for fidelity by a second observer to estimate inter-observer reliability of the fidelity scores.

Assessment Methods

Our outcomes (or constructs) of interest are listed in Table 1. These constructs are quantifiable using well validated tests and measures. Our primary outcome is multisensory response and our secondary outcome is neurodevelopmental outcomes, including sensory adaptation, motor, language, tactile processing, and speech sound differentiation.

Table 1

Construct	Procedure(s)	Timing	Variable derived	Variable type
Multisensory response (Auditory-Tactile processing)	ERP to simultaneous puff + speech sound	Pre and post intervention (near discharge)	Pre-Post change in amplitude at frontal scalp locations in 176-377 ms time window IMP: Percentage of time spent in near-typical neural activation	continuous continuous
Sensory reactivity and adaptation to environment	ITSP	9-12 months	Behavioral neurological threshold score (typical or atypical) Raw score in sensory processing domain	nominal continuous
Language	Bayley III	22-26 months	Composite standard score Scaled standard scores for expressive and receptive components	continuous
Motor	Bayley III Neurological Exam	22-26 months 9-12 months 22-26 months	Composite standard score Scaled standard scores for fine and gross components	continuous
Tactile processing **Functional tactile connectivity**	ERP to calibrated air puff ERP to calibrated air puff	Baseline/ After intervention Baseline/ After intervention	Amplitude of response in 171-240 time window at central and frontal scalp locations Absolute clustering coefficient of alpha band coherence network	continuous continuous
Speech Sound differentiation	ERP to 6 speech sounds	Baseline/ After intervention	Difference in absolute amplitude between /du/ and /gu/ in frontal scalp locations in 250-350 ms time window	continuous

Primary Outcome Measures

Sensory processing measurement by ERP:

ERP Recording: A high-density array of 128 electrodes embedded in soft sponges (Hydrocel Sensor Net, EGI, Inc., Eugene, OR) will be used to record ERPs with a sampling rate of 1000 Hz, filters set to 0.1-400 Hz (Figure 2a). Recording of brainwaves will be controlled by Net Station (v. 4.3; EGI, Inc., Eugene, OR). E-Prime (v. 4.0, PST, Inc., Pittsburgh, PA) software will control stimulus delivery.

Stimulus-presentation paradigm: The ERP procedure involves blocks of trials from four conditions: multisensory (simultaneous speech sound-puff), puff alone, speech sound alone, and sham puff in a randomly generated sequence. To prevent habituation, no more than 2 repetitions of a condition occur in a row, with inter-trial intervals varying randomly between 2000-2500 ms.

The “light touch” stimulus is an air puff emanating from a nozzle positioned above the skin of the palmar surface of the right hand secured in a mold holder (Figure 2b). Another mold holder connected to the second nozzle is placed 15 cm away at midline (sham condition). The pressure

at the skin surface is 5 psi, or less than the pressure of the smallest microfilament used to test for neuropathies.[55] The entire test session generates 60 trials per condition and lasts 8-10 minutes. The speech sound condition is a computer-generated woman's voicing of one of six syllables (i.e. /ba/, /da/, /ga/, /bu/, /du/, /gu/) delivered in a free field setting using a microphone placed at midline 15 cm. The speech stimuli are computer-synthesized consonant-vowel syllables from the "transition-only" stimulus series. The five-formant syllables were synthesized on a Klatt (cascade) synthesizer, so that the amplitude of individual formants was modulated as a function of the respective formant frequencies. The central frequencies of the steady-state portion of the formants were kept constant across the different consonants and only varied as a function of the vowel sounds. The syllables are presented at 65 dB SPL(A). More than the 2 key stimuli (/bu/ and /gu/) are presented to prevent habituation. The stimulus presentation paradigm simultaneous allows collection of multi- and uni-sensory conditions and the replication of prior-identified time windows and scalp regions that quantify individual differences in multi- or uni-sensory processing.

Preparation and analysis of ERP data: The recorded data will be filtered using a 0.3-40 Hz bandpass filter and segmented on stimulus onset to include a 200-ms prestimulus baseline and a 500-ms post-stimulus interval. Electrodes will be referred to Cz and re-referenced offline to an average reference. Resulting segments will be screened for motor/ocular artifacts using standard algorithms in NetStation, followed by a manual review.

We will utilize the time windows and electrode clusters identified in our previously published and preliminary data (Section C1) to guide computation of the mean amplitude for each patient, condition and time point using NetStation statistical extraction tools.

Index of multisensory processing (IMP): We have already identified template maps over the cumulative 500ms post-stimulus time window using a topographic cluster analysis (i.e., Topographic Atomize & Agglomerate Hierarchical Clustering approach) (see Preliminary Data section) for full-term (FT) and preterm (PT) infants, which accounted for 96.3% of the global explained variance in ERP response to the MS stimulus. Different pairs of template maps have been identified for the 3 time windows corresponding to previously-identified microstates during the multi-sensory condition (127-175ms,176-377ms, 378-599ms). Using a within-participant spatial correlation process (i.e., cases are electrodes and the two variables are (a) participant's ERP and (b) template map value), we can identify which template map (FT or PT) best fits each time sample's observed topographical data for each participant.

This fitting procedure will be carried out for each of the 3 time windows. From this procedure, we calculate the percentage of time the topographical pattern of the participant's ERP to MS stimuli is most like the FT template map. We call this index the IMP. This index is expressed as a percentage from 100% (all time samples show nearly-typical activation) to 0% (no time samples show nearly-typical activation).

Secondary Outcome Measures

Baby Care Questionnaire (BCQ):
The BCQ is a parent questionnaire that assesses the degree of parental structure and parental attunement. The three sections include sleeping, feeding, and soothing. Parents are asked to indicate their feelings about each statement by circling strongly agree (SA), agree (A), disagree (D), or strongly disagree (SD). The tool is validated for parents expecting a baby through 24 months of age.

Hammersmith Neonatal/Infant Neurological Examination (HNNE/HINE):
The HNNE and HINE is a neonatal/infant neurological examination consisting of 34 items to assess tone, tone patterns, reflexes, movements, abnormal signs, and behavior.

Parenting Styles and Dimensions Questionnaire (PSDQ):
The PSDQ is a 32-item parent questionnaire that assesses parenting style based on a series of items scored by parents along a 5-point Likert-type scale.

Infant Toddler Sensory Profile (ITSP):
The ITSP is the most validated test for the behavioral evaluation of sensory processing. This parent-rated questionnaire has 48 questions, addressing five sensory processing sections (Auditory, Visual, Tactile, Vestibular, and Oral Sensory Processing) and a General measure. One variable from this instrument is the infant's neurological threshold (tendency to respond to sensory stimuli). 'Low neurological threshold' reflects situations when minimal sensory input causes a system to respond and 'high neurological threshold' reflects situations when a large amounts of stimulation is required before a response is registered. Typical, atypical high and atypical low ranges are provided for neurological threshold scores in the 7-12 month age band using published norms on 589 term-born American infants generating nominal variables.

Raw individual section scores are also provided. The ITSP has been used in large studies of preterm infants.

Bayley Scales of Infant and Toddler Development (Bayley III) — 3rd Edition:

The Bayley III is the gold standard for the evaluation of former NICU graduates, especially preterm infants. We will use the language and motor composite standard scores for CA. The Bayley is currently administered in the Follow-Up Clinic by trained examiners who undergo yearly retraining by a Gold-standard examiner.

Preschool Language Scales (PLS5) - 5th Edition

The PLS-5 is a language assessment tool administered by a trained professional in which children point or verbally respond to pictures or objects. It assesses both receptive and expressive language skills in young children.

Child Behavior Checklist (CBCL)

The CBCL is a 113-question standardized checklist used to detect social-emotional and behavioral concerns in young children. The form is filled out by parents and uses a 3-point Likert-type scale.

Analysis Plan

Power/Sample Size

A total sample size of 230 (115 in each group) will be recruited. Although attrition in past similar work has been much lower, we estimate power under an assumption of 10% attrition (i.e., 180). Because pilot studies afford effect size estimates with wide confidence intervals due to small sample sizes, power analysis results were conducted for the effect size available from pilot data and lowest effect size that the after-attrition sample size affords. Feasibility of the expected effect size is then evaluated. Power analyses are based on primary variables (multi- and uni- sensory processing). If between-group differences occur on the putative covariates (i.e., pretreatment variables related to the outcomes) and they are associated with putative mediators or outcomes, the pretreatment variables will be statistically controlled after ensuring that the homogeneity of slopes assumption has been met. Pretreatment ERP variables will be statistically controlled regardless of the effect size of between-group differences to improve effect size estimates. Missing data will be handled using multiple imputation.

Statistical Analysis

Our first aim is to demonstrate that preterm infants receiving a standardized, parent-supported, auditory-tactile intervention in addition to standard of care in the NICU will have more typical cortical multisensory processing at discharge to home and better sensory adaptation and motor and language outcomes than infants receiving only standard of care.

For Aim 1, the between-group differences will be tested on ERP-measured multisensory processing after completion of intervention, sensory reactivity and adaption at Year 1, and motor and language ability at Year 2.

When the dependent variable is continuous, we will use ANOVAs when controlling for pretreatment variables and independent sample t-tests when there is no need for a covariate. When testing the nominal form of the sensory reactivity and adaptation outcome, we will use logistic regression.

Statistical power: Using PASS software, the estimated power using a 1-tailed test for an effect size based on the pilot data (Hedges *g* = .74) with a sample size of 180 is over 99%. Even if the actual effect size is *g = .35,* we will have over 80% power to detect the effect under the proposed conditions. Detecting the required minimum effect size is probably for a number of reasons.

First, the expected effect size for the proposed treatment on multisensory processing should be larger than that derived from the pilot because the treatment used in the pilot

(i.e., skin-to-skin contact *without* the suck-contingent presentation of parent's voice) and theory strongly suggests the multisensory treatment will produce a larger effect than a unisensory treatment. Secondly, a medium main effect size of the proposed treatment on neurodevelopmental outcomes is expected because multisensory processing is moderately to strongly associated with later neurodevelopmental outcomes.

Our second aim is to test the role of multisensory responses at discharge in mediating intervention effects on later sensory adaptation, and motor and language outcomes.

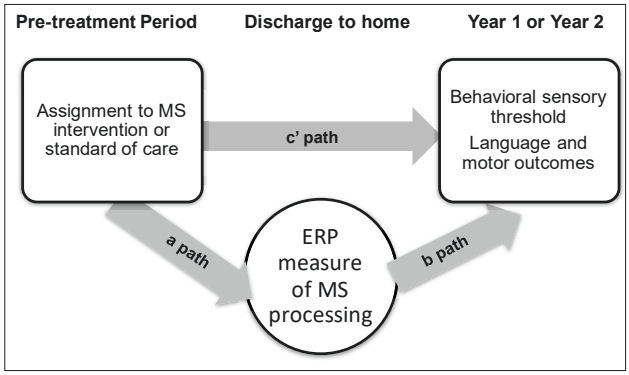
Mediation models are presented in Figure 3. The indirect effect (the product of the a path coefficient * the b path coefficient) will be tested for significance using the bias-corrected bootstrap method. The a path is the main effect of treatment on the ERP measure of multisensory processing. The b path is the association of the ERP measure of multisensory processing with the outcome controlling for the treatment group.

Statistical power: Using simulation data, an indirect effect produced by paths each with at least the mid-point between small and medium effect sizes (0.26; i.e., at least 6.8% of the variance of the criterion variable accounted for in each path) will be detected with over 80% power when using a sample size of 180. For example, if paths a and b each have an effect size of 0.26, a significant indirect effect will be detected with 80% power with 148 participants, far fewer than the proposed sample size of 180.

In the power analysis for Aim 1, we indicated that a medium effect of the treatment on multisensory processing is expected (i.e., the a path for Aim 2). We have no pilot data for the b path because it is the association of multisensory processing with neurodevelopmental outcome *controlling for the treatment*. The proposed study is the needed RCT to estimate the effect size of the b path. However, a zero-order correlation between multisensory processing and motor and language Bayley standard scores was 0.41 to 0.42, respectively, in the previous correlational study on preterm infants. These are considered between medium and large effect

sizes. Finally, multisensory processing was associated with more typical behavioral thresholds to sensory stimuli in the home as evaluated on the Infant Toddler Sensory Profile in a previous correlational study with preterm infants. Thus, the expectation for an indirect effect that is at least the mid-point between small and medium is quite realistic.

Figure 3



Our third aim is to explore the role of m responses in mediating intervention effects on later motor and language outcomes.

Plan: An analogous analysis procedure as was proposed for Aim 2 will be used to test Aim 3. The putative mediators are the two unisensory processing (i.e., speech processing or touch) variables. The putative mediator is speech processing for the language outcome and the putative mediator is touch processing for the motor outcome.

Statistical power: The power analysis for Aim 2 applies to Aim 3. The expectation for an indirect effect that is at least the mid-point between small and medium is realistic. With regards to a path, the sample-size-adjusted standardized mean difference on speech processing effect size from the contingent parent-voice vs control (a treatment that is a subset of the proposed treatment) contrast was large (i.e., Hedges *g* = 1.06). The proposed treatment will provide more nurturing touch than infants in control group and nurturing touch has been associated with better touch perception experience in our preliminary studies. The zero order correlation speech perception to later language was very large (R^2 = .39) in the previous correlational study of preterm infants.

Data management

Data will be stored using Research Electronic Data Capture (REDCap), which is a secure, web-based application designed to support data capture for research studies by building and managing online surveys and databases. Support for REDCap is available through Emory

University. In some cases, paper versions of assessments and forms may need to be used. If this happens, these completed forms will be kept in a locked cabinet within the lab space. Additionally, some participant data such as the parent recordings and the EEG data, will be kept in a password protected shared drive supported by Emory University (e.g., Lab-Specific Sharepoint/OneDrive/Amazon Web Service) that only designated study personnel (determined by the PI) have access to. Finally, each research participant will be assigned a code. All data collection procedures and all participant data storage will utilize this code. Only the PI and designated study personnel will have access to the code key.

Patient and public involvement

This study is based on our previous work and the NIH call for applications regarding multisensory processing and interventions. Neither patients nor public advisory boards were involved in the conception, design or implementation of this study.

Ethics and dissemination

Informed consent will be obtained from the parent/guardian in accordance with the IRB protocol.

Data management: Data will be stored using Research Electronic Data Capture (REDCap), which is a secure, web-based application designed to support data capture for research studies by building and managing online surveys and databases. Support for REDCap is available through Emory University. In some cases, paper versions of assessments and forms may need to be used. If this happens, these completed forms will be kept in a locked cabinet within the lab space. Additionally, some participant data such as the parent recordings and the EEG data, will be kept in a password protected shared drive supported by Emory University (e.g., Lab-Specific Sharepoint/OneDrive/Amazon Web Service) that only designated study personnel (determined by the PI) have access to. Finally, each research participant will be assigned a code. All data collection procedures and all participant data storage will utilize this code. Only the PI and designated study personnel will have access to the code key. In compliance with Emory's record retention policy, data will be stored for at least six years from study completion and consent forms will be stored for at least 25 years following study completion or in compliance with federal and Emory regulations.

Assuming acceptance, the results of this study will be disseminated via peer-reviewed publications and conference presentations.

Discussion

Previous studies prove that early sensory experiences shape brain development in former preterm infants. A few associative studies have demonstrated improved neurodevelopmental outcomes with supportive, targeted sensory input (ie STS or infant directed speech).

To our knowledge, this is the first RCT study to design and test a MS intervention using brain-based measurements in order to elucidate the causal effects of the MS intervention on neural processing changes to mediate neurodevelopmental outcomes. This study provides a critical link in further understanding interactions between brain development, plasticity, environment input, and subsequent neurodevelopment in this particularly vulnerable population of infants.

References:

Listed in the published article: Nell et. al., BCM Pediatrics March 2019. Randomized Controlled Trial Protocol to Improve Multisensory Neural Processing, Language and Motor Outcomes in Preterm Infants. https://bmcpediatr.biomedcentral.com/articles/10.1186/s12887-019-1455-1#Sec36