A Randomized Controlled Trial to Improve Mother-Infant Synchrony Among Women with Childhood Adversity

NCT04818112

Protocol & Statistical Analysis Plan Version 1.6 01/31/2024

Protocol for

"A Randomized Controlled Trial to Improve Mother-Infant Synchrony among Women with Childhood Adversity"

Protocol Number 2009050756
National Clinical Trial (NCT) Identified Number: NCT04818112
Principal Investigator: Aleeca F. Bell, PhD RN CNM

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STATEMENT OF COMPLIANCE

This trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56) will be followed.

- National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials will complete Human Subjects Protection and ICH GCP Training.

- All applicable policies and guidelines from University of Arizona's Research, Discovery, and Innovation programs (HIPAA Privacy Program, Research Laboratory & Safety Services, and Human Subjects Protection Program) will be followed.

In summary, the protocol, informed consent form(s), recruitment materials, and all participant materials have been submitted to site research committees and to the University of Arizona's Institutional Review Board (IRB) for review and approval. Approval of protocol elements and the consent forms were obtained prior to participant enrollment. Additionally, any amendments to this protocol will be reviewed and approved by the University of Arizona's IRB prior to implementation. Furthermore, all changes to the consent form will be IRB-approved and a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent using a previously approved consent form.

Protocol Approval

Principal Investigator: Aleeca Bell, PhD, RN, CNM
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Biobehavioral Health Sciences Division
University of Arizona College of Nursing
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[redacted]

1.0 SUMMARY DOCUMENTS

1.1 Abstract

Title: A randomized controlled trial to improve mother-infant synchrony among women with childhood adversity

Background: This 4-year, single-site, randomized, attention-controlled clinical trial seeks to evaluate the efficacy of an infant-massage intervention (ATVV) to improve reciprocal communication patterns between mother and infant called mother-infant synchrony. Ideally, as a mother recognizes and is sensitive to her infant's cues, she adapts her nurturing behavior to maximize the infant's ability to respond with clearer cues and greater self-regulation. The quality of mother-infant (M-I) synchrony predicts healthy infant attachment and long-term physical, cognitive, and emotional development of the child. [7, 13, 18, 19] Additionally, the neuropeptide **oxytocin (OXT)** is critical in promoting M-I synchrony. [7-11] However, **childhood adversity** is common [1-4] and causes potent long-term dampening of the OXT system, potentially placing M-I synchrony and future development of the child at risk.

Hypothesis: We posit that those mothers with childhood adversity will have more difficulty with mother-infant synchrony and will benefit from interventions such as infant massage ATVV, which improve nurturing engagement through auditory, tactile, visual, and vestibular (ATVV) communication patterns which influence epigenetic regulation of the oxytocin (OXT) system. Our objectives are the following-

- **Aim 1- Evaluate the efficacy of daily ATVV over 3 postnatal months among mothers with childhood adversity. Compared to the Attention Control group (*n*=125), mother-infant dyads in the ATVV group (*n*=125) will exhibit:**
    - **H1:** Higher oxytocin function at 1, 2, and 3 postnatal months as compared to prenatal baseline and
    - **H2:** More mother-infant (M-I) synchrony at 3 postnatal months.
- **Aim 2- Identify molecular mechanisms in the OXT system underlying M-I synchrony (*N*=250).**
    - **H1:** Lower M-I synchrony will be associated with dampening of the OXT system in maternal peripheral blood and decreased OXT in infant saliva at 3-postnatal months.

Methods: The infant-massage intervention, ATVV (Auditory, Tactile, Visual, Vestibular), consists of infant-directed speech, moderate touch massage, eye to eye gaze, and horizontal rocking. The primary outcome, M-I synchrony, is collected 3 months post birth in 250 first-time mothers (and their full-term infants) randomized to apply ATVV daily from birth to 3 months (n = 125) or receive infant care education in an Attention Control group (n = 125). M-I synchrony is measured using 3-minutes of video-recorded interaction to micro-code shared gaze (primary outcome), affect (primary outcome), speech, and touch. OXT level is a known biomarker of synchrony. Secondary outcomes include a comprehensive analysis of the OXT system including identification of a potential epigenetic role of the oxytocin receptor gene (OXTR). We analyze maternal blood in pregnancy and at 1, 2, and 3 months post birth (and infant salivary OXT). We aim to identify molecular mechanisms in the OXT system underlying M-I synchrony in maternal blood and discover molecular profiles. Regression based methods assess ATVV effects on and relations among M-I synchrony and OXT measures.

Impact: This study will advance understanding of ATVV's efficacy to improve M-I synchrony and the underlying epigenetic mechanisms to promote synchrony in an understudied population. Because this early life and home-based intervention is easy to teach and perform, it has high translational potential for practice. Furthermore, this study design serves as a model for testing in other at-risk groups. The social and economic cost savings associated with reducing the potent impact of adverse events on maternal and infant outcomes cannot be underestimated.[2, 5, 141-142]

1.2 Synopsis

Title: A randomized controlled trial to improve mother-infant synchrony among women with childhood adversity

Study Design: A 4-year single-site, randomized, two-group, attention-controlled clinical trial
Hypothesis: Mothers with childhood adversity will have more difficulty with mother-infant synchrony, due in part to a dampened oxytocin system, and will benefit from the infant-massage intervention applying nurturing engagement through auditory, tactile, visual, and vestibular (ATVV) communication patterns which will influence epigenetic regulation of the oxytocin (OXT) system.
Intervention: A multisensory infant-massage consisting of auditory, tactile, visual, and vestibular (ATVV) stimuli administered by the mother to the infant one time each day for 3 months.
Comparison: Attention-control mothers receiving same amount of temporal attention as received by the intervention group.
Objectives and Working Hypotheses: Aim 1- Evaluate the efficacy of daily ATVV over 3 postnatal months among mothers with childhood adversity. Compared to the Attention Control group (*n*=125), mother-infant dyads in the ATVV group (*n*=125) will exhibit: H_1 - Higher oxytocin function at 1, 2, and 3 postnatal months as compared to prenatal baseline and H_2 - More mother-infant (M-I) synchrony as measured by reciprocal gaze, affect, speech, and touch at 3 postnatal months. **Aim 2**- Identify molecular mechanisms in the OXT system underlying M-I synchrony (*N*=250). H1- Lower M-I synchrony will be associated with dampening of the OXT system in maternal peripheral blood and decreased OXT in infant saliva at 3-postnatal months.
Primary Outcome: Mother-infant Synchrony as measured by time spent engaging in synchronous eye-to-eye gaze and positive affect.
Secondary Outcomes: Mother-Infant Synchrony as measured by time spent engaging in synchronous positive speech and affectionate touch. Functioning of maternal OXT system as measured by epigenetic marks in the OXT receptor (OXTR) gene, expression of the OXTR gene, quantity of OXTR protein produced, and quantity of OXT peptide in maternal peripheral blood and OXT peptide in infant saliva.
Inclusion Criteria: Pregnant women are eligible if they are

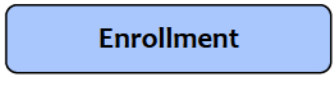
- healthy (gestational diabetes is acceptable),
- greater than or equal to 18 years old,
- expecting their first child (previous miscarriage(s) and abortion(s) acceptable),
- speak and read English or Spanish,
- score greater than or equal to 2 on the Adverse Childhood Experience (ACE) survey,
- expect to deliver a healthy infant,
- expect to deliver a full-term infant (greater than or equal to 37 weeks and 0/7 days), and
- expect to deliver a singleton infant.

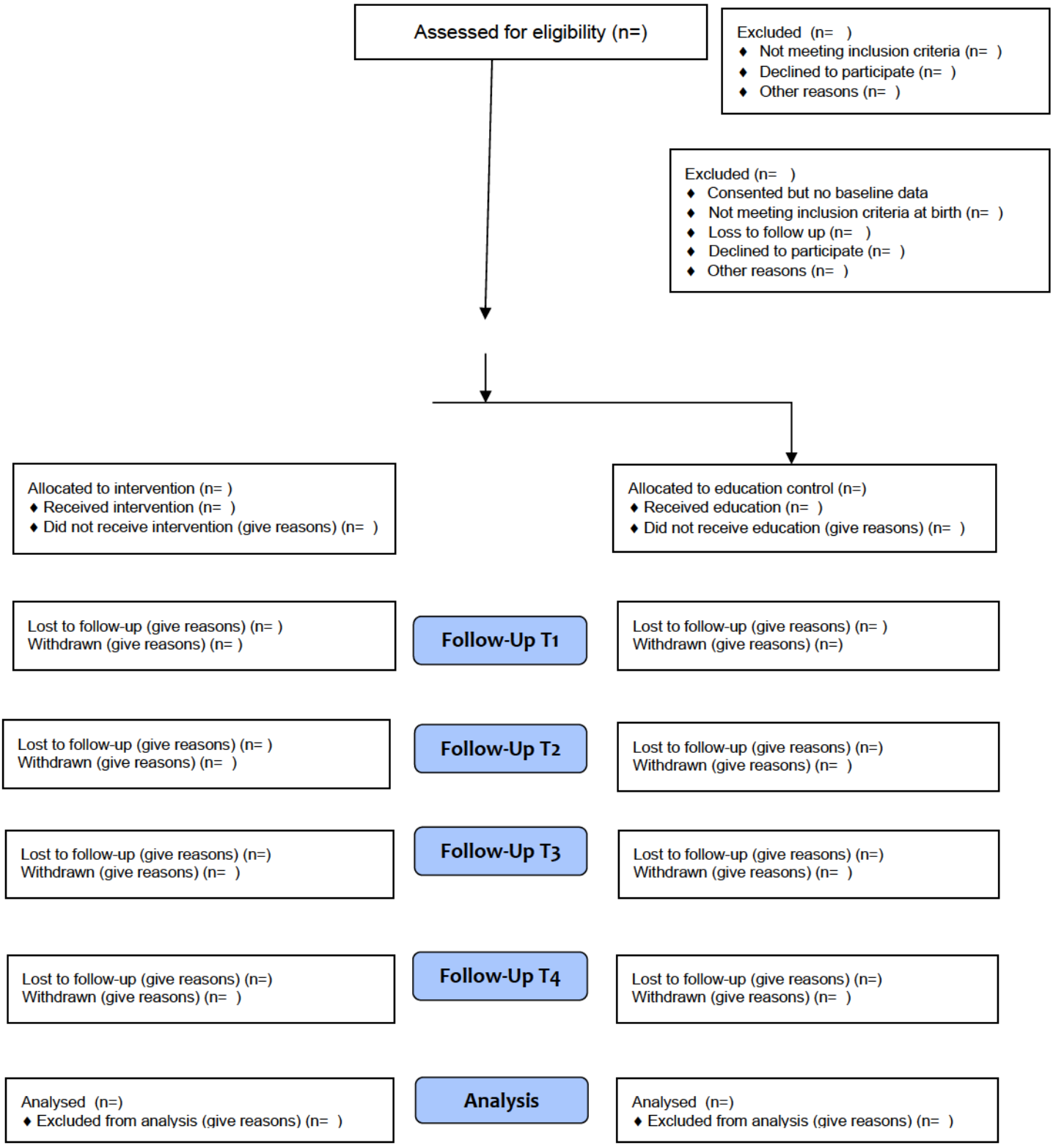
Exclusion Criteria: Pregnant women are ineligible if they

- have given birth to $\geq$ 1 child
- have no access to a cell phone during the first 3 postnatal months,
- carrying multiple fetuses,
- taking illicit drugs,
- ever diagnosed with bipolar,
- ever diagnosed with autism,
- ever diagnosed with psychosis,
- infant diagnosed as small for gestational age (SGA) defined by body mass, head circumference, and body length less than the 10^{th} percentile for the gestational age at birth,
- infant diagnosed with a chromosomal anomaly,
- infant diagnosed with a congenital anomaly,
- infant has a serious illness which would interfere with mother-infant interaction

Length of Time Required for Participation in Study: 6 - 9 months
Initiation Date: 23 June 2021
Initial Enrollment Date: 12 July 2021
Final Data Collection Date: projected 31 March 2024
Project Completion Date: projected 31 January 2025

1.3 Flow Diagram




Assessed for eligibility (n=)
Excluded (n=)
♦ Not meeting inclusion criteria (n=)
♦ Declined to participate (n=)
♦ Other reasons (n=)
Excluded (n=)
♦ Consented but no baseline data
♦ Not meeting inclusion criteria at birth (n=)
♦ Loss to follow up (n=)
♦ Declined to participate (n=)
♦ Other reasons (n=)
Allocated to intervention (n=)
♦ Received intervention (n=)
♦ Did not receive intervention (give reasons) (n=)
Allocated to education control (n=)
♦ Received education (n=)
♦ Did not receive education (give reasons) (n=)
Lost to follow-up (give reasons) (n=)
Withdrawn (give reasons) (n=)
Follow-Up T1
Lost to follow-up (give reasons) (n=)
Withdrawn (give reasons) (n=)
Lost to follow-up (give reasons) (n=)
Withdrawn (give reasons) (n=)
Follow-Up T2
Lost to follow-up (give reasons) (n=)
Withdrawn (give reasons) (n=)
Lost to follow-up (give reasons) (n=)
Withdrawn (give reasons) (n=)
Follow-Up T3
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Withdrawn (give reasons) (n=)
Lost to follow-up (give reasons) (n=)
Withdrawn (give reasons) (n=)
Follow-Up T4
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Withdrawn (give reasons) (n=)
Analysed (n=)
♦ Excluded from analysis (give reasons) (n=)
Analysis
Analysed (n=)
♦ Excluded from analysis (give reasons) (n=)

1.4 Schedule of Events for "Randomized Controlled Trial to Improve Mother-Infant Synchrony among Women with Childhood Adversity"

Event / Timepoint & Location	Instruments / Outcome Measures	Recruitment (last trimester) Clinics	Baseline (33wks ± 2wks ga) College of Nursing	Birth (prior to discharge) TMC, BUMC or Zoom	Birth (after discharge) Phone/text	T1 (28 days - 7days/+14 days) College of Nursing	T2 (56 days -7 days/+14 days) College of Nursing	T3 (84 days -7 days/+14days) College of Nursing	T4 (6 mos ± 4 wks) phone
Recruitment/Screening		x							
Informed Consent			x						
Randomization				x					
ATVV/Attn Control Training				x	x	x	x	x	
Text Responses (daily)					x	x	x	x	
Text Reminder (monthly)					x	x	x	x	
Phone Call (weekly)					x	x	x	x	
Intervention Fidelity Videos						x	x	x	
REDCap Questionnaires	Screening Questionnaire (phone)	x							
	adverse childhood experiences amb* ACE (phone)	x							
	Demographics Questionnaire		x						
	maternal attachment amb MAAS		x						
	resilience amb BRCS		x				x		
	emotional support amb PROMIS-ES		x				x		x
	stess mindset amb SMM		x				x		
	perceived stress amb PSS-10		x			x	x	x	x
	depressive symptoms amb PHQ-8		x			x	x	x	x
	anxiety amb GAD-7		x			x	x	x	x
	PTSD amb DSM-5 (PCL-5)		x						
	autonomic reactivity amb BPQ-ANS		x					x	x
	parenting received amb PBI-M		x						
	parenting received amb PBI-P		x						
	history of adversity amb ATES		x						
	Childbirth & Infant Survey			x					
	Follow Questionnaire					x	x	x	
	birth experience amb CEQ					x			
	birth experience amb PTSD-childbirth					x			
	parenting amb PSI-SF					x	x	x	x
	Followup Questionnaire at T4								x
	Research Participant Perception Survey								x
Peripheral blood draw	epigenetic marks amb % DNA methylation at CpG sites (n=6)		x			x	x	x	
	OXTR expression amb qRT-PCR of mRNA		x			x	x	x	
	OXTR total protein amb Western Blot		x			x	x	x	
	OXT peptide amb ELISA assay		x			x	x	x	
Infant Saliva	OXT peptide amb ELISA assay					x	x	x	
Mother-Infant Synchrony Videos	Affect Synchrony							x	
	Gaze Synchrony							x	
	Vocal Synchrony							x	
	Touch Synchrony							x	
*amb = as measured by									

2.0 INTRODUCTION

2.1 Background/Scientific Rational

2.1.1 Childhood Adversity

Childhood adversity (defined as abuse, neglect, or household dysfunction experienced up to age 18) exerts potent mental and physical burdens on individuals by altering nervous, endocrine, and immune systems well into adulthood, and acts as a dose-response risk factor for the leading causes of disease.[2, 5] Childhood adversity is commonly experienced, under-reported, and exerts an economic toll.[53, 141-142] The original Adverse Childhood Experiences (ACE) study of > 9,000 US adults demonstrated > 50% of adults experienced at least 1 ACE.[2] The largest most diverse ACE study to date (*n*=249,934) across 23 states revealed 62% of respondents had at least 1 ACE, 25% had at least 3 ACEs, and significantly higher ACEs reported by minorities and those with low income.[3] Narrowing the prevalence of ACE types to women across 3 population studies reveal that 27-34% reported emotional abuse, 15-20% reported physical abuse, and 16-32% reported sexual abuse.[1,3,4] Additionally, animal models of early-life adversity (i.e., poor maternal care), framed within the Developmental Origins of Health and Disease paradigm (DOHaD),[54] demonstrate that offspring who were exposed to early life adversity grow up to exhibit poor maternal care towards their offspring.[55]) Women with childhood adversity may be at higher risk for poor M-I synchrony. [7-11]

2.1.2 Childhood Adversity and the Oxytocin System

Framed within the Developmental Origins of Health Disease (DOHaD) paradigm, offspring exposed to early life adversity (i.e., poor maternal care) grow up to exhibit poor maternal care towards their offspring.[54] Rat pups receiving poor maternal care (low licking and grooming) exhibited epigenetic modifications in the stress response system that persisted into adulthood.[ref 55] Similarly, rat pups receiving low maternal licking and grooming exhibited OXT receptor gene (*OXTR*) modification, lower OXTR expression, and lower OXT levels that persisted into adulthood resulting in increased anxiety and stress;[45, 51] however, an intervention to improve the quality of maternal care then resulted in improved OXT function.[56] In newborn voles a one-week intervention mimicking poor maternal care, versus usual maternal care, resulted in *de novo* higher *OXTR* methylation (in blood) correlating with lower OXTR gene expression and fewer OXTR binding sites (in the brain), showing that not only can *OXTR* methylation respond quickly to maternal care but that peripherally measured methylation reflects brain function.[143] In these DOHaD animal models, epigenetic modification of systems that regulate a sense of safety versus fear, such as the OXT system, are the underlying mechanisms regulating maternal behavior. Similarly, human studies also show a link between the quality of early parenting and OXT levels of the child well into adulthood.[6, 34, 57-59, 151] The relationship between OXT level and observed maternal nurturing behavior may be different in mothers with, versus without, a history of childhood adversity.[60] While epigenetic effects from childhood adversity are found in genome-wide and candidate gene studies on stress hormones,[61] evidence of *OXTR* epigenetic effects is limited to 3 studies showing increased *OXTR* DNA methylation (i.e., dampening) after poor maternal care in early life,[50] in adolescent anxiousness,[62] and in adult psychosis[49] after childhood adversity.

2.1.3 Mother-Infant Synchrony

Mother-Infant (M-I) synchrony, our primary outcome, is a coordinated and fluid process involving affectionate "motherese" speech, touch, gaze, and affect.[12-14] When a mother is sensitive, responsive, and adaptive to her infant's cues through nurturing behavior, the infant responds with greater self-regulation and alertness, and vice versa. Both mother and infant experience the physical benefits of secure attachment: less stress reactivity and a calmer autonomic nervous system.[73-75] M-I synchrony is mutually rewarding to mothers and infants emotionally, behaviorally, and physiologically.[19] Constructs that describe healthy M-I interaction (such as M-I synchrony and responsive caregiving) have been well-researched for decades as essential prerequisites for shaping the early architecture of healthy brain circuits that enhance more complex brain development.[17] Responsive caregiving is a protective factor that lessens the effects of cumulative adversity on health and safeguards against life-long socioeconomic health disparities.[15, 16] Poor mother-infant (M-I) synchrony exerts a strong effect on impairing the social, emotional, cognitive, and physiologic development of children.[7, 13, 18, 19] Impairments include behavior problems, low empathy, attachment insecurity, poor

social adaptation in peer groups, cognitive deficits, psychopathologies, and poor autonomic regulation and stress reactivity, in both low- and high- risk cohorts.[7, 13, 18, 19]

2.1.4 Mother-Infant Synchrony and the Oxytocin System

A high functioning OXT system may be important for mothers because the most likely biologic correlate of M-I synchrony is the neuropeptide OXT, known for its key role in promoting nurturing maternal behavior, attachment, social affiliation, empathy, stress reduction, and positive mood.[7-11, 63, 64] OXT appears to influence behavior by acting on the nervous system as a metaphor for psychological safety.[65] Maternal OXT acts peripherally supporting birth and lactation, and in multiple species acts centrally in the regulation of M-I attachment.[9, 66] Feldman and others report M-I attachment and synchrony are associated with increased peripheral OXT levels, *OXTR* single-nucleotide polymorphisms, increased brain activation in OXT regions, and administration of exogenous OXT.[33, 36, 64, 67] Feldman also found a significant correlation of 0.30 between lower plasma OXT and lower parental bonding that both mothers and fathers experienced as children.[34] However, no investigation has extended these OXT system findings to *OXTR* epigenetic mechanisms. Evidence to support a role for *OXTR* epigenetic regulation in M-I synchrony shows an association between increased *OXTR* DNA methylation and psychosocial behaviors: depression, anxiety about attachment, social phobia, callous-emotional traits, autism spectrum disorder, anorexia-nervosa, slower processing of facial emotion, and lower brain activation in areas of social cognition.[38-44, 46-48, 52, 68, 69] The oxytocin system is highly plastic in early life[70, 71] when the *OXTR* can be epigenetically programmed for vulnerability to psychopathologies grounded in defense, fear and anxiety.[62] The quality of early parenting that an infant receives can affect their OXT levels well into adulthood.[6, 34, 57-59, 72] While our proposal is not a transgenerational study design and will not collect blood to measure epigenetic outcomes in the infant, evidence suggests that early life experiences become embedded into the child's genome;[61, 71] thus, our findings on infant OXT levels from the proposed study may warrant follow-up to determine our behavioral intervention's effect on epigenetic modification of the child's long-term OXT system.

2.1.5 ATVV Intervention

ATVV (Auditory, Tactile, Visual, Vestibular stimulation) is a 15 minute multisensory behavioral intervention consisting of infant-directed affectionate "motherese" speech, moderate touch massage, eye to eye gaze, and horizontal rocking.[24, 76, 77] Ideally, ATVV should be administered by mothers soon after birth;[29, 30, 78] when experience-dependent neural plasticity is high in maternal and infant brains.[22, 23] These early face-to-face interactions provide critical stimuli for maturation of the "social brain".[7] Meta-analyses report that the most promising interventions are the ones that help mothers engage with their infant, and provide opportunities for practicing play and responsive talk.[16, 20, 21] In administering ATVV, mothers learn to be comfortable recognizing and adapting their behavior when the infant is signaling cues to engage or disengage. ATVV is an enjoyable daily opportunity for mothers to practice modeling M-I synchrony by using eye to eye gaze, affectionate "motherese" speech and soothing touch.[78] Maternal nurturing and attachment behavior improved in two RCTs with ATVV and Kangaroo Care (where pre-term infants are held skin-to-skin);[28, 79] however, ATVV has added value beyond the touch element due to the engaged responsive caregiving element not found in Kangaroo Care. ATVV improves maternal sensitivity to preterm infant cues and responsiveness during play,[29, 30] greater maternal confidence,[80] lower maternal distress,[28] and enhance preterm infant behavioral development,[25-27] thus supporting a need to extend these findings to full-term infants.

2.1.6 Will ATVV be Associated with Improved OXT function?

The ATVV intervention is likely to improve OXT function in mothers and infants. OXT will likely increase by the soothing effect of ATVV, as shown by numerous studies on touch and OXT.[81, 82] Nurturing social touch stimulates the reward response and increases OXT levels.[81] Specifically, pleasant affiliative touch activates C-tactile afferent nerve fibers that mediate the release of OXT.[81] In three separate RCTs with full-term infants (< 48 hours old) our team showed that with just one administration of ATVV (compared to controls) mothers experienced increases in maternal OXT, while infants had lower values of cortisol and demonstrated autonomic stability, which OXT helps to regulate. Additionally, infants modified their behavior from sleep to alertness - the optimal state for facilitating M-I synchrony.[83-85]

2.1.7 Will ATVV be Associated with Improved Mother-Infant Synchrony?

Dr. Feldman has developed a methodology of micro-coding maternal and infant behaviors to measure M-I synchrony, and has demonstrated a clear link with peripheral OXT levels.[7, 12, 19, 31, 32] Using video recordings of mother-infant dyads interacting, behavioral interactions are micro-coded and quantified second-by-second to characterize the synchrony of mother and infant speech, touch, gaze, and affect. Using these micro-coded M-I behaviors, we are able to objectively measure patterns of dynamic M-I communication, conveying maternal affection and sensitivity to cues, as well as infant responsiveness and readiness to interact.[31] Assessment of these behaviors in only a 3-minute window yields rich detail of synchrony. Over the first postnatal months, more coordinated episodes of synchrony take on a larger proportion of the parent-infant interaction, and by 3-months of age full-term infants can engage in a clear, measurable, temporal structure of M-I synchrony.[18, 31, 32, 74, 102, 104] Studies by Feldman demonstrate that parents with higher plasma and salivary OXT levels display more synchrony with their infants,[34] as well as more coordinated neural circuits for empathy and social cognition.[86, 87] Higher levels of micro-coded M-I synchrony are associated with higher OXT levels in both the parent and the infant, and with higher correlations between parent and infant OXT levels.[33] Similarly, administration of intra-nasal OXT to the parent results in higher parent OXT levels in the context of higher synchrony; and importantly results in higher infant OXT levels, and more correlated levels between the parent and the infant in more synchronous interactions.[67] With this solid foundation of research linking OXT peptide levels with micro-coded M-I synchrony, the next critical step is to further identify molecular mechanisms of synchrony by linking M-I synchrony to *OXTR* epigenetic marks and gene expression.

We posit that mothers with childhood adversity will have more difficulty with M-I synchrony, due in part to a dampened OXT system, and will benefit from interventions that improve nurturing engagement by epigenetic regulation of the OXT system. Most interventions are costly, complex, time-consuming,[20, 21] and miss an important opportunity of intervening during early M-I experience-dependent neural brain plasticity.[22, 23] We aim to fill this gap by testing the efficacy of an easy to learn, early behavioral intervention to improve OXT system function and M-I synchrony.

2.2 Safety Considerations

This study will recruit pregnant women, a vulnerable population (45 CFR 46.204). While pregnant women, mothers and their healthy full-term infants are vulnerable populations, the infant massage intervention includes behaviors that mothers typically use when interacting with their infants and is low risk. All protocol elements have been submitted to the University of Arizona Institutional Review Board which has determined that there is no more than minimal risk associated with participating in this study for pregnant women, their fetuses, or their infants (45 CFR §46.404/21 CFR §50.51). (See IRB approval letter dated September 28, 2020 in Appendix A.)

2.2.1 Expected Risks

Physical risks associated with the maternal blood draw may include discomfort (common risk), bleeding or bruising (occasional risk), fainting (rare risk), and infection (rare risk). There is a biohazard risk to staff in handling blood and saliva specimens. There is a risk of COVID19 infection transmitted from asymptomatic individuals. The risks associated with giving blood and saliva samples are very low, especially in a study that is not conducting genome-wide association analyses and is not able to identify medical problems or diseases. However, we are not able to know all the risks from taking part in genetic research studies. Additionally, some women may be uncomfortable or anxious when completing questionnaires about mood, adversity, or health. Furthermore, there is potential risk associated with loss of privacy or confidentiality.

2.2.2 Amelioration of Risks

2.2.2.1 Amelioration of Physical Risks

To minimize discomfort and risks associated with the maternal blood draw, we employ experienced phlebotomists

trained in conducting venipuncture. To minimize risk from handling blood and saliva specimens, all research team members are required by OSHA to have annual training in Bloodborne Pathogens and Laboratory Safety at the University of Arizona. Physical risks to the infant participants are minimal. Typically, mothers describe the massage as an enjoyable experience for them and their infant. For over 35 years, the ATVV intervention has been administered safely to more than 800 infants, including premature infants with severe brain injury. It is documented in the literature that ATVV has a positive effect on the premature infant.

2.2.2.2 Amelioration COVID-19 Risks

All research personnel are trained in safety measures specific to COVID19. Standard operating procedures describing safety measures taken by and on the behalf of personnel and research participants were submitted to Research, Innovation, and Impact initially for protocol-development activities which was approved on February 17, 2021, and then for research activities which was approved on April 6, 2021. (See SoP Conducting a Clinical Research Project during a Pandemic.)

2.2.2.3 Amelioration of Emotional Discomfort or Distress

Self-report questionnaires include measurement of symptoms of depression, general anxiety, and adverse experiences which may cause some women to become uncomfortable or anxious when completing these questionnaires which encourage self-reflection regarding mood, adversity, or health. Participants are encouraged to inform the research team member if they feel uncomfortable completing any questionnaire or question. If continuing to complete the questionnaires causes discomfort, they may choose to skip certain questions, complete the survey at home, complete it later, or withdraw from the study without consequence.

Additionally, our team has extensive experience with developing interview guides on sensitive topics and has designed the content and ordering of questions to minimize distress. However, we have created an emergency process which will be put into action in the unlikely circumstance that distress continues or escalates during the interviews. The specific emergency plan that will be implemented will be based on the presence or absence of suicide risk. (See SoP Adverse Events).

2.2.2.4 Amelioration of Privacy Risk

Electronic data will be stored on two secure systems that use encryption - Research Electronic Data Capture (REDCap) and University of Arizona Box Health (UA Box Health). All study laptops and tablets will use password encryption for access to the laptop or tablet. All data outside of these systems will have unique anonymous codes or in the case of paper data, be secured in a locked file cabinet in a locked room. Laboratory specimens (labeled with unique anonymous codes) will be processed and stored in freezer systems within locked keycard access rooms at the UA College of Nursing, Biological Core Laboratory.

2.2.2.5 Compensation for Research-Related Injury

Participation in this research study possesses no more than minimal risk for injury or adverse effects. Therefore, no compensation funds in the event of research related injury have been set aside. In the past 35 years of NIH-funded research with the ATVV, there has not been an adverse event directly related to the ATVV intervention, even when infants with brain injury were enrolled. This study will enroll low-risk healthy mothers and infants. Infants are excluded from the study if born with a significant congenital anomaly or medical condition which inhibits mother-infant bonding.

2.3 Ethical Considerations

2.3.1 Observation of Abuse or Neglect

Researchers and professionals who work with families with children under the age of 18 are responsible for the welfare of children. If the research team suspects an infant has been physically or emotionally abused or neglected, following the guidelines set by the Arizona Department of Child Safety (DCS), it is our mandatory responsibility to report it to the Arizona DCS. Mothers will be informed in writing via the consent form of our responsibility to report suspected child neglect or abuse. (See SoP)

2.3.2 Participation of Children in Research

The University of Arizona requires that the assent form, signed by the mother of the infant from whom the saliva is obtained, and aggregate data be retained until the child reaches 24 years of age (6 years after the child reaches 18 years of age in the state of Arizona). Please see disposition of data in section 7.26.

3.0 OBJECTIVES AND ENDPOINTS

3.1 Study Overview

We will test the ATVV, a multisensory behavioral intervention, where mothers learn to engage with their infant while administering Auditory (infant directed speech), Tactile (massage), Visual (eye to eye gaze), and Vestibular (rocking) stimulation.[24] We build on ATVV's success with preterm infants and their mothers (e.g., improved maternal sensitivity, lower maternal distress, and enhanced infant behavior and development) to determine benefits for full-term M-I dyads.[25-30] We will target first-time mothers with childhood adversity. Mothers randomized to ATVV will administer it once daily for the first 3 postnatal months. At 3 months, when full-term infants reliably exhibit clear cues and maintain alertness, we will apply a rigorous behavioral measure of M-I synchrony developed by a member of our team using frame-by-frame video of micro-coded M-I behavior quantifying reciprocal speech, touch, gaze, and affect.[31, 32, 73] Micro-coded behavior yields rich detail of interaction patterns known to influence long-term developmental outcomes.[7, 18, 19] Our feasibility pilot showed significant and strong correlations between ATVV frequency and improved M-I synchrony over one month.

We will also assess ATVV's effect on maternal and infant peripheral OXT levels,[7, 19] a known biomarker of M-I synchrony. We, and others, have shown that dampening in the OXT system (i.e., abnormally low OXT blood and saliva levels) is evident in poor M-I synchrony and maternal behavior,[33-37] yet little is known about OXT's epigenetic regulation of synchrony or maternal behavior in humans. Thus, we will target regions of the **OXT receptor gene (*OXTR)*** where DNA methylation is linked to maternal behavior in rodents, psychosocial behaviors in humans, and in our pilot study to M-I synchrony.[38-52] We will examine whether our OXT measures emerge early to predict efficacy of ATVV and quality of M-I synchrony at 3 months. We analyze maternal blood in pregnancy and at 1, 2, and 3 months post birth (and infant salivary OXT). We aim to identify molecular mechanisms in the OXT system underlying M-I synchrony in maternal blood and discover molecular profiles. Regression based methods assess ATVV effects on and relations among M-I synchrony and OXT measures.

3.2 Aims

To meet these objectives, we have designed a 4-year, single-site, randomized, attention-controlled clinical trial with the following aims:

Aim 1: Evaluate the efficacy of daily ATVV over 3 postnatal months among mothers with childhood adversity.
We hypothesize that compared to the Attention Control group (n=125), mother-infant dyads in the ATVV group (n=125) will exhibit:

- H1: Higher oxytocin (OXT) function (i.e., decreased oxytocin receptor (OXTR) gene methylation at candidate sites, and increased OXTR mRNA, OXTR protein, and OXT in maternal plasma, and increased OXT in infant saliva) at 1, 2, and 3 postnatal months.

- H2: More synchronous eye-to-eye gaze [primary], positive affect [primary], speech, and touch at 3 postnatal months.

Aim 2: Identify molecular mechanisms in the OXT system underlying mother-infant synchrony (N=250).

- H1: We hypothesize that lower mother-infant synchrony will be associated with dampening of the OXT system, evidenced by increased OXTR methylation at candidate sites, and decreased OXTR mRNA, OXTR protein, and OXT in maternal plasma, and decreased OXT in infant saliva at 3-postnatal months.

3.3 Outcomes

The two primary outcomes are mother-infant synchronous gaze and mother-infant synchronous affect. These and the secondary outcomes are listed in Table 2. Constructs and Operational Measures.

Table 2. Constructs and Operational Measures

Construct	Operational Measure	Time*			
		B/I	1	2	3
Outcomes					
OXTR epigenetic marks	Maternal plasma for % DNA methylation from candidate CpG sites -1001, -959, -934, -924, -901, and -860. Methylation relates to social, maternal nurturing behavior, & mRNA levels[38-44] CpG sites selected based on their implication in previous studies[46-50, 69] and cost-effectiveness in co-targeting additional regions using the selected assay.	☐	☐	☐	☐
OXTR expression	Maternal plasma for mRNA level using qRT-PCR to report total *OXTR* gene expression (this reflects DNA transcribed [copied] into RNA, before translation produces protein).	☐	☐	☐	☐
OXTR protein	Maternal plasma for total protein level of OXTR (amount of OXT receptors available to bind with OXT peptides) using Anti-OXTR antibody and Western Blot (gold standard).	☐	☐	☐	☐
OXT peptide	ELISA assay to report OXT peptide level from maternal plasma and infant saliva. In our pilot, we collected sufficient infant saliva (1.5-2.0 ml) for the kit's sensitivity of 15pg/ml OXT detection. Antenatal and postnatal OXT measures are stable between groups.[140]	☐	☐	☐	☐
Mother-Infant Synchrony	3-minute video-recording of mothers freely interacting with their infant and micro-coded for mother and infant gaze [primary], affect [primary], vocalization and touch.				☐

***Baseline (B), In-hospital (I), and postnatal months T1, T2, T3; **Available in Spanish**

3.3.1 Mother-Infant Synchrony

Mother-Infant (M-I) synchrony is a coordinated and fluid process involving affectionate "motherese" speech, touch, gaze, and affect.[12-14] Three months is the earliest, most reliable age that M-I synchrony can be assessed with micro-coding.

3.3.1.1 Micro-coding Behaviors

M-I synchrony is measured by micro-coded behaviors quantified from frame by frame video recordings of the mother's and her infant's contributions to the interaction.[7, 12, 31, 32, 73] Behavior categories for mothers and infants (individually and in synchrony) are gaze, affect, vocalization and touch. Each category has mutually exclusive codes. For decades, observations of M-I interaction have used video to reliably assess the quality and nature of M-I interaction.[13] Feldman has shown that synchrony and reciprocity are individually stable from infancy to adolescence,[148, 150] and across situations,[102, 149] suggesting that filming does not obscure the nature of the M-I relationship. Our experience is that mothers rapidly become used to the camera. Micro-coded behaviors have been validated in studies showing associations with maternal and infant risk conditions, OXT and cortisol measures, maternal brain response to infant cues, social adjustment, cognitive function, and autonomic regulation.[7, 12, 19, 63]

3.3.1.2 Micro-coding Parent Behaviors

Parent Gaze: assesses the direction of parent gaze and includes the following codes of gaze to infant's face, to infant's body, to object or environment, and gaze aversion (indicating that parent gazes away from the infant but gaze is not focused on other objects or the environment). Parent Affect: the parent's expressed affect is coded on the basis of facial expressions, body tone, movements, and other non-verbal signals and includes positive, neutral, and negative affective expression. Parent Vocalizations: the parent's vocal output is coded along four codes of 1) "motherese" speech which is infant-directed speech typically high-pitched with a sing-song rhythm; 2) "typical" adult speech to the infant in a normal range and regular rhythm; 3) adult speech to other adult; and 4) no speech. Parent Touch: includes six codes of affectionate touch (loving touch such as hugging, kissing, stroking, or light finger-tip touching); touch of infant

extremities (touch of infant's hands or feet often with another object); functional touch (touch that has a functional goal such as wiping the infant's mouth); proprioceptive touch (includes touch that changes the infant's position in space (e.g., pulling the infant to a sitting position); stimulatory touch (indicating touch that intends to stimulate and increase arousal); and no touch.

3.3.1.3 Micro-coding Infant Behaviors

Infant Gaze is coded similar to parent's gaze along the following codes: gaze to parent, gaze to object or environment, and gaze aversion. Infant Affect is coded as high positive (laugh, giggle, smile), positive (smile, bright face), negative (sad face, occasional whimper), high negative (cry, strong whining), or neutral (none of the above). Infant vocalizations include positive (such as positive babbling, cooing, or giggles) and negative (such as fussing and crying). Infant Touch includes intentional, accidental, and no touch.

3.3.1.4 Categories of Synchrony

Two variables are created as measures of synchrony via conditional probabilities (parent in behavior X while infant in behavior Y). Affect Synchrony: episodes in which parent and infant simultaneously express positive affect. Gaze Synchrony: episodes in which mother and child both look at each other's face. Vocal Synchrony: mother expresses "motherese" vocalization while infant emits positive vocalizations. Touch Synchrony: mother touches infant affectionately when mother and infant share social gaze.

3.3.1.5 Coding Procedures

At the study session, once the infant is in an alert state, mothers are instructed to freely interact with their infant during 4 minutes of video recording. When coding, the first minute is ignored and coding occurs on the final 3-minutes. Interactions are micro-coded frame by frame on a computerized system (Noldus, Waggenigen, The Netherlands), consistent with previous research on parent-infant synchrony.[31, 102-103] Coders of the videos are blinded to group condition. They will prepare the videos for coding, code the data, and transfer data into a form ready for analysis. Coders are trained to 90% inter-rater reliability, and 20% of videos will be double coded for inter-rater reliability. Coding of mother-infant synchrony has been validated in multiple studies and measures of synchrony have shown to correlate with maternal and infant risk conditions, oxytocin and cortisol levels, maternal brain response to infant cues, and to predict better social adjustment, cognitive functions, and autonomic regulation in research spanning birth to adolescence.[7, 19, 63]

3.3.2 Oxytocin System

To understand the biology underlying M-I synchrony, and the efficacy of ATVV, we are comprehensive in analyzing the OXT pathway: epigenetic regulation and expression of the receptor gene, expression of the receptor protein, and peptide level from maternal blood, and peptide level from infant saliva. Our approach expands upon most synchrony studies limited to only OXT peptide level. Samples will be initially processed at the College of Nursing (CON) Biological Core Laboratory within 2 hours of collection. Sample ID, collection time, volume, time received, and date of extraction will be recorded and entered into data storage software for specimen tracking. Processed samples will be labeled and stored at -80C in the lab's freezer bank until needed for the project's analyses. Freezers are in a secure key card access room and are continuously monitored with a malfunction alarm system.

3.3.2.1 OXTR DNA methylation from maternal plasma

Methylation will be measured at the University of Arizona's Genetic Core using bisulfite conversion, followed by high-throughput next-generation sequencing of PCR amplicons. Briefly, bisulfite conversion will be performed using the EZ DNA Lightning Kit (Zymo Research, Irvine, CA; D5031). After conversion, DNA will be PCR amplified using previously published primers TSL101F (5'-TTGAGTTTTGGATTTAGATAATTAAGGATT-3') and TSL101R (5'-biotin-AATAAAATACCTCCCACTCCTT ATTCCTAA-3').[39] These primers amplify a region between -1001 and -856 (relative to the translation start site) allowing us to measure methylation levels of CpG sites at -1001, -959, -934, -924, -901, and -860.[38-

[44)] Primers will be modified with 5' linker sequences, as previously described,[(105)] and a two-stage PCR process will be used to incorporate sample-specific barcodes and Illumina sequencing adapters into each amplicon.[(106)] Deep sequencing (>1000 sequences/amplicon/ sample) of amplicons will be performed using and Illumina MiSeq (Illumna, Inc., San Diego, CA) instrument, and percent methylated cytosine at each position along the OXTR amplicon will be evaluated by analysis of sequence data, allowing for high resolution analysis of percent methylation. Efficacy of bisulfite conversion and sequencing error rates will be evaluated using cytosine positions that are not part of CpG sites, and through methylated and unmethylated standards. Raw reads will be mapped to expected amplicon sequences in a bisulfite conversion-aware manner.[(107)] Methylation will be quantified at each CpG based on the distribution of methylated and unmethylated bases aligned to that position. Additional exploratory CpG sites have been selected based on their implication in previous studies[(46-50, 69)] and cost-effectiveness in co-targeting additional regions using the selected assay.

3.3.2.2 OXTR mRNA expression from maternal plasma

Total *OXTR* RNA will be measured at the CON Biological Core Laboratory and extracted from whole blood using the simplyRNA Blood Kit, including a DNAse treatment step. RNA will be reverse transcribed using a High-Capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific). Target gene expression (*i.e.*, OXTR; Hs01041213_m1) will be normalized to the geometric mean of Glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Hs99999905_m1), Beta-2 microglobulin (B2M; Hs00187842_m1) and hypoxanthine phosphoribosyltransferase 1 (HPRT1; Hs02800695_m1). All assays will be *Taqman* gene expression assays, synthesized by Life Technologies, and will include exon-exon junctions, to reduce background genomic DNA amplification. Quantitative PCR will be conducted on an ABI ViiA7 real-time PCR instrument, employing TaqMan® Fast Advanced Master Mix (Life Technologies). All assays will be performed in technical triplicates, and fold changes in gene expression will be assessed using the comparative CT method.

3.3.2.3 OXTR protein expression from maternal plasma

OXTR protein will be measured at the CON Biological Core Laboratory and isolated using NE-PER™ Nuclear and Cytoplasmic Extraction Reagents (ThermoFisher, 78833). Protein will be separated on a NuPAGE™ 4-12% Bis-Tris protein gels (ThermoFisher, NP0342BOX) with constant voltage 130V for 0.5 hours and transferred for 2hrs onto PVDF membranes. Membrane will then be incubated one hour in 5% blocking solution, then overnight with rabbit anti-OXTR antibody (Abcam, ab181077) at a 1:200 dilution in TBST. Anti-rabbit secondary antibody (Cell Signaling; 7076S) will then be used at a 1:20,000 dilution in TBST. β-actin will be detected using mouse anti-β-actin antibody (Sigma-Aldrich; A5316) at a 1:2000 dilution in 1% blocking solution overnight at 4 °C followed by anti-mouse secondary antibody (Cell Signaling; 7076S) at a 1:5000 dilution in 1% blocking solution for 2 hrs. Western blots will be developed using SuperSignal™ West Pico PLUS Chemiluminescent Substrate (ThermoFisher; 34580), detected using a BioRad ChemiDoc™ MP System and quantified using Quantity One software. Standard curve for OXTR protein will be constructed by making standard solution with different concentrations.

3.3.2.4 OXT peptide from maternal plasma

OXT peptide will be measured at the Laboratory for the Evolutionary Endocrinology of Primates (LEEP). Blood samples will be drawn into pre-chilled EDTA (1mg/mL blood) tubes and placed on ice. Tubes are centrifuged at 1600 xg for 15 minutes at 4°C. Plasma will be transferred to a pre-chilled 1.5 ml eppendorf plastic tube and stored first at -20°C for at least 24 hours, then at -70°C for long term storage. Samples are sent to LEEP to be analyzed in duplicate by enzyme immunoassay (EIA) using the Oxytocin ELISA kit (Enzo; ADI-901-153A-0001).

3.3.2.5 OXT peptide from infant saliva

OXT peptide will be measured at LEEP. Saliva samples will be collected using Salimetric's swab method for infants (used in our pilot). A 90mm x 6.5 mm polymer swab (able to withstand chewing) is held firmly in the infant's mouth for 90 seconds. Tubes are centrifuged at 500-1000 rpm for 5 minutes at 4°C. The supernatant is aliquoted into 1.5 mL tubes and frozen at -80. Samples are sent to LEEP to be analyzed in duplicate by enzyme immunoassay (EIA) using the Oxytocin ELISA kit (Enzo; ADI-901-153A-0001).

4.0 STUDY DESIGN

The single-site study design is a 4-year, randomized, two-group, attention-controlled clinical trial. Women (N=250) will be screened for childhood adversity, enrolled in pregnancy to obtain baseline OXT data. Soon after birth (pre-hospital discharge), M-I dyads who continue to be eligible are randomized into the intervention group (*n*=125) and taught to administer the ATVV intervention daily for 3 months or randomized into the attention control group (*n*=125) and taught safe infant care. There are a total of 5 study visits (3rd trimester, soon after birth, 1st postnatal month, 2nd postnatal month, 3rd postnatal month), plus a follow-up phone call at 6 postnatal months to assess quality of parenting using a self-report questionnaire.

This is a randomized controlled trial (RCT) comparing data between the ATVV and Attention Control groups. Random assignment is centrally controlled by our statistician using a computer-generated list of random numbers (0=intervention and 1=control), balanced so that half the M-I dyads are in the ATVV group. Mothers will be asked to not disclose their group assignment to mothers in the postnatal unit.

4.1 Study Population

The study population sample and setting is perinatal women giving birth at either Tucson Medical Center (TMC), Banner University Medical Center (BUMC), or an alternative site. As the region's largest birthing facility, TMC had 5582 live births in 2019.
Of the births at TMC, 89% were full-term (> 37 0/7 weeks), 99% were > 18 years, 39% were first-time mothers, 59% were Latina White, 10% were non-Latina White, 8% were American Indian, 4% were Black, and many lived in zip codes with income below the poverty level. The BUMC (located adjacent to the College of Nursing) had 1733 live births in 2019. Of the births at BUMC, 90% were full-term (> 37 0/7 weeks), 90% were > 18 years and 31% were first-time mothers. Additionally, 79% of birthing women at BUMC self-identified as White, 7% as Black, and 4% as Latina. Both TMC and BUMC are within Pima County, where in 2018 Latina women represented 48% of births and non-Latina White women represented 39% of the births, thus we expect about half of our sample will be Latina.

4.1.1 Inclusion Criteria

Pregnant women are eligible if they are adults > 18 years old, giving birth to their first child (will be primiparous), speak and read English or Spanish, score > 2 on the Adverse Childhood Experiences (ACE) questionnaire, have access to a cell phone during the first three months after delivery, and expect to deliver a healthy full-term, singleton infant (> 370/7 weeks) without serious conditions that would impact normal development (e.g. intra-uterine growth retardation, chromosomal or congenital anomalies, or serious illness). After giving birth, M-I dyads can remain in the study if the infant is full-term without serious conditions that would impact normal development (e.g. intra-uterine growth retardation, chromosomal or congenital anomalies, small for gestational age (SGA) or M-I interaction.

4.1.2 Exclusion Criteria

Pregnant women are excluded if they are younger than 18 years old, are giving birth to their second child (primiparous) or higher (multiparous), are unable to speak or read English, score less than 2 on the Adverse Childhood Experience (ACE) survey, do not have access to a cell phone during the first 3 postnatal months, are carrying multiple fetuses (e.g., twins), take illicit drugs, have ever been diagnosed with bipolar, autism, or schizophrenia, delivers a preterm infant (less than 37 0/7 weeks), or delivers an infant with a serious condition that would impact normal development (e.g. chromosomal or congenital anomalies, Small for Gestational Age (SGA) defined by body mass, head circumference, and body length less that the 10th percentile for the gestational age at birth, or serious illness).

4.1.3 Lifestyle Considerations

The participants are free to engage in activities as usual while participating in this clinical trial. However, we request that they not share how to conduct the infant massage.

4.1.4 Strategies for Recruitment

4.1.4.1 Waiver of Authorization and Letter

Using a Waiver of Authorization and site permission to identify potentially eligible women through the Electronic Health Records systems of the recruitment sites (TMC and BUMC), potential participants are approached by clinic staff during their prenatal visits at the recruitment sites. If agreeable, the Recruiter introduces the study. When an eligible woman is interested, the Recruiter invites her to complete a 10-item ACE survey to assess level of childhood adversity. During a pandemic, many pregnant women receive care through telehealth visits rather than in-person visits. The research team member pre-screens using an eligibility checklist and then mails potentially eligible pregnant women a letter introducing the study. The content of the letter is dictated by the sites.

4.1.4.2 Recruitment Materials in Prenatal Clinics

After having obtained appropriate approvals, recruitment materials are placed in those clinics providing face-to-face visits. See Appendix B for examples of the Recruitment Materials. For providers or their delegates asking patients if they would be willing to discuss the study with our research team, we obtain the contact information, prescreen, and call the interested potential participants. A telephone script is followed by research personnel to describe the study and confirm eligibility including completing the 10-item ACE survey. If the woman scores $\geq$ 2 on the ACE and meets the other inclusion/exclusion criteria including expressing interest in reviewing the consent form, we will mail or email (their choice) an informed consent to review. The consent form indicates that we will use the results of the ACE instrument in the study. We call again to answer any study questions and ask if they are interested in participating. If they are agreeable, we consent in REDCap. Women are scheduled for a baseline data collection study visit in pregnancy where they continue the consent process to ensure they have understood the requirements of participation.

4.1.4.3 Referrals, Prenatal Classes, and Community Organizations

We network with other research studies recruiting from OB/GYN which refer patients who have given permission to be contacted by other research studies. Additionally, we recruit via community organizations such those offering prenatal classes. We present a one-minute description of the study and provide our study contact information on a power point slide. See Appendix B for Recruitment Materials.

4.1.5 Screen Failures

If a mother scores less than 2 on the ACE, we will remove all 13 elements of identification and retain only the ACE score without the corresponding answers to the 10 questions. We would like to determine if the inclusion/exclusion criteria such as the requirement to have access to a cell phone, screens out any portion of the population with high ACE scores. Additionally, this study has a second level of screening which occurs at the birth of the baby. If a participant delivers a baby not meeting the inclusion criteria such as full term (37 weeks 0/7 days) and healthy, they are withdrawn from the study and are not randomized. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE) which are retained with study records. Because there are no modifiable factors associated with screen failure, we do not rescreen participants.

4.1.6 Strategies for Retention

We expect an 80% retention rate. [redacted] previous R01 studies demonstrated 85% retention with women administering ATVV to their newborn preterm infant after hospital discharge for 2-months and demonstrated 72% retention for 2-3 months in another study. In this proposal, considering 20% attrition and collecting data through 3-postnatal months, we expect to acquire complete data in 24 months; thus, we require enrolling 3-4 women per week and retaining 2-3 women per week.

4.1.6.1 Track Enrollment and Retention Rates

As part of our quality control process, we monitor with monthly excel graphs our targeted versus actual enrollment and retention rates. We meet each week to review these two graphs and then discuss whether we should continue forward with the plan, discuss how to tweak the current course, or set a new course of action.

4.1.6.2 Establish Relationships

To maintain a culturally sensitive research team in this study, we hired Latinx (bilingual English and Spanish) team members. Additionally, our overall approach to enhance retention is to frequently reach out to mothers to develop a strong trusting relationship that reinforces how valuable their time is and how much we appreciate their participation. We will send holiday and birthday cards, and in addition to the automatic, daily, push-out texts we will send personal texts. We've learned that mothers prefer phone or text communication versus email or mail.

4.1.6.3 Participant Guidance Materials

We combine what we learned between our feasibility pilot and the RCT studies that Co-I Dr. White-Traut has conducted with preterm infants. We provide mothers with large colorful magnets which map out the study visits and expectations associated with participating in the study. See Appendix A. Additionally, we have appointment cards to provide to the mothers when we visit them in the hospital after birth of their baby. These appointments cards are used for all 3 post-natal visits.

4.1.6.4 Reminder Calls, Multiple Contact Numbers, and Flexible Scheduling

Staff send a text message and/or call one week before, two days before, and the day of a scheduled study visit for confirmation. When a participant cannot be reached for one week at the last known phone number, previously provided contacts are asked for assistance in locating her. Flexible scheduling of appointments and rescheduling if necessary, will accommodate mothers' schedules. At each monthly study visit, we will provide positive uplifting feedback to mothers in both groups on their experience with either ATVV and/or maternal caregiving in general.

4.1.6.5 Compensation

Mothers will be provided compensation for their time totaling $300: $50 at enrollment, $50 at birth, $50 at T1, $50 at T2, and $100 at T3. There is no compensation for the phone call at 6 months. Additionally, transportation fees are compensated at $4 per visit or if the participant is traveling via their own vehicle, the College of Nursing maintains a reserved parking spot for research participants.

4.2 ATVV Intervention

4.2.1 Description of ATVV Intervention

The ATVV (Auditory, Tactile, Visual, Vestibular stimulation) is a 15 minute multisensory behavioral intervention consisting of infant-directed affectionate "motherese" speech, moderate touch massage, eye to eye gaze, and horizontal rocking.[24, 76, 77] The ATVV intervention provides clinicians a low-cost, easily accessible intervention for mothers to implement that has been well researched by our team with preterm infants.[25-30] The Pathways Foundation, whose educational materials are approved by the American Academy of Pediatrics, includes ATVV in their education for parents with preterm infants (https://pathways.org/growth-development/massage-30-10-5/). ATVV is an enjoyable daily opportunity for mothers to practice modeling M-I synchrony by using eye to eye gaze, affectionate speech and soothing touch.

During the 15-minutes in which ATVV is administered, multisensory stimuli are presented in gradual progression:[24, 76, 77] "motherese" speech which is infant-directed speech typically high-pitched with a sing-song rhythm for 30 seconds, followed by massage for 10 minutes, with eye to eye gaze added as the infant becomes alert (ideally at the beginning of the intervention), and ending with rocking for the remaining 5 minutes. Importantly, "motherese" is maintained throughout the entire 15-minute intervention as long as disengagement cues are not exhibited. M-I engagement is attempted throughout the intervention; thus, mothers learn to identify, interpret and adapt to their infant's developmentally appropriate cues. The ATVV is applicable throughout the study period because it can be given even if the infant is too young for eye to eye gaze. Regardless of whether the infant is 1, 6, or 12 weeks old, ATVV is always offered contingent on infant cues to promote self-regulation and is withdrawn if the infant exhibits persistent disengagement cues.[24, 76]

4.2.2 Administration of Intervention

ATVV is administered by mothers soon after birth;[29, 30, 78] thus, filling a gap for simple interventions to promote engagement between mother and infant in early infancy when experience-dependent neural plasticity is high in maternal and infant brains.[22, 23] In administering ATVV, mothers learn to be comfortable recognizing and adapting their behavior when the infant is signaling cues to engage or disengage. ATVV is an enjoyable daily opportunity for mothers to practice modeling M-I synchrony by using eye to eye gaze, affectionate "motherese" speech and soothing touch.[78]

Mothers will be instructed to administer ATVV for 3 months, once daily at any time of their choosing, as long as it is in-between feedings when the infant is ready to interact but not too hungry (resulting in fussiness) and not too full (resulting in sleepiness). Daily use allows for ATVV to frequently reinforce positive M-I interaction. While twice versus once daily (in comparison to controls) yields more significant improvements in biologically at-risk preterm infants,[23, 27, 29, 30] our pilot showed that once versus twice daily is more acceptable for mothers of healthy full-term infants. A research team member will teach the intervention with the assistance of the ATVV Intervention Manual for Parents, and the online ATVV teaching video targeting newborns. The ATVV is easily learned within one hour and shown to be highly acceptable by mothers in the literature and in our pilot (> 90% agreement with the ATVV Reliability Checklist.[97] In addition to taking home the ATVV manual, mothers can access our online teaching videos with infants at three different ages (newly born, 6 weeks, and 3 months old).

4.2.3 Concomitant Activities

We will not mention the use of oils or other massage techniques during instruction. If we are asked about their use during the weekly phone calls, we will discourage their use. Additionally, a breastfeeding guide is included in the hospital materials, but questions regarding breastfeeding are referred to their primary care provider or other resources.

4.3 Intervention Compliance

To monitor mothers' fidelity and dosage of ATVV once daily, we will use text messaging through Twilio, an electronic communications platform within REDCap. To have access to a phone capable of text messaging is an inclusion criterion to participate in the study. Participants may tire of responding to daily texts, but our daily text is extremely brief requiring only a keystroke to respond. If a phone becomes lost, we will provide a temporary one. Each evening, Twilio will text the following-

- Please tell us how many minutes you gave your baby the Massage Plus today. Choose between 0-15 minutes.
- Please tell us the level of stress you may have experienced today on a scale of 0-10.

The question on stress is included because the attention-control mothers also receive the identical text message and stress is a covariate. See section 4.5. We will monitor REDCap daily, and call mothers if we identify noncompliance two days in a row. Second, we will ask during the weekly phone calls if anyone else besides the mother gave the massage. At each weekly phone call and monthly study visit, we will review how often mothers use the intervention (self-report vs REDCap data) and discuss solutions to barriers of fidelity (e.g. lack of time, fussy or sleepy infant). We will also discuss who else may be giving the massage to their baby.

4.4 Attention-Control Comparison

4.4.1 Description of Attention-Control Group.

The Attention Control group is designed to provide a similar amount of research staff attention while in the hospital after birth, during weekly phone calls, and at 3 postnatal study visits but with the focus on didactic education (content distinctly different from the intervention). The Attention Control education protocol has been used for over 15 years in three of our previous RCTs, demonstrating acceptability to mothers, easy implementation, and rigor in isolating the impact of ATVV.[28-30, 78] In our Attention Control, mothers will receive education on safe infant care including content on diapers, infant clothing, blankets, bathing, sleep positions, sleep habits, holding the baby, safety of infant equipment, breastfeeding, formula, and age-appropriate toys. The research member will use a prepared script and record in the Attention Control Record the information discussed. Since the ATVV intervention mothers will be contacted by a weekly phone call, the research member will also contact the Attention Control mothers by weekly calls to reinforce education. For both groups, weekly calls will include standard milestones in infant development.

4.4.2 Administration of Attention-Control Group.

The Attention-Control group is administered by the Research Assistant. An Attention-Control manual similar in appearance to the ATVV manual but differing in content is provided to mothers while they are in the hospital prior to discharge. Review of the infant care material while in the hospital is of equivalent time as required to instruct the ATVV Intervention group. The Attention-Control group also receives weekly phone calls to discuss topics of their request regarding infant care and development. We maintain focus on care and away from the mother-infant relationship. Questions regarding breastfeeding are referred to their primary care physician or other resources.

At the monthly visits and weekly phone calls with mothers in the attention control group, we will reinforce education on safe infant care (including content on diapers, infant clothing, blankets, infant care including bathing, sleep positions, sleep habits, holding the baby, safety of infant equipment, breastfeeding, formula, and age-appropriate toys). We will also include standard milestones in infant development for both groups.

4.4.3 Concomitant Activities

Equivalent to the ATTVV Intervention group, we will not mention the use of oils or other massage techniques during instruction. If we are asked about their use during the weekly phone calls, we will discourage their use. A breastfeeding guide is included in the hospital manual (and provided to the ATVV Intervention group), but questions regarding breastfeeding are referred to their primary care provider or other resources.

4.5 Covariates

We will control for key covariates that may influence outcomes (Table 3). We include a range of instruments to assess psychosocial factors experienced as an adult that may impact the OXT system and parenting behavior.[39, 110, 112, 128-130] To avoid an OXT spike during/after breastfeeding, we postpone blood draws to > 30-minutes after breastfeeding ends (a valid method used by us and others when studying OXT in breastfeeding mothers).[131] Measuring M-I synchrony < 3 months of age has lower validity, thus we will use the Parenting Stress Index as a valid proxy for quality of parenting [126,127] expecting it be associated with synchrony.

Table 3. Covariates

Construct	Operational Measure	B /I	1	2	3
Covariates					
Demographics Characteristics	**Maternal:** health status, age; weight; birth events; return of menses; birth control; mental health history; drug/alcohol use; marital status & father's involvement; education level; income **Infant:** health status; age; gestational age at birth; infant feeding method; infant's caretakers;	☐	☐	☐	☐
Maternal Adversity**	**Adverse Childhood Experiences** (ACE): well-established 10-items on verbal, physical, and sexual abuse; emotional and physical neglect; household challenges (e.g., substance abuse, mental illness, prison). 71% response rate & 93-99% response rate to individual questions.[2] Distribution of scores (*N*=248,934)[3] 0=38%, 1=24%, 2=13%, 3=9%, ≥ 4=16%. Stable over time: test-retest kappa = 0.6-0.7[108] Maternal ACE ≥2 associated with infant developmental delay (N=311, p<0.05).[109]	☐			
	Adverse Adult Experiences: 8-item modified version of the 10-item ACE providing a similar measure of adversity in adults > 18 years and associated with mental health.[112]	☐			
Maternal Mental Health**	**Post-traumatic stress disorder** (PTSD): Mini International Neuropsychiatric interview (MINI) Module H, V. 7.0.2 for DSM 5, 2016: 7-items[113] Concordance between PTSD in MINI versus Structured Clinical Interview for DSM-III-R Patients: Inter-rater reliability kappa=0.78, sensitivity=0.85, specificity=0.96, test-retest kappa=0.73.[114]	☐			
	Depressive symptoms: Patient Health Questionnaire (PHQ-9): 9-items using DSM-IV depression criteria.[115] Scores range 0-27 [115] Diagnostic validity from 8 primary care & 7 obstetrical clinics. Scores > 5 mild symptoms. Scores > 10: 88% sensitivity & 88% specificity for Major Depressive Disorder (MDD). Cronbach's α=0.86 & 0.89. Scores ≥ 10: 7-14 increased odds of MDD diagnosis.[116]	☐	☐	☐	☐
	Anxiety: Generalized Anxiety Disorders (GAD-7): 7-items[117] Scores range 0-21[117] > 5 mild, > 10 panic disorder, social anxiety & PTSD (66-89% sensitivity & 80-82% specificity).[118] α=0.89 in pregnant and non-pregnant.[119, 120]	☐	☐	☐	☐

Parenting Stress**	**Parenting Stress Index (PSI-SF)**: 36-items[123,124] 3 domains: parental distress, parent-child dysfunctional interaction, & difficult child. Appropriate from 0 - 3 years. Associated with maternal intrusiveness & sensitivity (elements of synchrony).[125] Test-retest=0.85, α=0.91.[123]		□	□	□

***Baseline (B), In-hospital (I), and postnatal months T1, T2, T3; **Available in Spanish**

4.6 Minimization of Bias

4.6.1 Randomization and Blinding

This is an RCT comparing data between the ATVV and Attention Control groups. Random assignment is centrally controlled by our statistician using a computer-generated list of random numbers (0=intervention and 1=control), balanced so that half the mother-infant (M-I) dyads are in the ATVV group. Block randomization is used to randomize participants into two groups of 125. The algorithm will consider blocks of size 2,4,6 and 8 and constrain the number of blocks of each size so that the total number of participants is 250. The block labels will be shuffled and assignments to the control and intervention groups will be evenly split at random within blocks. Additionally, to reduce allocation bias we will use sealed allocation and an audit trail. The database manager will create and retain a pdf version with time and date "seal" for the allocation sequence. The database manager will also encrypt/hide the contents of the cells containing the allocation. Allocation will be made by temporal sequence of 1) date and time of notification, then 2) date and time of birth. A specified member of the research team will input the date and time of birth of the infant to "unseal"/reveal the allocation. The date and time of unsealing provides an audit trail. The research team and PI are blinded to the allocation sequence. The educator of the ATVV Intervention group and the educator of the Attention Control group will not have access to the randomization files and will be notified of allocation at the time of initiation of scheduling the hospital visit.

4.6.2 Division of Responsibilities

The Attention Control Educator ([redacted]) teaches infant care to the Attention Control women before hospital discharge. Similarly, the ATVV Intervention Educator ([redacted] teaches ATVV to the ATTV women before hospital discharge. Instruction for both group conditions take approximately 30 minutes. To prevent bias, only Attention Control Educators will work with the Attention Control group, and only [redacted] will work with the ATVV group. The Program Coordinator assists with baseline data collections as needed to maintain this division of responsibility. However, personnel management and participant-language needs necessitate some flexibility.

4.7 Power Analysis

Our final sample size at the 3-month endpoint is estimated to be at least 100 per treatment arm, after a projected 20% attrition from enrollment of 250 women. This sample size will achieve 80% power to detect as significant a 0.44 standard deviation difference in primary outcomes (M-I synchrony gaze and affect) between treatment arms assuming α=0.025 and a 2-sided test. Similar interventions on a related outcome (maternal sensitivity), and preliminary data from members of our team (on OXT level), showed between group effect sizes ranging from 0.28 to 0.62. In addition, the standardized differences in M-I synchrony ranged between 0.12 and 0.7 comparing at-risk (i.e., premature infants, maternal depression) and normal comparison groups at 3 and 9 months, supporting our choice of 0.4 as a feasible and clinically meaningful effect size. For aim 2 we have 80% power to detect modest correlations of 0.22 and larger assuming a conservative α=0.01.

4.8 Statistical Analysis Plan

Our primary statistical approach is regression based, using a general linear model to conduct bivariate correlations, multiple linear regression, analysis of variance or covariance, as well as tests of mediation and/or moderation and a mixed-effect model for repeated measures (MMRM) recommended for primary analysis of clinical trials with continuous endpoints.[136] We will screen measures for violations of assumptions and use robust standard errors or power transformations if assumptions are not met. All regression-based analyses will be performed using either SAS software, version 9.4 (SAS Institute Inc., Cary, NC), or Stata, version 15.1[137] We will also adopt a discovery-based analysis of patient sub-groupings. This analysis will be based on an unsupervised clustering of all patients across all molecular measurements, with two primary outcomes. We will identify (1) patterns of molecular expression linking different

methylation profiles to gene and protein expression to evaluate the types of molecular signatures that discriminate sample groupings, and (2) sets of clinical covariates that are associated with sample sub-groups to determine the clinical and phenotypic factors that describe and differentiate sample clusters. These analyses will be performed using R software.

4.8.1 Evaluate Efficacy of ATVV

Aim 1: Evaluate the efficacy of daily ATVV among mothers with childhood adversity on behavioral and physical measures of M-I synchrony in a randomized study with an Attention Control group. The goal of our analysis is to determine treatment arm differences at the study endpoint (3 postnatal months), using a 2.5% type I error rate and two-sided test for our primary outcomes. We will use 5% type I error rate and Benjamini Hochberg correction among related groups of secondary outcomes.[147] We will estimate treatment effects with and without key covariates known to be associated with M-I synchrony (e.g., mental health, adversity, infant feeding method, infant health status, and 1-month Parenting Stress Index) which will increase the power of the test. For infant outcomes, we will include sex as a covariate and test for treatment effect differences by sex using an interaction term. Our statistical method for the physical measures, measured at baseline (late pregnancy) and monthly intervals (1-, 2-, and 3- postnatal months), will be MMRM regressing the outcome on indicators for treatment, time, and their interaction. For the physical measures the goal is to determine treatment arm differences in change from baseline to the study endpoint. In contrast, our method for the behavioral outcomes, measured once at the study endpoint, will be a general linear model regressing the outcome on an indicator for treatment arm. We will conduct intention to treat (ITT) and per protocol analyses (defined as completing 80% or more of daily ATVV sessions) and compare our findings. While missing data will be minimized through careful procedures, some missing data are inevitable with longitudinal studies. In order to retain all randomized participants for ITT, we will use multiple imputation to predict missing outcomes with all available measures, and model aggregated results across imputation datasets.[138] For physical measures where we have repeated measurements, we will compare multiple imputation results to the full information maximum likelihood approach available through MMRM. We will use inclusive models with auxiliary variables related to missingness, among covariates collected at baseline, if needed to support the missing at random assumption.[138, 139] Sensitivity analyses will assess the robustness of our findings if we find imbalanced baseline characteristics in spite of randomization, characteristics associated with attrition, or non-ignorable missing data. We will conduct fidelity and dose response analyses in addition to our main effects models. Dose (frequency of ATVV sessions) will be collected daily for three months from the treatment arm participants and can be used to explore the patterns of adherence over time as well as optimal dose associated with OXT system changes by month.

4.8.2 Identify OXT-System Mechanisms Associated with M-I Synchrony

Aim 2: Identify molecular mechanisms in the OXT system underlying M-I synchrony. We will examine the time change pattern of OXT elements to understand the form of their trajectory (e.g., ascending or descending, linear, quadratic) using locally weighted regression (lowess) graphs, stratified by treatment arm. We will also assess the form of hypothesized relationships between elements (i.e., *OXTR* methylation negatively related to *OXTR* mRNA, OXTR protein, and OXT peptide - which are positively related to each other) stratified by treatment group and time. We will model these time change patterns with MMRM and use interactions of time, intervention, and the OXT element to establish if aspects of the OXT system and/or their interrelationships change over time and if that change is influenced by the ATVV intervention. Ultimately, we will assess if the behavioral measure of M-I synchrony is associated with the OXT system and its components, including methylation at base pairs and time trends across repeated measurements. We will examine covariates such as childhood adversity and cumulative stress for their substantive contribution to understanding the relationship between M-I synchrony and OXT functioning, including effect modification with interaction terms. While we will test for heterogeneity in random coefficients through MMRM, we will further explore population heterogeneity using bioinformatics methods, described below, which will provide a complementary, person-centered approach to understanding how subgroups inferred from the OXT indicators are related to personal characteristics, dose, and outcomes.

Molecular Profiles: To address heterogeneity in molecular profiles we will perform a clustering analysis to identify potential subgroups with differing patterns of associations within the OXT system. We will use k-means clustering, doing a search for the optimal number of clusters k by repeating clustering several times for each k over a wide range and using consensus clustering statistics to determine the values of k that yield highly reproducible clustering results. This will yield sample sub-groups that faithfully capture the heterogeneity in the data set without over-fitting clusters to noise. We can then profile the subgroups using the key covariates, treatment arm, and MI synchrony using descriptive and bivariate inferential statistics. In addition, we can examine the different molecular profiles that discriminate different sub-populations to determine putative molecular mechanisms linked to different outcomes observed in the covariate analysis. We will evaluate such differences both qualitatively, using heatmap visualizations, and quantitatively, based on differences between cluster centroids. This will allow us to generate hypotheses about biomarkers that might distinguish individuals most likely to benefit from the intervention. These combined methods will inform us about the efficacy of the ATVV intervention, increase our understanding of the OXT system associated with M-I synchrony, and support our future research to enhance targeted or tailored intervention efforts.

5.0 Quality Management

5.1 Primary Outcome: Mother-Infant Synchrony

All M-I dyads will be video recorded freely interacting with each other for 4 minutes (coding occurs on the last 3 minutes). Video recording occurs when the infant is alert and ready to interact, > 30 minutes after the end of a breastfeeding or formula feeding, and data collection order may be adjusted if the baby is sleepy or fussy. As per Feldman's standardized procedure, we will use two cameras on tripods to zoom in on the mother's and infant's faces separately; during coding the software synchronizes the two recordings. To avoid distracting the M-I dyad, research members are outside of the dyad's room and able to view remotely on a laptop the real-time recordings. Later, videos will be coded for M-I synchrony by team members blinded to group assignment. Coders are trained to 90% inter-rater reliability, and 20% of videos will be double coded for inter-rater reliability.

5.1.1 Double Coding Mother-Infant Synchrony Videos

The PI will train (blinded to group assignment) research personnel coding video-recordings for mother-infant synchrony to > 90% inter-rater reliability by the end of their training period. Additionally, a collaborator's laboratory [redacted] will provide a service of coding 100% of the study's video recordings for reliability of the mother-infant synchrony measure. Collaborators will follow the UA IRB approved protocol for safe and secure handling of specimens and video recordings labeled with unique anonymous codes. Collaborators will not have access to participant identifiers. Data will be shared using the UA Box Health cloud-based secure system. [redacted] The University of Arizona will code 20% of the videos for reliability.

5.1.2 Consultation Meetings

[redacted]

5.2 Reliability of ATVV Instruction

The PI is fully trained to criterion (> 90% agreement with the ATVV Reliability Checklist) on the intervention. [redacted] will train [redacted] to criterion establishing >90% reliability. [redacted] will teach the ATVV to

mothers before they are discharged from the hospital after birth. Inter-rater reliability will be periodically measured by [redacted] for 20% of videorecorded sessions and maintained above 90%.

5.3 Fidelity of ATVV Methodology

At each study visit (1, 2, and 3 months), we will retest parental reliability (ATVV Reliability Checklist) and instruct mothers how to adapt the ATVV with their maturing infant.

6.0 Data, Safety, & Monitoring Plan

This randomized controlled trial has Institutional Review Board (IRB) approval from the University of Arizona (UA). The risks associated with this study are minimal and the potential for adverse events are low. The proposed study meets the NIH criteria for a phase II clinical trial. The ATVV multisensory massage intervention will occur in 125 mother-infant dyads to primarily determine its efficacy at improving mother-infant interaction, compared to an attention control group of 125 women. While pregnant women, mothers and their healthy full-term infants are vulnerable populations, the infant massage intervention includes behaviors that mother typically use when interacting with their infants and is low risk. For over 30 years, we have applied the ATVV intervention to over 800 infants (who were primarily vulnerable preterm infants) and have never had to terminate the ATVV intervention because of physiologic instability. Data collection methods are also low-risk, involving questionnaires, maternal venipuncture, buccal swab for infant saliva, and a video recording of mother-infant interaction. Additionally, our only contact with pregnant women is for enrollment, and one study visit when they will complete questionnaires and allow collection of one maternal blood sample. Given the low risk of this study, our DSM plan will use a Safety Monitoring Committee (SMC) to allow for an independent committee to review data. According to the Policy of the National Institute of Nursing Research (NINR) for Data and Safety Monitoring of Extramural Clinical Trials, an SMC is "a group of two or more experts, who are independent of the protocol, which reviews data from a particular study." Generally, one of these experts is a biostatistician.

6.1 Monitoring Entity

The Principal Investigator (PI) holds primary responsibility for data safety and monitoring. The plan includes a SMC with two committee members, outside of the University of Arizona, who are independent from the study protocol, have experience in clinical trials, and do not have conflicting interests. [redacted]

[redacted]

[redacted]

6.2 Procedures for Monitoring Study Safety and to Minimize Risk

To promote study safety, there are weekly research team meetings for the initial 6-months, and bi-weekly team meetings after the initial 6-months. Team meetings (with the PI, program coordinator, research assistants, and select co investigators) includes monitoring of the following items-

- recruitment,
- retention,
- attrition,

- performance of the study site,
- reliability and fidelity of the intervention,
- confidential data collection and data management,
- quality and integrity of data, and
- participant safety.

The research team members identify potential safety issues and solutions.

The SMC meets semiannually to review data safety and monitoring. The SMC will convene more often if necessary. The SMC reviews materials provided to them by the PI on the following –

- study progress,
- study procedures and protocol,
- performance of the study site,
- confidential data collection and data management,
- quality and integrity of data, and
- participant safety.

The SMC conducts an audit of the following –

- randomly selected 25% of participant consent/parental permission forms to assure compliance with IRB requirements;
- randomly selected 25% of participant data records to assure reliability of data entry including assessing all variables that are missing, invalid or inconsistent;
- monthly quality assurance reports; and
- unanticipated problems and adverse events (see 6.4).

6.2.1 PI Responsibilities

The PI is responsible for coordinating SMC activities and includes the scheduling of meetings and providing appropriate materials to be presented to the SMC. Prior to the first meeting, the PI documents that members do not have conflicts of interest. All discussions and recommendations are summarized and distributed via meeting minutes. The PI develops the SMC meeting agenda, supplies the SMC members with data they require, and verifies reports and recommendations of the SMC.

6.2.2 SMC Responsibilities

The SMC advises the PI on any identified issues and may dictate modification of study procedures if there are concerns with adherence to recruitment protocol, quality and integrity of data collection and data management, and participant safety.

6.2.3 Reports

Within 10 days of each SMC meeting, a report will be prepared by the PI, confirmed by members of the SMC, and submitted to the institution's IRB and the funding agency (NINR) as required by reporting mechanisms. Reporting also includes an annual report to IRB, regulatory and sponsoring agencies as requested, and appropriate reports in the event of an adverse event(s). The SMC is the entity responsible for submitting necessary reports to NINR.

6.3 Description of Data Management

6.3.1 Data Integrity Procedures

The integrity of the intervention and quality of the data will be monitored primarily by the PI, Aleeca Bell, PhD, RN, CNM, who is an expert in the safe clinical care of perinatal women. [redacted] Random assignment is centrally controlled by our statistician as described in 4.6.1. The Recruiter will obtain informed consent in pregnancy. Dr. Bell will train all research team

members with attention to educating members on participants' autonomy, how to avoid applying undue pressure for enrollment, and participants' right to withdraw at any time. All procedures are written, and team members will be required to review written policies.

6.3.2 Security Measures

Data will be securely stored on two secure electronic systems that use encryption: Research Electronic Data Capture (REDCap) and University of Arizona Box Health (UA Box Health). **REDCap** will be primarily used to store informed consent and parental permission PDFs, demographics, health data extracted from medical records, and completed questionnaires. Within REDCap we use Twilio for daily text messaging with participants. **UA Box Health** will be used to store video-recordings, behavioral coding of the content within video recordings, and results of laboratory analyses. All electronic data outside of these systems will have unique anonymous codes.

All study laptops and tablets will use password encryption. A second password will be required to open study databases. The College of Nursing (CON) network is located behind a campus administered firewall. The CON systems are on a "private IP" net and access from outside is tightly controlled at the Firewall. Traffic from Outside to Inside network is blocked and only ports required for normal operations and special purpose ports for applications/services are allowed for outside access. There is also an Intrusion Detection System, and it is regularly monitored for security breaches. Wireless network traffic is isolated from Wired network traffic.

In addition to electronic data, hard copy data, such as informed consents and parental permissions, will be locked in a cabinet within the PI's locked office within the CON. Separate from identifiable data, is another locked cabinet within a locked CON office for data with unique anonymous codes. Only the PI and program coordinator will have access to these locked file systems. Laboratory specimens (labeled with unique anonymous codes) will be processed and stored in freezer systems within locked keycard access rooms at the UA College of Nursing, Biological Core Laboratory. Freezers are continuously monitored with a malfunction alarm system. The Biological Core Laboratory will also conduct the analyses of oxytocin receptor gene expression and oxytocin receptor protein. Samples will be transported on dry ice to the UA Genetic Core for analysis of oxytocin receptor gene methylation. Samples will be transported on dry ice to the UA Laboratory for the Evolutionary Endocrinology of Primates (LEEP) for analysis of oxytocin peptide.

6.3.3 Data Handling Practices

Medical record data from recruitment sites (Banner University Medical Center and Tucson Medical Center) will be viewed in the electronic systems and entered directly into REDCap/Box-Health using password encrypted laptops or tablets. Pre-screening procedures (as described in the section below on data confidentiality) will not use paper but will be entered in a password protected EXCEL file stored on the encrypted UA Box Health. PHI collected on paper (e.g., informed consents and parental permissions) will be kept in a locked cabinet in a locked office at the College of Nursing; PDFs are created and uploaded into REDCap. The scanner in the College of Nursing suite is encrypted. Data from questionnaires and information from mothers collected at study sessions will also be entered directly into REDCap. Blood and saliva specimens are labeled with unique codes without identifiable information and hand delivered on ice by a research team member from the data collection room (2nd floor of the College of Nursing) to the Biological Core Laboratory (4th floor of the College of Nursing) for processing and secure storage. Electronic video recordings of mother-infant interaction at the final data collection session will be labeled with unique codes without identifiable information and stored on the encrypted UA Box Health for coding of data. Complying with federal guidelines, raw data (including video recordings and biological specimens) will be destroyed three years after submission of our final NIH report.

6.3.4 Quality Assurance Measures

To ensure quality of data and compliance with IRB requirements, the following procures will be followed:

• The PI will audit the informed consents/parental permissions in each of the first 20 enrolled participants and then 20% of enrolled participants throughout the study period.

• The PI and [redacted] will train to criterion the ATVV to research personnel ([redacted]) and check inter-rater reliability in the first 10 enrolled participants and 20% of all sessions throughout the study period.

• The PI will audit the first 10 sequential data collection visits and then 20% of all sessions throughout the study period.

• The PI will train (blinded to group assignment) research personnel coding video-recordings for mother-infant synchrony to > 90% inter-rater reliability by the end of their training period. [redacted] will audit 20% of all video-recordings and recode them to maintain reliability.

6.3.5 Data Confidentiality

To minimize risk related to loss of confidentiality, the PIs and other authorized study personnel will have IRB required training in recruitment, informed consent procedures, human subjects Research, HIPAA, and data management using the institution's training modules and certifications. No information about participants during the research will be disclosed to others without a participant's written permission, except if necessary to protect their rights or welfare or if required by law. Only aggregate data will be published or discussed in conferences, and no individual information will be included that would reveal a participant's identity. Secure electronic systems (REDCap and UA Box Health) are described above in the section on data security.

6.4 Identifying, Reviewing, and Reporting Adverse Events and Unanticipated Problems

6.4.1 Definitions

6.4.1.1 Adverse Events

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether considered intervention related or not. According to the UA IRB, an **adverse event** (AE) is further explained as an untoward physical or psychological occurrence in a human subject participating in research which occurs during the study having been absent at baseline or, if present at baseline, appears to worsen. The event may be any unfavorable outcome, including abnormal laboratory result, symptom, disease, or injury.

6.4.1.2 Serious Adverse Events

Adverse events classified as serious include those resulting in death, life-threatening injury, hospitalization, or prolongation of hospitalization, persistent or significant disability, or a congenital anomaly or birth defect. Events not meeting the above criteria but requiring intervention to prevent one of these outcomes are also considered serious adverse events.

6.4.1.3 Unanticipated Problems

According to the UA IRB, **unanticipated problems** involving risks to subjects or others refers to a problem, event or information item that is unexpected, given the nature of the research procedures and the subject population being studied. Potential risks associated with participating in this study have been delineated in section 2.2.

6.4.2 Procedure to Classify an Adverse Event

AEs are classified into the following three categories-

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities. Because this study has been classified by the IRB as research involving no more than minimal risk (Appendix A – Approval Documents), we will not document mild adverse events.

- **Moderate** – Events result in a low level of inconvenience or concern with the intervention. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

In the past 35 years of NIH-funded research with the ATVV, there has not been an adverse event directly related to the ATVV intervention, even when infants with brain injury were enrolled. This study will enroll low risk healthy mothers and infants. Infants are excluded from the study if born with a significant congenital anomaly or birth defect. If a specific safety concern is identified by research team members during enrollment, the weekly phone calls, or monthly study visits, (either for the infant and/or the mother) team members will

- document the event or problem using the Adverse Event Report form,
- follow the protocol as outlined in section 6.2 on minimizing risk which indicates to meet and discuss any AE as a team, and
- report severe events to the PI per instructions at the top of the AE Report.

If there is an immediate safety concern for the mother and/or the infant, the team member consult with the PI. The PI will convene a meeting with the SMC for any moderate or serious adverse events or unanticipated problems. The NIH Guidance on Reporting Adverse Events to IRBs for NIH Supported Multicenter Clinical Trials will be followed. The UA IRB has established policies for 1) reporting unanticipated problems and 2) handling complaints and non-compliance with human subject protection regulations. We will adhere to UA IRB instructions on how to report problems and adverse events, found at https://rgw.arizona.edu/compliance/human-subjects-protection-program/guidance-researchers. The IRB lists events that require reporting within 5 business days of the PI becoming aware (e.g. serious adverse events or serious unanticipated problems), events that require reporting within 15 business days of the PI becoming aware (e.g. adverse events or problems that indicate a greater risk of harm than previously known), and events that do not require reporting (e.g. adverse events or problems not associated with greater harm than previously known).

In summary, this is a minimal risk protocol for injury or adverse events. We have never had an adverse event with the premature infants in our research. For full term infants, there is no anticipated physical risk. Safety assessments and monitoring are incorporated into the project. If a physical risk occurs, the case will be reviewed by the SMC and guidelines established for future review.

6.5 Procedures for multi-site studies to ensure compliance with the DSM plan

This is not a multi-site research project. [redacted] Collaborators will follow the UA IRB approved protocol for safe and secure handling of specimens and video recordings labeled with unique anonymous codes. Collaborators will not have access to participant identifiers. Data will be shared using the UA Box Health cloud-based secure system. Collaborators must complete comprehensive training and be invited to collaborate on UA Box Health.

6.6 Assessment of external factors that may impact safety or ethics of the study

We have not identified any external factors, such as developments in the literature, that may impact the safety of participants or ethics of the study. Infant massage is a common practice worldwide, and our expanded infant massage version (which includes attention to infant cues, eye contact, talking and rocking) is typically enjoyable for mothers and infants. We check intervention fidelity at each monthly study visit, as well as ask mothers how the intervention is going at each weekly phone call. If external factors are identified, the PI and SMC will discuss with NINR appropriate changes to protocol.

6.7 Advanced plans for interim and/or futility analysis as appropriate

This study is minimal risk and the need to execute a futility plan is extremely low. A futility analysis will be based on recruitment, retention, and adverse events. The SMC will monitor recruitment, retention, and occurrence of adverse events through biannual meetings with the PI and if needed through meetings convened by the PI within 48 hours of a serious adverse event. The SMC will develop action plans to remediate areas of concern and provide recommendations.

7.0 Project Management

7.1 Timeline

The proposed research will require approximately four years to complete data collection. We will conduct preliminary analysis on available outcome data at the beginning of years 2 and 3. We have applied and received a no-cost-extension for final laboratory analyses, statistical analyses, and dissemination of findings. The quality of acquired data will be monitored frequently.

Table 4. General Timeline

	Year 1 by quarter				Year 2 by quarter				Year 3 by quarter				Year 4 by quarter			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Staff hiring & training, data set-up	□	□														
Data set up, entry, management, & cleaning	□	□	□	□	□	□	□	□	□	□	□	□	□	□		
Recruitment		□	□	□	□	□	□	□	□	□	□	□				
Data collection		□	□	□	□	□	□	□	□	□	□	□				
Blood & saliva processing		□	□	□	□	□	□	□	□	□	□	□				
Lab analysis of OXTR measures								□					□	□		
Lab analysis of plasma & saliva OXT peptide													□	□		
Coding of M-I synchrony videos							□	□		□	□	□	□			
Statistical analysis					□	□	□	□	□	□	□	□	□	□	□	□
Preparation/dissemination of findings/reports											□	□	□	□	□	□

Table 5. Detailed Timeline with No-Cost-Extension for Final Months of the Study

	10/1 2023	11/1 2023	12/1 2023	1/1 2024	2/1 2024	3/1 2024	4/1 2024	5/1 2024	6/1 2024	7/1 2024	8/1 2024	9/1 2024	10/1 2024 to 1/31 2025
Baselines end	*												
Births end 2-10 weeks later		*	*										
T1 data collections end				*	*								
T2s data collections end					*	*							
T3s data collections end						*	*						
Bio samples to labs	*	*	*	*	*	*	*						
Collect birth data	*	*	*	*	*	*	*	*					
Video coding	*	*	*	*	*	*	*	*					
6-month data collections end								*	*				

Lab results									*	*	*		
Database cleaning	*	*	*	*	*	*	*	*	*	*	*		
Stat analysis							*	*	*	*	*	*	*
Manuscripts										*	*	*	*

7.2 Personnel

7.2.1 Aleeca Bell, PhD, RN, CNM

Dr. Aleeca Bell, PI, assumes scientific responsibility for the overall conduct of the research; develops project procedures and supervises staff training, data collection, database maintenance, and all other aspects of the study; monitors budgetary expenditures; conducts regular meetings among study collaborators; and prepares reports, papers, and presentations of results. Dr. Bell will co-train to criterion the ATVV intervention to research personnel and check inter-rater reliability in a percentage of the sessions. Dr. Bell will train the staff (blinded to group assignment) who code videos for measuring mother-infant synchrony.

[redacted]

7.2.5 Other Investigators

[redacted]

[redacted]

7.3 Settings, Facilities, and Equipment

7.3.1 University of Arizona: College of Nursing (CON):

The PI (Dr. Bell) has a private locked faculty office located in the Division of Biobehavioral Health Sciences. The project's research team will occupy locked offices adjacent to the CON Behavioral Core Laboratory (see description below). Research staff offices allow for confidential research activities. The research team will have shared access to all facilities in the Behavioral Core Laboratory. Data collection will occur in the CON Behavioral Core Laboratory. A waiting area for study participants is available upon entering the laboratory. We will utilize a conference room dedicated to completing informed consent and self-report surveys, teaching women either the intervention or attention control education, checking intervention fidelity, and video recording mother-infant interaction. We will utilize the clinical measurement room to collect infant saliva and for venipuncture to collect maternal blood. In the CON Biological Core Laboratory, laboratory personnel will prepare all study samples for storage. Laboratory personnel will also conduct oxytocin receptor (OXTR) protein and OXTR gene expression analyses.

7.3.2 University of Arizona: Genetics Core:

The University of Arizona Genetics Core (UAGC) will conduct the project's OXTR gene DNA methylation analysis using Illumina MiSeq for deep sequencing. The UAGC offers access to state-of-the-art resources and services to help investigators, educators, students and the biotech community conduct and promote research in the fields of genetic and genomics.

7.3.3 University of Arizona: Laboratory for the Evolutionary Endocrinology of Primates (LEEP):

The Laboratory for the Evolutionary Endocrinology of Primates (LEEP) is an endocrinology lab with enzyme-immunoassay capabilities in the Emil W. Haury Anthropology building. LEEP houses all equipment required to perform the project's plasma and saliva oxytocin assays led by [redacted]

7.3.4 University of Arizona: Statistics Laboratory Facilities:

The Statistics Consulting Laboratory (Stat Lab), includes Co-I [redacted] Stat Lab personnel work closely with the University of Arizona Genetics Core to ensure an efficient, secure, and reliable infrastructure for data management and analysis of genetic data. This coordinated approach streamlines the integration of high-dimensional sequencing data.

REFERENCES