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PROTOCOL TITLE:

Pilot Study of Nurse-Administered Touch and Biobehavioral Stress Responses of Preterm Infants (Pilot NAT-BIO Study)

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VERSION NUMBER/DATE:

Version 2.1 / 11.08.2021

REVISION HISTORY

Revision #	Version Date	Summary of Changes	Consent Change?
1.1	02.05.2021	Pre-approval revision: clarify data storage, clarify IRB reporting of AEs	No
1.2	02.10.2021	Pre-approval revision: clarify nursing involvement in intervention and saliva collection	No
1.3	02.22.2021	Pre-approval revision: change data retention period in section 27-3 from 3 to 6 years	No
1.4	03.29.2021	Pre-approval revision: (1) change to pilot study with up to 20 infants, (2) change from sequential to randomized cross-over with one version of intervention, (3) remove measure of behavioral responses	Yes
1.5	03.30.2021	Pre-approval revision: clarify PI	Yes
1.6	04.02.2021	Pre-approval revision: (1) change title of parent demographic form, (2) add optional photographs to procedure	Yes
2.0	07.14.2021	Post-approval revisions: (1) aims – add neurobehavioral assessment 2) change inclusion ages and add exclusion for special isolation; (3) recruitment – add flier, add option for phone discussion, add infant/nurse compensations; (4) procedures – change infant age at time of observations, change to bare-handed touch, change to "nurse-only" caregiving (5) measures – change from	Yes

		NCH monitors to Holter monitors, change timing of saliva collections, change saliva collection to swabs, add neurobehavioral assessment a 35 weeks; (6) data – add collection of pain score from electronic health record and Neonatal Infant Stressor Scale; (7) remove Deborah Steward from DSMB	
2.1	11.08.2021	Post-approval revisions: (1) change from nurse-collected to researcher-collected saliva and nurse-applied to researcher-applied electrodes, (2) add behavioral sleep measure, (3) change from cellulose microsponges to medical swabs for saliva collection.	Yes

1.0 Study Summary

Study Title	Pilot Study of Nurse-Administered Touch and Biobehavioral Stress Responses of Preterm Infants (Pilot NAT-BIO Study)
Study Design	Randomized cross-over clinical trial
Primary Objective	Determine the effect of a nurse-administered touch intervention (NaTI) on biobehavioral stress responses of preterm infants during essential nursing care.
Secondary Objective(s)	(2) Identify factors to increase feasibility of the NaTI as a nurse-administered, integrated part of essential, procedural care, (3) Determine associations between biobehavioral responses and early neurobehavior.
Research Intervention(s)/ Investigational Agent(s)	NaTI (NaTI): NaTI is a systematic touch intervention consisting of 3 static, brief touches lasting a total duration of 2.5 minutes provided by bedside nurses at specified points during essential care. The intervention is provided with bare hands (i.e. gloves are not worn).
IND/IDE #	N/A
Study Population	Preterm infants born 27 to 30 weeks post-menstrual age; NICU nurses
Sample Size	Up to 20 infants; up to 20 nurses
Study Duration for individual participants	Infant participation on each of 2 days will last approximately 6 hours from the attachment of electrodes for skin conductance responses and heart rate/heart rate variability to the removal of these electrodes. Infant participation will include data collection on two consecutive days for a total duration of 24-48 hours. Infant neurobehavior will be assessed at 35 weeks post-menstrual age. The assessment takes 20-30 minutes. Total infant participation will last from the time of the first observation to neurobehavioral assessment (approximately 5-7 weeks), depending on anticipated discharge and clinical stability for the neurobehavioral assessment. Nurse participation will last approximately 15 minutes per survey completion.
Study Specific Abbreviations/ Definitions	**NaTI**: nurse-administered touch intervention **SC**: standard care **NAPI**: Neurobehavioral Assessment of the Preterm Infant **AIM**: Acceptability of Intervention Measure **IAM**: Intervention Appropriateness Measure **FIM**: Feasibility of Intervention Measure **SID**: study-specific identification **Observation period**: period of active study participation for infants lasting approximately 6 hours and beginning with the attachment of electrodes before the scheduled care episode during which data collection will occur and ending with the following scheduled care episode when electrodes are removed.

2.0 Objectives

2.1 The objective of the proposed study is to determine the effect of a nurse-administered touch intervention (**NaTI**) on preterm infants' stress responses during essential nursing care, measured by changes in biobehavioral stress responses (vital signs, skin conductance

responses [**SCR**s], heart rate variability [**HRV**], behaviors, and cortisol)[1,2]. We will accomplish this objective through the following specific aims:

- **Aim 1 (primary): Determine the effect of a NaTI on biobehavioral stress responses of preterm infants during essential nursing care.**
- **Aim 2: Identify factors to increase the feasibility of the NaTI as a nurse-administered, integrated part of essential, procedural care.** We will examine nurse-related factors that may affect the feasibility of intervention implementation in clinical practice for every episode of essential care.
- **Aim 3 (exploratory):** Determine associations between biobehavioral responses and early neurobehavior.

2.2 We hypothesize that the NaTI will attenuate activation of the physiologic stress response systems.

3.0 Background

3.1 Infants born preterm experience neurodevelopmental impairments in cognition[3], motor function[3], and language acquisition[4,5]. In the United States, 50% of cerebral palsy cases and 8% of learning disabilities are attributable to preterm birth[6]. While many preterm infants receive early intervention services after neonatal intensive care unit (**NICU**) discharge, these services are costly and not available to all eligible infants[7-9]. Interventions during NICU hospitalization may improve neurodevelopment and reduce the human, societal, and economic burdens of neurodevelopmental impairment in preterm infants.

Stress exposure in the NICU is associated with abnormal brain development[10-12] and cognitive, motor, and behavioral neurodevelopmental impairments[13-16]. Preterm infants experience an average of 7.5-17.3 painful procedures[17] and 22.97 acute stressors[16] per day of hospitalization. Seemingly benign procedures (e.g. diaper changes, repositioning) are among the most frequent stressors[16,18] and are commonly clustered together during essential nursing care. Essential nursing care is a frequent, repeated stressor, typically occurring every 3 or 4 hours during the infant's hospitalization. Most episodes (86%) of essential care begin with an abrupt rather than a gradual approach[18], without time for infants to acclimate. Because essential care is repeated, frequent, and abrupt, interventions targeting how essential care is provided may affect preterm infant stress responses and improve outcomes.

Essential nursing care activates the sympathomedullary (**SAM**) pathway and hypothalamic-pituitary-adrenal (**HPA**) axis, resulting in measurable biobehavioral effects[2,19-22]. SAM pathway activation increases sympathetic nervous system (**SNS**) activity and suppresses parasympathetic nervous system (**PNS**) activity in response to painful procedures[23,24] and essential nursing care[21,22,25,26]. Stress responses resulting from SAM pathway activation can be measured as changes in vital signs (heart rate [**HR**], respiratory rate [**RR**], oxygen saturation [**SpO2**])[27], HRV[27], and SCRs[24]. The release of catecholamines from the adrenal medulla in response to SNS stimulation[28] causes stimulation of the cardiac sinoatrial node and measurable changes in HR, RR, SpO2, and HRV[27]. HRV represents the beat-to-beat variations in HR, as measured by the R-R interval, and is sensitive to inputs from the SNS and PNS[29,30]. SCRs, produced by the filling and emptying of palmar and plantar sweat glands in response to SNS stimulation[24], are valid measures of SAM pathway activation in

the first weeks of life in infants born <34 weeks post-menstrual age (**PMA**)[31]. Further, activation of the HPA axis causes a release of cortisol from the adrenal cortex[28]. Cortisol can be quantified from saliva[28], providing a non-invasive measure that is highly correlated with blood levels[32,33]. Salivary cortisol levels increase in preterm infants in response to bathing[26], routine handling[34], and painful procedures[34] and decrease in response to pain management interventions[34]. Early, repeated activation of the immature SAM pathway and HPA axis during essential caregiving may cause aberrant changes in the form of neural programming of these stress response systems, altering their function and contributing to NDI[35]. Neonatal HRV, for example, is associated with childhood neurodevelopment[36,37] and may be an important end-point for powering future studies. Thus, we will determine associations between HRV, as well as HR, SCRs, and cortisol, and infant neurobehavior at 35 weeks PMA.

Activation of the SAM pathway and HPA axis in response to essential caregiving may be a consequence of the mismatch between the preterm infant's neurologic expectation for touch and his/her NICU reality. The fetus *in utero* experiences comforting touch from the surrounding amniotic fluid, uterine containment, and external touches to the mother's abdomen[38]. Conversely, much of the touch experienced by preterm infants in the NICU is procedural or painful[39-42]; preterm infants receive little comforting touch during their hospitalization[18,41-43]. During the last weeks of gestation, neuronal circuits to support the peripheral touch receptors that formed early in gestation become connected to the limbic system, giving social and emotional meaning to touch[44]. These circuits are affected by touch experiences in the NICU[44,45], potentially leading to touch aversion from comfort touch deprivation[38]. Thus, preterm infants may begin to associate any touch with early noxious touch experiences in the NICU, resulting in a poorer tolerance for future interaction and touch[46].

While noxious touch from essential caregiving induces a physiologic stress response[21,22,47], comforting touch enhances PNS activity[48], increases HRV[48], decreases cortisol levels[49,50], decreases behavioral responses to pain[51], and improves neurodevelopment[48]. Maternal skin-to-skin contact, during which mothers hold their diaper-clad infants between their breasts, reduces stress responses of preterm infants during painful procedures[52,53] and increases PNS activity[54]. However, maternal presence in the NICU is minimal[55], hindered by family and job responsibilities and access to transportation[56]. On average, mothers are present for 50% of the days of their infant's hospitalization and provide skin-to-skin care for 0.2 days each week[55]. With limited maternal presence, preterm infants receive little comforting touch[18,41-43]. Nurses provide 87.8% of the direct contacts experienced by preterm infants, but only 3.5% of these contacts are comforting touch[57]. Clinician-administered touch interventions (i.e. massage therapy[48,58] and Gentle Human Touch[49,50,58,59]) are effective in attenuating preterm infant stress responses and promoting neurodevelopment. However, these interventions require training and are typically delivered for only a few minutes each day for a few days[48,60,61]. Comforting touch provided during specific painful procedures such as heel lance[51,62,63] and endotracheal suctioning[62,64] is effective in reducing the stress responses of preterm infants. A case study revealed that 5 seconds of comforting touch to gently awaken a preterm infant at 28 weeks PMA prior to essential care was associated with fewer stress behaviors during the care episode and faster recovery of HR and SpO2 after the care[65].

Much research on preterm infants' stress responses is limited by a narrow focus of measures and insufficient consideration of infant age. Many studies focus on behavioral responses and measures of either SAM pathway[21-24] or HPA axis[66-68] activation. Studies including a broad spectrum of measures needed for comparisons across studies are lacking. Also, many studies include multidimensional indices of pain/stress, scoring infants on behavioral cues, physiologic measures, and/or demographic characteristics (e.g. PMA)[69]. Such indices (e.g. Premature Infant Pain Profile, Neonatal Infant Pain Scale), are difficult to compare across studies and are complicated by developmental factors (i.e. age[69]) and subjective interpretation of scores[70]. We will build on previous research by measuring a broad spectrum of objective stress responses, including measures of SAM pathway and HPA axis activation.

Previously conducted randomized controlled trials (**RCT**s) testing the efficacy of comforting touch have a high risk for bias, resulting in low[62] or very low[70] quality evidence based on the Grading of Recommendations Assessment, Development and Evaluation system. Moreover, heterogeneity among trials makes it difficult to accurately determine effects[48,62]. Much of the bias in these studies results from failure to measure contextual factors (e.g. infant age, previous pain exposures)[70] or lack of blinding[48,62,70]. While it may be impossible to conceal group assignment from caregivers in a touch RCT, we will blind outcome assessors and analysts. Importantly, studies of touch interventions for painful procedures commonly use multidimensional indices of pain/stress[62,63,70] that include subject measures of behavioral responses and may include infant age, making comparisons across infants difficult as previously described[69] and increasing performance bias. We designed this study to reduce the performance bias affecting previous studies by collecting data on context of care, blinding outcome assessors and analysts, using objective stress response measures that can be compared across studies, and following published procedures for quantifying behavioral responses in research studies[71].

We are aware of only one study testing the effect of comforting touch during essential care. In a small study (n=12) of preterm infants, comforting touch was effective in reducing scores on the Premature Infant Pain Profile, a multidimensional index that includes behavioral observations and vital signs[72]. However, this study used two caregivers to provide the intervention, which cannot be feasibly implemented for every episode of essential nursing care during the infant's hospitalization[73]. In addition, infant ages ranged from 24 to 32 weeks PMA at birth, timing of the intervention was not standardized, and the study included only one multidimensional outcome measure[72]. To test the NaTI, an intervention intended for delivery by one nurse, we will standardize intervention delivery and measure multiple objective outcomes.

3.2 Recent work by our study team shows that preterm infants are frequently exposed to stressors associated with essential nursing care and that standard care (**SC**) includes very little comforting touch[74,75]. PI Nist found that infants born 28-31 weeks PMA experienced a median of 24 skin-breaking procedures (e.g. heel lance, venipuncture, venous cannulation) and 30 invasive procedures (i.e. skin-breaking procedures plus entrances into a body cavity) during their first 2 weeks of life[76]. Preterm infants also experienced numerous non-painful stressors during essential care, including a median of 109 diaper changes, 104 gavage feedings, and 79 episodes of gastric aspiration[77]. Because infants experienced these

stressors during clustered essential nursing care, the manner in which this essential care is delivered is critical.

In a RCT, Co-I Pickler tested the effect of a touch intervention provided during preterm infant feedings[75]. Over 195 mean gavage feedings, infants in the experimental group received a touch experience on average 57% of the time versus 16% for infants in the control group; the touch varied from 6-15 minutes based on feeding duration. Importantly, some control group infants received no touch during feedings. Infants receiving greater amounts of touch achieved feeding milestones sooner[78] and had improved neurobehavior at discharge[79]. These data demonstrate that comforting touch does not occur as part of routine caregiving, even though the frequency and essential nature of these activities would suggest their importance to optimal development[80]. We will build on this work by measuring acute stress responses and standardizing the duration of touch.

3.3 The NaTI is a theory-guided, systematically delivered intervention provided during essential nursing care. The Neonatal Stress Embedding Model provides the theoretical foundation for this study and posits that NICU stress exposure activates the SAM pathway and HPA axis; repeated activation of these immature systems in the preterm infant results in aberrant programming, affecting brain structure and neurodevelopment[35]. We will test the short-term effect of the NaTI on the stress response systems and will build on this work in a future study by examining the effect of routine implementation on neurodevelopment. We hypothesize that the NaTI will attenuate activation of the SAM pathway and HPA axis (Fig 1). To determine the effect, we will measure a broad spectrum of stress responses (HR, HRV, SRCs, cortisol), advancing the work of previous researchers who measured infant responses to specific procedures (e.g. heel lance[51,62,63], endotracheal suctioning[62,64]) using a small number of biobehavioral measures. In contrast to other tested interventions, the NaTI requires no specialized training and a minimal time commitment (<5 minutes) from nurses and can be integrated as part of on-going essential care. The NaTI addresses how routine, necessary care is delivered. While the positive effects of comforting touch are well known, comforting touch is not a routine part of essential nursing care.

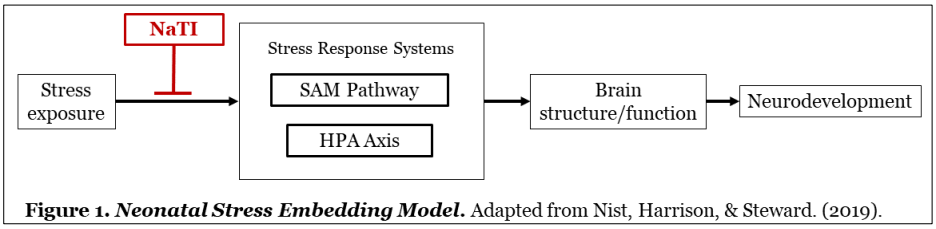

Figure 1. ***Neonatal Stress Embedding Model.*** Adapted from Nist, Harrison, & Steward. (2019).

Routine comforting touch interventions may promote positive long-term outcomes for preterm infants by buffering the effect of noxious touches and providing the necessary, neurologically expected touch that promotes normal sensorineural development. In this study, we will test the preliminary effect of a systematic NaTI provided during essential nursing care on infant stress responses. This study is based on the scientific premise that comforting touch, provided through a systematic NaTI, meets the preterm infant's neurologic expectation for touch and increases PNS activity, attenuating activation of the SAM pathway and HPA axis, and reducing the aberrant programming of these stress response systems.

4.0 Study Endpoints

4.1 Data collection to accomplish aims 1 & 2 will last the duration of two observation periods, one each on two consecutive days. Infant participation will last approximately six hours for each observation period. Participation will begin approximately three hours prior to a scheduled care episode (i.e. data collection period) when monitoring electrodes are attached and will end approximately 2.5 hours after the scheduled care episode (i.e. data collection period) when the next care episode begins and study electrodes are removed. Infants will receive a neurobehavioral assessment at approximately 35 weeks PMA. The timing of this assessment will depend on anticipated discharge and clinical stability. Infant participation for the entire study will last between 5-7 weeks, depending on infant PMA at birth and timing of the neurobehavioral assessment.

4.2 The NaTI will be stopped for infants experiencing bradycardia, defined as HR less than 100 beats per minute for 10 seconds, or oxygen desaturation, defined as oxygen saturation less than 85% for 10 seconds. To prevent the overstimulation of infants during intervention delivery, the NaTI will be stopped for tachycardia, defined as HR greater than 200 beats per minute for 10 seconds. NaTI delivery may resume with the next planned intervention point in the care but will be discontinued for the remainder of the care for two or more episodes of bradycardia, oxygen desaturation, or tachycardia occurring during intervention delivery as defined above. A few infants may not tolerate the handling required to complete the neurobehavioral assessment. The Neurobehavioral Assessment of the Preterm Infant (**NAPI**) is intended to be administered in a specified, fixed sequence to minimize infant handling and potential distress. While we expect that infants at 35 weeks PMA will not find the assessment distressing, for those who exhibit signs of distress (e.g. bradycardia, oxygen desaturation, inconsolable crying), the assessment will be terminated.

5.0 Study Intervention/Investigational Agent

5.1 Description. The NaTI is a systematic touch intervention consisting of three static, brief touches provided by bedside nurses at specified points during essential care. To provide the NaTI, nurses will cradle the top of the infant's head with one hand, using their other hand to contain the infant's lower body. Nurses will provide the touch for one minute at the start of the care to allow infants time to acclimate to the stimulation, 30 seconds after the diaper change, and one minute at the conclusion of the care to provide a gradual end to the care and facilitate infant recovery and sleep. Nurses will provide the touches using bare hands (i.e. no gloves) to provide infants with human skin-to-skin contact and will perform hand hygiene before and after each touch. Nurses often provide discretionary comforting touch, commonly referred to as hand containment or facilitated tucking, in the same manner as the NaTI as part of their routine care (NCH Patient/Family Care Policy 30:23). The NaTI differs from discretionary comforting touch in that it is provided at specific points during the care and for a prescribed length of time. A member of the research team will be present during the care, perform timing for the intervention, and provide verbal instructions for nurses to begin and end the NaTI.

6.0 Procedures Involved*

6.1 ***Study Design. Aim 1.*** Infants will be assigned to standard care or standard care with the addition of the NaTI in a randomized cross-over manner to determine the effect of a NaTI on biobehavioral stress responses during essential nursing care. We define essential nursing care as a scheduled episode of clustered caregiving activities related to health monitoring, hygiene, and clinical needs that includes at least a diaper change and feeding. We will enroll up to 20 infants for this pilot study to collect complete, usable data on 16 infants.

Aim 2. To identify factors to increase the feasibility of the NaTI as a nurse-administered, integrated part of essential, procedural care, we will conduct a survey study of nurses who have provided the NaTI to enrolled infants. We will specifically ask nurses to describe barriers and facilitators of implementation.

Aim 3. We will use a longitudinal cohort design to determine associations between biobehavioral responses and early neurobehavior. We will collect biobehavioral stress response data during the two observed episodes of care and will administer the NAPI to each enrolled infant at approximately 35 weeks PMA.

6.2 ***Procedures. Aims 1.*** Infants will be enrolled during the first 10 days of life. We will collect demographic and clinical data from the infant's electronic health record (**EHR**) after enrollment and throughout the hospitalization. Because stress responses may differ based on postnatal age[81], data collection for stress responses will occur between 10 and 20 days of life. To eliminate variance in cortisol due to diurnal patterns, we will perform all observations at the same time of day for all infants, between 1000 and 1300. Observations will occur on two consecutive days unless infants are not available due to off-unit or other scheduled procedures. In this case, the second observation will occur on the next day that the infant is available. Infants will be assigned to receive standard care or standard care with a NaTI in a randomly assigned sequence. For the two observed care episodes, we will require that nurses provide this care exclusively to limit confounding by the participation of additional caregivers. With assistance from the bedside nurse, the researcher, who is a Registered Nurse, will attach monitoring electrodes for vital signs, HRV, and SCRs during the care episode prior to the observed care to minimize stress responses from handling and monitoring prior to the baseline period (Fig 2). Nurses commonly attach monitoring electrodes as part of their clinical practice.

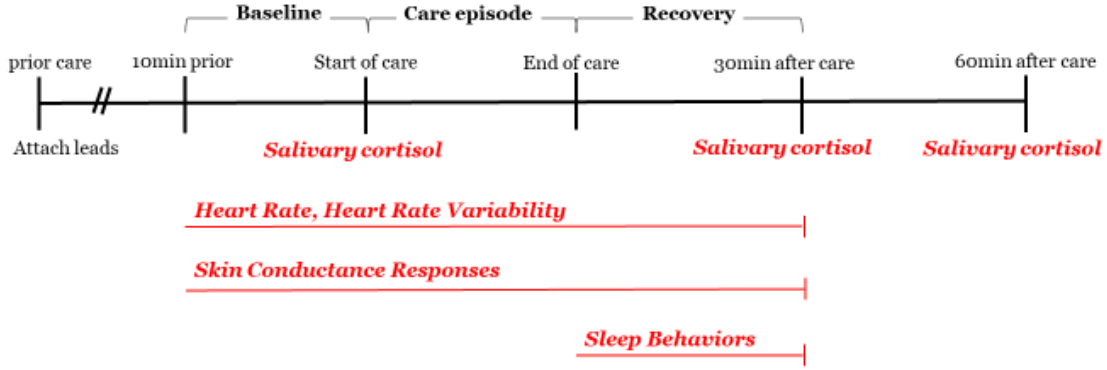

Figure 2. Timeline of study measures.

Measures, Aims 1:

- ***SAM pathway activation.*** *Heart rate* will be measured continuously during the observed care episode, using external monitors. HR, a measure of SNS response, is our primary outcome measure because nurses can use HR in clinical practice to identify SAM pathway activation. *Heart rate variability,* specifically high frequency HRV (**HF HRV**), provides an index of PNS activity and is generally reduced in response to internal or external stressors[82]. Continuous electrocardiogram (**ECG**) data during baseline, care episode, and recovery will be collected using external Holter monitors. Electrodes for ECG data will be applied by the researcher with assistance from the bedside nurse during the care prior to each observed care. Raw ECG waveform data will be imported into Co-I Harrison's MARS® Ambulatory ECG Analysis and Editing System (General Electric, Inc.) and processed according to recommended protocols[82,83]. During processing, artifact will be eliminated with final calculations based on normal sinoatrial node-initiated complexes. Analysis will include HF HRV calculated in one-minute epochs averaged for each phase (i.e. baseline, care episode, recovery). *Skin conductance responses* will be measured by attaching two disposable electrodes with isotonic electrode gel (Biopac Systems, Goleta, CA) to one foot approximately three hours prior to data collection (i.e. during the episode of care prior to the observed episode of care). Electrodes are connected to an amplifier to record SCRs during the entire observed care. At the beginning and end of each period (baseline, care episode, recovery), we will record ambient (i.e. room or incubator) and infant skin temperatures; ambient and skin temperatures <24ºC and <32ºC, respectively, may affect SCRs[84,85].
- ***HPA axis activation.*** *Salivary cortisol* will be measured from saliva samples taken immediately prior to the start of care (baseline), 30 minutes after the care has ended (post-care), and 60 minutes after the care has ended (recovery; Fig 2) using medical sponges gently introduced into the infant's mouth.. We have previously used these sponges for saliva collection from preterm infants without causing infant distress. A member of the research team, who is a Registered Nurse, will collect saliva samples with the assistance of the bedside nurse. Saliva collection is non-invasive and may be collectedas part of clinical practice (test code XSCRT according to the NCH Laboratory Medicine website; see protocol Appendix). We will batch process samples and quantify cortisol levels using a commercially available enzyme-linked immunosorbent assay (**ELISA**; Salimetrics, State College, PA).
- ***Recovery.*** We will measure infant *recovery* by recording episodes of bradycardia, tachycardia, oxygen desaturation, or other monitor alarms during the 30-minute recovery period following the episode of essential nursing care. We will observe sleep behaviors to determine if the infant is awake, asleep, or drowsy during this 30-minutes recovery period using the Anderson Behavioral State Scale (ABBS). Behavioral data will be collected through observation without disturbing or handling the infant.
- ***Context of care.*** We will record the duration of the caregiving episode and the procedures included. We will use the acute scale of the Neonatal Infant Stressor Scale (**NISS**)[88] to quantify the procedural stressors experienced by infants during the observed care episode. We have previously modified this measure to account for

the procedural stressors commonly experienced by preterm infants[77]. Additionally, we will observe and record all direct touches experienced by the infant for one hour prior to the caregiving episode. Direct touches are those defined as direct physical contact between a caregiver and infant and might include parent skin-to-skin care, interventions for apneic episodes, and adjustment of a respiratory interface. We will use these data to compare the context of care for infants between assigned conditions and will examine moderation of infant responses to care episodes by this context.

- ***Demographic and clinical data.*** We will collect *maternal demographic information and clinical data* including age, race, prenatal medications, labor/delivery complications and *infant demographic and clinical data* including sex, PMA, birthweight, clinical comorbidities, and medications at enrollment and throughout hospitalization from the EHR and through parent report. We will record PMA based on first trimester ultrasound, if available, as this method of dating is most accurate[69]. Otherwise, we will use the best obstetric estimate, second/third trimester ultrasound, or postnatal assessment. In addition, we will record the pain score documented by nurses during the observed care. We will retrospectively collect data from the EHR on the number of *invasive procedures* (e.g. lab draws, intubations, intravenous line insertion) experienced by enrolled infants from the time of birth through the observed care episode(s). Finally, we will collect clinical data from the EHR to calculate a Score for Neonatal Acute Physiology (**SNAP**)-II, a validated measure of *illness severity* for preterm infants that includes clinical data from the first 12 hours of life (blood pressure, temperature, oxygenation indices, serum pH, urine output, and presence of seizures)[89]. Finally, we will quantify the amount of *parental skin-to-skin contact* that infants have received from the time of birth through the observation period(s).

Optional infant photographs. With parental consent, we will take photographs of infants for use in future scientific and educational presentations. We will not disclose any information about these infants, but they may be identifiable by their photographs. Photographs will be stored on OSU's password protected research server. Parents may further opt to provide an email address where photographs may be sent for their personal use.

Procedures. Aim 2. Nurses providing the NaTI to infants will be invited to participate in a survey study. Those who choose to participate will complete a demographic questionnaire once and a NaTI survey, Acceptability of Intervention Measure (**AIM**), Intervention Appropriateness Measure (**IAM**), and Feasibility of Intervention Measure (**FIM**) each time he/she provides the NaTI for a study infant. Nurses will be assigned study identification numbers (**SIDs**) to track participation and to link nursing data to infant data. We will provide phone/text reminders for nurses to complete and return the questionnaires.

Measures, Aim 2:

- ***Demographic questionnaire.*** This questionnaire will include baseline data on participating nurses such as age, sex, race, nursing education, and nursing experience.

- ***NaTI survey.*** This investigator-developed survey uses Likert-style ratings and open-ended questions to evaluate nurses' experiences, attitudes, perceptions, and suggested improvements for the NaTI.
- ***Acceptability, appropriateness, and feasibility measures.*** Nurses will complete the Acceptability of Intervention Measure (**AIM**), Intervention Appropriateness Measure (**IAM**), and Feasibility of Intervention Measure (**FIM**)[90]. Each measure includes four Likert-style questions evaluating the degree to which respondents like the intervention, find the intervention useful, and believe that the intervention can be practically implemented.
- ***Preliminary cost.*** We will estimate preliminary cost of the intervention as part of the feasibility assessment using the nursing time required to deliver the intervention and nursing time and interventions required to respond to infant physiologic instability during the recovery period.

Procedures. Aim 3. At approximately 35 weeks PMA, we will administer the NAPI to each enrolled infant. If discharge is anticipated prior to 35 weeks PMA, we will coordinate with clinical staff to perform the assessment prior to the infant's discharge. For infants who are not clinically stable based on nursing judgment or are requiring invasive ventilation or continuous positive airway pressure (**CPAP**) at 35 weeks PMA, we will delay the neurobehavioral assessment until the infant is clinically stable. Neurobehavioral assessments will be coordinated with the clinical staff so as not to interfere with clinical operations and will occur prior to a scheduled episode of essential nursing care.

Measures, Aim 3:

- ***NAPI.*** The NAPI is a neurobehavioral assessment for preterm infants between 32 weeks PMA and term equivalent age [91]. The assessment includes seven "clusters" – motor development and vigor, scarf sign, popliteal angle, alertness and orientation, percent asleep ratings, irritability, and vigor of crying – that, together, define the developmental maturity of the preterm infant [92]. A recently published systematic review of neonatal assessments concluded that the NAPI had excellent content validity and adequate reliability and construct and criterion validity [93]. The NAPI can be used for evaluative or discriminative purposes and has published preterm infant norms [93]. The NAPI is able to discriminate between extremely low birthweight and very low birthweight infants and is significantly correlated with neurodevelopmental assessment scores at 18 and 30 months corrected age [94]. Moreover, previous research found that acute stress and degree of prematurity predicted scores on two clusters of the NAPI [95]. The NAPI is administered approximately one hour prior to a feeding and in a specified, invariable sequence to minimize the variance in infant responses attributable to inconsistencies in test administration [91]. Co-I Nist has previously used the NAPI in her studies of preterm infants[74].

6.3 ***Infant participants.*** To protect the *privacy of infants and their parents*, we will meet with bedside caregivers prior to contacting parents to ensure that it is an appropriate time to discuss the study. If parents indicate at any time that they are

not interested in participation, we will make no further contact. To *protect the confidentiality of infants and their data,* enrolled infants will be assigned a SID that will be used to label all data and samples. No other identifying information will be included on data forms. The key linking identifying information and SIDs will be encrypted and stored on an electronic password-protected encrypted research server at the OSU CON. The key linking names and SIDs, electronic and paper data forms, and consent forms will be stored in separate locations. Electronic access to the research server is restricted to the OSU CON local area network or virtual private network by a Cisco ASA 5510 firewall. Paper data forms and consent forms will be stored in locked filing cabinets in a locked office at the OSU CON. Access to the key, data, and consent forms will be limited to research team members who have been approved by the Institutional Review Board (**IRB**) and are compliant with all required training in the protection of human subjects.

To *minimize risks to the physical safety* of infants during the study, we are using only non-invasive methods to measure biobehavioral responses. HR and HRV will be measured using three infant-sized electrodes attached to the infant's chest. Two additional electrodes attached to the infant's foot are required to measure SCRs. We will use the BIOPAC EL512 electrodes designed specifically for infants and will use moistened gauze to remove the electrodes after the observation period to minimize the risk of skin irritation. Infants with skin conditions that preclude the attachment of additional sensors will be excluded from the study. To minimize infant agitation, we will use medical swabs attached to a handle to collect saliva for salivary cortisol analysis. These swabs are similar to those used for oral care in the NICU and are not distressing to infants.

For the *safe delivery of the NaTI*, we have developed specific criteria to determine when the intervention should be stopped. The NaTI will be stopped for infants experiencing bradycardia, defined as HR less than 100 beats per minute for 10 seconds, or oxygen desaturation, defined as oxygen saturation less than 85% for 10 seconds. To prevent the overstimulation of infants during intervention delivery, the NaTI will be stopped for tachycardia, defined as HR greater than 200 beats per minute for 10 seconds. NaTI delivery may resume with the next planned intervention point in the care but will be discontinued for the remainder of the care for two or more episodes of bradycardia, oxygen desaturation, or tachycardia occurring during intervention delivery as defined above. Additionally, nurses will provide clinical interventions, including increasing supplemental oxygen, based on their clinical judgement. All adverse events will be recorded and reviewed to determine expectedness, relatedness, and severity.

Nurse participants. We will *protect the confidentiality of data* for nursing surveys by assigning each participating nurse a SID that will be used to label all data. The key linking nurse identities to SID will be encrypted and stored on an electronic password-protected encrypted research server at the CON. The key linking names and SIDs, electronic and paper data forms, and consent forms will be stored in separate locations. Electronic access to the research server is restricted to the OSU CON local area network or virtual private network by a Cisco ASA 5510 firewall. Paper data forms and consent forms will be stored in locked filing cabinets in a

locked office in the OSU CON. Access to the key, data, and consent forms will be limited to research team members who have been approved by the IRB and are compliant with all required training in the protection of human subjects. Surveys will request nurse feedback on the intervention and will not include sensitive data. Nurses always have the option to skip questions that they do not wish to answer.

6.4 *Infant data* will be generated from the study procedures (e.g. SCRs, HR, HRV, behaviors, neurobehavioral assessments) and will be collected from parents of participating infants, bedside monitors, and EPIC (e.g. demographic and clinical data). *Nurse data* will be collected from nurses on study forms.

Table 1. Data Categories and Source

Participant	Data Item	Data Source
Infant	Heart rate	Research activity
	ECG data for heart rate variability	Research activity
	Skin conductance responses	Research activity
	Salivary cortisol	Research activity
	Behavioral states	Research activity
	NAPI	Research activity
	Infant Data Form (attached)	Research activity, bedside monitors, EPIC
	Mother Demographic Form (attached)	Parent
Nurse	Demographic Form (attached)	Nurse
	NaTI Survey (attached)	Nurse
	Acceptability, Appropriateness, and Feasibility for Nurses Form (attached)	Nurse

7.0 Data and Specimen Banking*

7.1 With parental consent for infant data and nurse consent for nurse data, all data will be stored indefinitely on the OSU CON's secure research server or in locked filing cabinets within a locked office in the OSU CON. After the required data retention period, the encrypted key linking SIDs with participant identities will be deleted. The resulting dataset will contain only de-identified data. We will not store samples collected during this study long-term. There will be no samples remaining after this study is complete.

7.2 Data may be used for future unspecified research with parental and nurse consent, which will be provided with the initial consent for this study. Future studies will be related to this research and will undergo IRB review prior to the use of any data collected during this study, as required. Data will not be available for use outside of this research team.

8.0 Sharing of Results with Subjects*

8.1 Individual results will not be shared with participants. If parents are present at the time of the neurobehavioral assessment, we will explain the range of responses possible for preterm infants and will describe mature versus immature responses. Parents of participating infants and nurse participants may request aggregate data to be distributed at the conclusion of the study by writing to Marliese Nist, Newton Hall, 1585 Neil Avenue, Columbus OH 43210. Study results will also be disseminated through peer-reviewed publications and internal and external research presentations.

9.0 Study Timelines*

9.1 Participation for infants each day will last approximately 6 hours and will begin when electrodes to measure SCRs are attached to the infant during the care episode prior to the observed care. Infant participation to measure biobehavioral responses to care will last approximately 24-48 hours and will include two 6-hour observation periods as described above, one each on two consecutive days. If a participating infant is not available on the second consecutive day due to an off-unit or scheduled procedure, data collection will resume on the next possible day. Total infant participation in the study will last between 5-7 weeks, depending on PMA at birth and the timing of the neurobehavioral assessment planned for 35 weeks PMA. Nurse participation is expected to last 15-20 minutes or the time required to complete the demographic questionnaire and study questionnaires. Nurses may be asked to complete additional sets of questionnaires if they provide the NaTI more than once.

We expect that infant enrollment will require four months. Nurse participants will be enrolled at the time of infant data collection for infants.

Preliminary data analysis should be complete 9 months after enrollment of the first infant participant.

10.0 Inclusion and Exclusion Criteria*

10.1 ***Infant participants.*** We will request a partial HIPAA waiver for activities preparatory to research to periodically screen the NICU census for eligible infants. We will first screen the census to identify infants born in the specified age range (i.e. 27-30 PMA). Infants who are eligible by PMA at birth will be further screened for eligibility based on inclusion and exclusion criteria.

Nurse participants. Eligible nurse participants will be identified by the research team during infant data collection as those caring for infants receiving the NaTI. No screening of nurse participants will be required.

10.2 ***Infant participants.*** Infants will be included if they are (1) born between 27-30 weeks PMA, (2) born to mothers who are English-speaking and able to provide informed consent, and (3) no more than 10 days old at the time of enrollment. Infants will be excluded if they (1) are diagnosed with Grade III/IV intraventricular hemorrhage or other neurologic abnormality (e.g. seizure disorder) affecting sensory perception or motor function, (2) are diagnosed with a congenital anomaly requiring surgery during the neonatal period, (3) are receiving scheduled steroids or vasopressors, (4) have skin conditions that preclude the attachment of sensors, (5)

are diagnosed with neonatal abstinence syndrome or born to mothers with known illicit drug use, except marijuana, during pregnancy, (6) are diagnosed with chromosomal abnormalities, or (7) require special isolation that includes universal gloving for potentially infectious pathogens.

Nurse participants. Eligible nurses will be those providing care to infants receiving the NaTI. There are no additional inclusion or exclusion criteria for nurses.

10.3 ***Infant participants.*** All infant participants will be preterm infants.

Nurse participants. Nurse participants will be nurses working in the NICU. There may be pregnant nurses in the sample of nurse participants.

11.0 Vulnerable Populations*

11.1 ***Infant participants.*** Participants in this study will be preterm infants born 27-30 weeks PMA. We will not enroll extremely preterm infants of uncertain viability. The study procedures pose no more than minimal risk to infant participants and may have potential, if slight, benefit for infants. We will obtain written informed consent from parents prior to the initiation of any study procedures. Parents will be given adequate time to consider participation in the study prior to providing written informed consent. Infant participants will not provide assent, as infants are developmentally unable to do so.

Nurse participants. Nurse participants may include pregnant women. This is a minimal risk study for nurse participants and poses no physical risk to a pregnant nurse's fetus. Nurse participants will provide written informed consent prior to participation.

12.0 Local Number of Subjects

12.1 ***Infant participants.*** All infants will be enrolled from the NCH NICUs. We will enroll up to 20 infants to collect complete, usable data from 16 infants.

Nurse participants. Nurses will be those employed in the NCH NICUs. We will invite all nurses who provide care to infants receiving the NaTI to participate in the survey study, providing up to 20 total opportunities for nurse responses. Assuming 80% participation, we expect up to 16 nurse responses.

13.0 Recruitment Methods

13.1 ***Infant participants.*** After identifying an eligible infant, study staff will meet with parents in the NICU or in the mother's postpartum hospital room to introduce the study. Study staff will wait until parents have visited the NICU to see their infant and receive a medical update prior to attempting to contact parents. Study staff will check with bedside caregivers in the NICU or the postpartum unit to ensure that it is an appropriate time to meet with parents. During the first meeting, study staff will provide parents with an informational flier that outlines the study purpose and procedures. We will ask parents if it is a good time to discuss their infant's eligibility for a research study and will conduct any follow-up discussion either in-person or over the phone based on parental preference. Study staff will provide a thorough overview of the study, including the study purpose, procedures, potential

risks and benefits, and the voluntary nature of participation and will inform parents that they may withdraw from the study at any time. Parents will have until their infant reaches two weeks of age to decide whether or not to participate in the study. For this minimal risk study, written informed consent will be required from one parent. If parents are unmarried without a paternity affidavit, consent will be obtained from the mother according to Ohio law.

Nurse participants. Prior to beginning infant enrollment, we will provide unit-based education for staff to introduce the intervention and the nursing survey study. Nurses will be individually approached at the time of infant observation. Study staff will provide an overview of the nursing survey study, including a discussion of the study purpose, surveys, potential risks, and the voluntary nature of the study. Nurses will have until the end of their shift to decide whether or not to participate in the study. At the time that consent is obtained, we will explain to nurses that they may be asked to complete additional surveys if they provide the NaTI again. They will be told at the time of consent and reminded, if they are asked to complete additional surveys, that study participation is completely voluntary. Study staff will further inform nurses that their decision to participate in the study will have no effect on their employment.

13.2 All infant participants will be hospitalized patients in the NCH NICUs. Nurse participants will be registered or licensed practical nurses employed in the NCH NICUs and providing care to infants receiving the NaTI.

13.3 Potential infant participants will be identified through periodic screening of the NICU census for recently admitted infants who meet inclusion criteria. Potential nurse participants will be identified during data collection for infants.

13.4 We will provide nurses employed in the NCH NICUs with an educational PowerPoint prior to the enrollment of infants. This PowerPoint will provide an overview of the study and will introduce nurses to the nursing survey study.

13.5 We will provide a small board book to participating infants at the time of the first observation and data collection. We will provide nurses with a $10 Target gift card at the time of data collection each time they agree to complete a set of surveys.

14.0 Withdrawal of Subjects*

14.1 Infant participants may be withdrawn from the study if they become ineligible for participation based on the inclusion and exclusion criteria between the time that written informed consent is obtained from parents and the time that data collection commences. Nurse participants will not be withdrawn from the study.

14.2 If infants are withdrawn from the study prior to data collection, we will inform parents that their infant is no longer participating in the study due to pre-established inclusion and exclusion criteria.

14.3 If infants are withdrawn from the study, previously collected data (i.e. demographic data) may be used but further data collection will cease.

15.0 Risks to Subjects*

15.1 ***Infant risks.*** We do not expect the static touches provided during the NaTI to increase the risk for physiologic decompensation beyond the risk that exists for essential nursing care. We expect that infants will experience physiologic regulation during these touches. However, the NaTI will extend the duration of the care episode by 2.5 minutes. Some studies of massage in preterm infants report no adverse events related to the intervention[96-98]. A few investigators have reported infrequent occurrences of bradycardia, apnea, or desaturation associated with massage[99,100]. In a study of tactile stimulation in preterm infants 26-32 weeks PMA, Fucile and Gisel (2010) paused the intervention for 1.1% of the administrations due to bradycardia, apnea, or oxygen desaturation. While these are possible risks in our study, we expect the occurrence of bradycardia, apnea, and oxygen desaturation to be infrequent, especially given the static nature of the NaTI in contrast to the stimulation of massage or tactile stimulation.

We will collect biobehavioral data from infants. *HR and HRV* will be collected from monitoring equipment similar to that used as part of clinical care in the NICU. The collection of HR and HRV data will require the attachment of three additional ECG electrodes to the infant's chest. If the infant's chest is not large enough to accommodate the additional electrodes, electrodes for clinical monitoring can be moved to the infant's back at the nurse's discretion. The additional ECG electrodes may cause mild erythema after removal, similar to the removal of electrodes used for clinical monitoring. *SCRs* will require the attachment of two additional electrodes with isotonic gel to the infant's foot. These electrodes may cause mild erythema after removal, similar to the removal of electrocardiogram electrodes used for clinical monitoring. *Salivary cortisol* will be collected three times during the observation period (prior to the start of the care episode, 30 minutes after the care episode, 60 minutes after the care episode) using small medical swabs similar to those used to provide oral care to preterm infants. The swab will be gently introduced into the infant's mouth and held securely using the attached handle. There are no expected risks to infants from the saliva collection. *Protected health information* (**PHI**), including maternal prenatal data and infant clinical data, will be collected from each infant's EHR. There is a small risk of loss of confidentiality.

Nurse participants. Nurses will be asked to complete a demographic questionnaire once and an investigator-developed NaTI survey, the AIM[90], the IAM[90], and the FIM[90] each time they provide the NaTI to an infant. There is a small risk of loss of confidentiality for nurses who agree to participate. Surveys request nurse feedback on the intervention and will not include sensitive data.

15.2 As part of the biobehavioral data collection, there may be risks to infant participants that are not foreseeable.

15.3 If a pregnant nurse chooses to participant in the nursing survey study, there are no risks to her fetus.

16.0 Potential Benefits to Subjects*

16.1 Infants may directly benefit from the comforting touches provided by the NaTI although we can neither offer nor guarantee this potential benefit. We hypothesize that these touches will attenuate activation of the infant's stress response systems.

16.2 There is no direct benefit to nurses who participate in the nursing survey study. The survey study provides nurses with the opportunity to describe their perception of the NaTI and their suggestions for NaTI revisions. In this way, nurses will have the opportunity to be part of the decision-making process for their practice.

17.0 Data Management* and Confidentiality

17.1 ***Aim 1.*** The primary aim is to determine the effect of a NaTI on biobehavioral stress responses of preterm infants during essential nursing care. Our primary analysis will compare mean values of biobehavioral stress responses during an episode of essential care between cohorts (comparison vs interventions conditions). We will begin by examining sequence effects. We will then compare the biobehavioral stress responses of infants assigned to each condition using linear mixed models to account for multiple observations within infants. Additional variables may be added if potential confounding variables are identified between conditions, although none are anticipated. Secondary analyses will consider total area under the curve (**AUC**) for each biobehavioral response variable to identify important differences in overall recovery between conditions. For the primary (i.e. comparison of means during care) and secondary (i.e. comparisons of AUC) analyses, we will examine differences in outcomes by important biological variables (e.g. infant sex) and will repeat the analysis, including interactions to identify possible moderation of the effect by infant sex, race, ethnicity, and PMA at birth.

Aim 2. We will use measures of central tendency and variance to describe nurses' Likert responses. We will identify barriers and facilitators of intervention implementation for each episode of essential care during hospitalization from narrative responses.

Aim 3. We will use correlation analysis and regression models to determine the associations between biobehavioral stress responses and neurobehavioral outcomes.

17.2 To protect the confidentiality of infant and nurse participants and their data, infant and nurse participants will be assigned a SID that will be used to label all data and samples. No other identifying information will be included on data forms. The key linking identifying information and SIDs will be encrypted and stored on an electronic password-protected research server at the OSU CON. The key linking names and SIDs, electronic and paper data forms, and consent forms will be stored in separate locations. Electronic access to the research server is restricted to the OSU CON local area network or virtual private network by a Cisco ASA 5510 firewall. Paper data forms and consent forms will be stored in locked filing cabinets in a locked office at the OSU CON. Access to the key, data, and consent forms will be limited to research team members who have been approved by the IRB and are compliant with all required training in the protection of human subjects.

17.3 We will verify 100% of the data collected from the EHR for 25% of infant participants against the source documentation (i.e. EPIC).

17.4 All data and samples will be transported to the OSU CON under a Data Transport Authorization from the NCH Privacy Office and stored at the OSU CON under Data Use and Materials Transfer Agreements. With parental consent for infant data

and samples and nurse consent for nurse data, data and samples will be stored indefinitely. Access to the data and samples will be limited to study staff who have been approved by the IRB, are compliant with human subjects protections training, and have been trained by the PI on data storage procedures. Data and samples will be transported only by the study PI.

18.0 Provisions to Monitor the Data to Ensure the Safety of Subjects*

18.1 A data safety and monitoring plan has been developed for this clinical trial.

Purpose. The purpose of our data safety and monitoring plan (**DSMP**) is to safeguard the welfare of human subjects participating in the clinical trial through the regular review of safety data related to the intervention. The DSMP ensures that the research staff and principal investigator (**PI**) follow specific guidelines with respect to accurate data management and the assessment and timely reporting of adverse events associated with the investigational study Nursing Touch and Biobehavioral Stress Responses of Very Preterm Infants. The DSMP for the proposed study incorporates the Policies on Data and Safety Monitoring specified by OSU, NCH, the IRB, the Office of Regulatory Knowledge and Support in the Center for Clinical and Translational Science, and the OSU CON Data Safety Monitoring Committee (**DSMC**).

Data Safety Monitoring Committee. The DSMC is comprised of tenured faculty from the OSU CON with expertise in the conduct and analysis of clinical trials in the acute care setting. Members of the DSMC include Dr. Mary Beth Happ, Distinguished Professor of Critical Care Research and Associate Dean of Research and Innovation; and Dr. Alai Tan, Research Associate Professor and biostatistician.

Members of the DSMC have no apparent conflicts of interest related to the proposed study and are not current collaborators of any study investigators. DSMC members will report arising conflicts of interest to fellow members and the study investigators. DSMC members who develop significant conflicts of interest will resign from the DSMC.

DSMC members who leave OSU or resign their position prior to the conclusion of the clinical trial will be replaced with a new member appointed by the study investigators.

Interim Monitoring Procedures. On a monthly basis, the PI will produce interim reports specifically focusing on missing data and entry errors. The progress of the study will be monitored biannually by the college DSMC. On a biannual basis, the PI will prepare a written report on the progress of the study that will include data on: enrollment, comparison of target to actual enrollment, information on race, ethnicity, sex, overall status of the study participants, protocol deviations, and adverse or serious adverse events. These data will be collected in a spreadsheet format and reviewed by Marliese Nist (Study PI). All reports will be reviewed by the DSMC on a biannual basis. After each report is reviewed, the Committee will recommend whether the study should continue, continue with modifications, or be terminated. PI reports and minutes from DSMC meetings will also be sent to the Office of Responsible Research Practices. Co-I Pickler, who has extensive

experience with DSMCs and the conduct of clinical trials, will assist the PI in developing these reports.

Procedures for Adverse Events

Identification of adverse events. Study staff will be at the bedside during infant data collection and will record the occurrence of adverse events in real time. Adverse events (**AE**s) will be reported immediately to the PI. Adverse events may also be identified by parents or neonatal intensive care unit staff, who are encouraged to alert the PI of these events. During the consent process, participating nurses and parents of participating infants will be informed of the minimal risk nature of this study and encouraged to report any AEs immediately to study staff. The PI will maintain an electronic record of AEs and will include a description of AEs in the written report submitted biannually to the DSMC. Given the short-term duration of infant participation in the study, AEs will be tracked from the time that infant participation begins following the attachment of sensors for data collection and will continue until the end of the recovery period (i.e. 30 minutes after the final direct touch of the care episode).

Definitions. Adverse Event: Any unfavorable and unintended sign, regardless of whether it is considered related to the intervention; also an "unanticipated problem" of any nature (e.g., psychological or social harm; designated as unrelated, definitely related, probably related, or possible related; see below). Serious adverse event (**SAE**): Any adverse event that is fatal or life threatening, is permanently disabling or requires prolongation of hospitalization. Life-threatening event: Any adverse event in which the subject is at immediate risk of death from the reaction as it occurs; does not include a reaction that, if it were to occur in a more serious form, might cause death. Unexpected event: Any adverse event that is not identified in nature, severity, or frequency in the study protocol or consent or the event was more serious than anticipated. Definitely Related: An adverse event that has a timely relationship to the administration of the investigational study procedure and follows a known pattern of response for which no alternative cause is present. Probably Related: An adverse event that has a timely relationship to the administration of the investigational study procedure and follows a known pattern of response, but for which a potential alternative cause may be present. Possibly Related: An adverse event that has a timely relationship to the administration of the investigational study procedure and follows no known pattern of response, but a potential alternative cause does not exist. Unrelated: An adverse event for which there is evidence that it is definitely related to a cause other than the investigational study procedure; in general no timely relationship to the administration of the procedure exists, or if so, the event does not follow a pattern of response and an alternative cause is present.

Grading scale for adverse event severity. Mild: Transient bradycardia (defined by clinical parameters established for each patient, usually heart rate <100 beats per minute), apnea, or oxygen desaturation requiring no intervention or an increase in supplemental oxygen. Bradycardia and oxygen desaturation are defined by clinical parameters established for each patient, usually heart rate <100 beats per minute or oxygen saturation <85% lasting 10 seconds. Erythema resulting from monitoring electrodes. Moderate: Bradycardia, apnea, or oxygen desaturation requiring an

increase in respiratory support (i.e. hand-bagging). Epidermal stripping (i.e. non-intact skin) resulting from monitoring electrodes. Severe: Physiologic decompensation requiring intubation or cardiopulmonary resuscitation.

Review of adverse events. The study investigators will provide an initial review of all AEs to the DSMC to determine their relatedness to the research and preliminary grading of severity. For each AE, the PI will record the onset, duration, intensity, treatment required, outcome and action taken. SAEs are not expected to occur during this study. Possibly, probably, or definitely related AEs, as determined by the study team, will be reported within 24 hours to the DSMC chair for further review. The DSMC will make the final determination on AE severity and relatedness.

Reporting of adverse events. Final evaluation of relatedness and severity of AEs will be determined by the DSMC. In accordance with NCH IRB Adverse Event Reporting Policy, an AE is reportable to the IRB within 5 business days if it meets all of the following criteria: (1) the event is unexpected, (2) the event is related or probably related to study participation, and (3) is serious and/or suggests that the research places subjects or others at a greater risk of physical or psychological harm than was previously known or recognized. Additionally, per NCH IRB policy, all protocol deviations or violations that harmed participants or indicate that others might be at increased risk of harm, breaches of confidentiality, and new literature that may affect willingness to participate are reportable events within 5 business days. AEs that must be reported annually with continuing review include: (1) minor protocol deviations that do not indicate an increased risk of harm, and (2) AEs that are not serious or unexpected. After review by the DMSC, all reportable AEs will be submitted by the PI to the NCH IRB. Reportable AEs will be forwarded to the NIH/NINR Program Officer by the NCH IRB. In addition, reportable AEs and the IRB acknowledgement letter and cumulative reportable AEs, SAEs, and unanticipated problems will be forwarded to the NIH/NINR Program Officer with the PI's Annual Progress Report. .

Assessment of External Factors. Because essential nursing care involves the handling of preterm infants, we do not expect 2.5 minutes of comforting touch provided during the care episode to increase the risk for AEs beyond what is expected during essential nursing care. The study investigators will meet monthly to discuss new developments and safety concerns. Prior to each monthly meeting, the PI will conduct a literature search to identify new developments that might affect the evaluation of risk for this study.

19.0 Provisions to Protect the Privacy Interests of Subjects

19.1 Study staff will approach parents for infant recruitment only after they have visited the NICU to see their infant and received a medical update. Study staff will check with bedside caregivers in the NICU or the postpartum unit to ensure that it is an appropriate time to meet with parents. During the first meeting, study staff will ask parents if it is a good time to discuss their infant's eligibility for a research study. We will contact parents no more than three times for enrollment unless parents request additional meetings and will cease all recruitment efforts if parents indicate

that they are not interested in the study. We will access the minimum necessary PHI to determine infant eligibility.

We will provide unit education to all staff prior to the enrollment of infants so that nurses will be familiar with the study prior to any efforts to recruit individual nurses. To recruit individual nurses, study staff will approach nurses at the time of infant observation for an enrolled infant. We will cease all recruitment efforts if nurses indicate that they are not interested in participation.

19.2 We will fully explain all study procedures prior to participant enrollment and will remind parents and nurses that they may withdraw from the study at any time without penalty. We will provide participants with an explanation of our data safety procedures to code data with a SID. Although we will not be collecting sensitive data, we will inform parents of participating infants and nurse participants at the time of data collection that they may leave questions blank if they are not comfortable answering them.

19.3 Parents of participating infants will provide some data for their infants (i.e. demographic data) and will provide written HIPAA authorization included in the consent form for the study team to collect data from the EHR. Data to address Aim 2 will be collected directly from nurses.

20.0 Compensation for Research-Related Injury

20.1 This is a minimum risk study for which we do not anticipate research-related injuries.

21.0 Economic Burden to Subjects

21.1 There will be no costs incurred by participants in the study.

22.0 Consent Process

22.1 ***Infant participants.*** Written informed consent will be obtained from parents of eligible infants either in the NICU or in the mother's postpartum hospital room. Study staff will approach parents any time after parents have visited their infant. Parents may complete the written informed consent document any time after the study team has discussed the study with parents and will have until their infant reaches 10 days of life to decide whether or not to participate in the study. For this minimum risk study, written informed consent will be required from one parent. If parents are unmarried without a paternity affidavit, consent will be obtained from the mother according to Ohio law. In addition to the initial discussion of the study, we will provide an explanation of the study procedures when we provide parents with the demographic data form and again when we collect infant data in the NICU if the parents are present.

Nurse participants. Written informed consent will be obtained from nurses who choose to participate in the study. Study staff will approach potential nurse participants at the time of infant observation. Nurses may provide written informed consent any time after the study has been fully explained and all questions answered by study staff and may have until the end of their shift to decide whether or not to participate. Nurses will be told during the initial consent process that they may be

approached again if they care for another enrolled infant during infant data collection but that they have the right to withdraw from the study at any time. If the same nurse is approached a second time for participation, study staff will remind him/her that participation is completely voluntary.

Informed consent from parents and nurses will be obtained following "SOP: Informed Consent Process for Research (HRP-090)".

Non-English Speaking Subjects – if known, skip if not known

- Study staff will recruit only English-speaking participants for the study.

Subjects who are not yet adults (infants, children, teenagers)

- Consent for infant participation will be obtained from one of the infant's biological parents or his/her legal guardian. Parents who are not yet adults are legally authorized to provide consent for their minor children. For this minimum risk study, written informed consent will be required from one parent/legal guardian. If parents are unmarried without a paternity affidavit, consent will be obtained from the mother according to Ohio law. Assent will not be obtained from infants, as infants are developmentally unable to provide assent.
- Consent for nurse participation will be obtained from nurses, who are legal adults and able to provide consent for themselves.

23.0 Process to Document Consent in Writing

23.1 Study staff will follow "SOP: Written Documentation of Consent (HRP-091)" to document consent in writing for infant and nurse participants.

24.0 Setting

24.1 Infant participants will be identified through periodic screening of the EHR. Infants will be recruited either in the NCH NICUs or in the mother's postpartum hospital room. Nurse participants will be recruited during their normal working hours in the NCH NICUs.

All study procedures will take place in the NCH NICUs. Surveys for nurse participants will be distributed in the NICU, but nurses may take these home and return them during their next scheduled shift.

Data storage and analysis and sample processing, storage, and analysis will take place at the OSU CON.

The study protocol will be submitted to the NCH and OSU IRBs. OSU is expected to cede review to NCH. IRB oversight for the study will be provided by the NCH IRB.

25.0 Resources Available

25.1 The NCH Neonatal Network admitted 372 infants born 27-30 weeks PMA in 2020, and the study team has extensive experience working with preterm infants in this age range and within the NCH NICUs. PI Nist, who is certified by the National Certification Corporation in neonatal intensive care nursing, worked for 10 years in the NCH main campus NICU, caring for preterm infants as a bedside nurse. For a study conducted between October 2017 and December 2018, this study team enrolled a sample of 73 preterm infants born 28-31 weeks PMA from the NCH Neonatal Network.

25.2 ***PI Nist*** (OSU) will oversee all aspects of the study, including ensuring compliance with regulatory, IRB, and organizational requirements. Dr. Nist will screen and recruit eligible infants and will obtain informed consent from parents. Working with the graduate research assistants, Dr. Nist will perform nurse training and on-site data collections. She will work with the Co-Is to clean and analyze physiologic data (i.e. HR, HRV, and SCRs). In cooperation with Co-I Pickler, Dr. Nist will oversee all salivary cortisol laboratory assays. Dr. Nist will work with the graduate research assistants to complete all data entry. She will coordinate efforts among the research team and plan and initiate disseminations efforts.

Co-I Harrison (OSU) will provide access to and training for the MARS® Ambulatory ECG Analysis and Editing System. Dr. Harrison will oversee cleaning and analysis of HRV data. In collaboration with the research team, Dr. Harrison will assist with data interpretation and dissemination efforts.

Co-I Pickler (OSU) will assist the PI in ensuring regulatory compliance and maintaining regulatory documentation for this clinical trial, including reviewing and reporting adverse events, as required. Dr. Pickler will provide oversight of intervention fidelity and will work with the PI to train laboratory personnel in the CON Stress Science Lab on protocols for the extraction of salivary cortisol from filter paper. Dr. Pickler will assist with data interpretation and dissemination efforts.

Co-I Shoben (OSU) will provide all statistical support for the project. Dr. Shoben will develop the randomization scheme for the cross-over trial and will assist in addressing data collection issues related to missing data or other unforeseen circumstances. Dr. Shoben will conduct the statistical analysis to address the aims. Dr. Shoben will participate in interpretation of study findings and dissemination efforts.

Co-Nelin (NCH) will serve as site PI for NCH and will act as liaison between OSU and NCH, assisting with the identification and recruitment of participants at the clinical site. Dr. Nelin will ensure the safety of all study participants and will assist with reviewing and reporting adverse events, as required. Dr. Nelin will assist with data interpretation and dissemination efforts.

Graduate Research Assistants will be hired to assist the PI in completing the day-to-day operations of the study, including on-site data collection, cleaning and analysis of physiologic data in preparation for statistical analysis, and data entry. The Research Assistants will perform HRV analysis under the supervision of the PI.

25.3 All members of the study team will be required to complete and maintain current CITI Program training as required by NCH and OSU. PI Nist will train all study staff on relevant standard operating procedures relevant to each person's assigned responsibilities and will document this training in study records.

25.4 All equipment and supplies need to perform study activities are available at NCH or OSU or will be purchased from the study budget.

26.0 Multi-Site Research*

26.1 Participant recruitment and enrollment will fall under the authority of the NCH IRB. We will enroll up to 20 infant participants from the NCH NICUs for the study. We will recruit all eligible nurses for participation. Eligible nurses are registered or licensed practical nurses providing the NaTI to infants, providing up to 20 total opportunities for nurse responses. Assuming 80% participation, we expect up to 16 nurse responses. Infant and nurse participants will be recruited from the NCH NICUs.

26.2 PI Nist is a PI at OSU and external collaborator at NCH. Dr. Nist will be responsible for all communication between NCH and OSU, including correspondence with the IRB. Communication between sites will be enhanced by monthly study meetings that will include all study staff at NCH and OSU.

26.3 Data and samples will not be stored at NCH and will be immediately transported to OSU for secure storage.

27.0 Protected Health Information Recording

1.0 Indicate which subject identifiers will be recorded for this research.

- ☒ Name
- ☐ Complete Address
- ☐ Telephone or Fax Number
- ☐ Social Security Number (do not check if only used for ClinCard)
- ☒ Dates (treatment dates, birth date, date of death)
- ☒ Email address, IP address or url
- ☒ Medical Record Number or other account number
- ☐ Health Plan Beneficiary Identification Number
- ☒ Full face photographic images and/or any comparable images (x-rays)
- ☐ Account Numbers
- ☐ Certificate/License Numbers
- ☐ Vehicle Identifiers and Serial Numbers (e.g. VINs, License Plate Numbers)
- ☐ Device Identifiers and Serial Numbers
- ☐ Biometric identifiers, including finger and voice prints
- ☐ Other number, characteristic or code that could be used to identify an individual
- ☐ None (Complete De-identification Certification Form)

2.0 Check the appropriate category and attach the required form* on the Local Site Documents, #3. Other Documents, page of the application. (Choose one.)

☒ Patient Authorization will be obtained. (Include the appropriate HIPAA language (see Section 14 of consent template) in the consent form OR attach the HRP-900, HIPAA AUTHORIZATION form.)
☐ Protocol meets the criteria for waiver of authorization. (Attach the HRP-901, WAIVER OF HIPAA AUTHORIZATION REQUEST form.)
☐ Protocol is using de-identified information. (Attach the HRP-902, DE-IDENTIFICATION CERTIFICATION form.) (Checked "None" in 1.0 above)
☐ Protocol involves research on decedents. (Attach the HRP-903, RESEARCH ON DECEDENTS REQUEST form.)
☐ Protocol is using a limited data set and data use agreement. (Contact the Office of Technology Commercialization to initiate a Limited Data Use Agreement.

***Find the HIPAA forms in the IRB Website Library, Templates.**

Attach the appropriate HIPAA form on the "Local Site Documents, #3. Other Documents", page of the application.

3.0 How long will identifying information on each participant be maintained?

Identifying information will be maintained with research records for six years after the study is closed, in accordance with NCH IRB requirements. After this time, identifiers will be removed from study records. Signed and dated consent documents and HIPAA authorizations will be maintained for six years after the study is closed, in accordance with NCH IRB requirements. All study records, consent documents, and HIPAA authorizations will be stored in a locked filing cabinet within a locked office at the OSU CON or on a password-protected research server at the OSU CON. Access to study records will be limited to IRB-approved study staff.

4.0 Describe any plans to code identifiable information collected about each participant.

Each participating infant and nurse will be assigned a SID to label paper data forms and electronic data. The key linking identifiers and SIDs will be encrypted and kept on a password-protected research server at the OSU CON in a separate folder from study data. Identifiers will be removed from this key six years after the study is closed.

5.0 Check each box that describes steps that will be taken to safeguard the confidentiality of information collected for this research:

X Research records will be stored in a locked cabinet in a secure location
X Research records will be stored in a password-protected computer file
X The list linking the assigned code number to the individual subject will be maintained separately from the other research data
X Only certified research personnel will be given access to identifiable subject information

6.0 Describe the provisions included in the protocol to protect the privacy interests of subjects, where "privacy interests" refer to the interest of individuals in being left alone, limiting access to them, and limiting access to their information. (This is not the same provision to maintain the confidentiality of data.)

Study staff will approach parents for infant recruitment only after they have visited the NICU to see their infant and receive a medical update. Study staff will check with bedside caregivers in the NICU or the postpartum unit to ensure that it is an appropriate time to meet with parents. During the first meeting, study staff will ask parents if it is a good time to discuss their infant's eligibility for a research study. We will contact parents no more than three times for enrollment unless parents request additional meetings and will cease all recruitment efforts if parents indicate that they are not interested in the study. We will access the minimum necessary PHI to determine infant eligibility.

We will provide unit education to all staff prior to the enrollment of infants so that nurses will be familiar with the study prior to any efforts to recruit individual nurses. To recruit individual nurses, study staff will approach nurses for participation at the time of infant observation for an enrolled infant. We will cease all recruitment efforts if nurses indicate that they are not interested in participation.

Confidential Health Information

1.0 Please mark all categories that reflect the nature of health information to be accessed and used as part of this research.

- ☒ Demographics (age, gender, educational level)
- ☒ Diagnosis
- ☒ Laboratory reports
- ☒ Radiology reports
- ☒ Discharge summaries
- ☒ Procedures/Treatments received
- ☒ Dates related to course of treatment (admission, surgery, discharge)
- ☒ Billing information
- ☒ Names of drugs and/or devices used as part of treatment
- ☒ Location of treatment
- ☐ Name of treatment provider
- ☒ Surgical reports
- ☒ Other information related to course of treatment
- ☐ None

2.0 Please discuss why it is necessary to access and review the health information noted in your response above.

Clinical information for infants is required to describe illness severity, treatment course, and diagnosis to determine study eligibility, differences in the effect of the intervention based on these variables, and differences between cohorts. Demographic information is needed to determine study eligibility and differences in the intervention's effect based on infant sex. Billing information is needed to determine insurance type, a proxy for socioeconomic status.

Demographic information for nurse participants will be collected directly from enrolled nurses. No PHI will be collected from nurses.

3.0 Is the health information to be accessed and reviewed the minimal necessary to achieve the goals of this research? ☒ Yes ☐ No

4.0 Will it be necessary to record information of a sensitive nature? ☐ Yes ☒ No

5.0 Do you plan to obtain a federally-issued Certificate of Confidentiality as a means of protecting the confidentiality of the information collected? ☒ Yes ☐ No

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Appendix

Salivary cortisol collection from NCH Laboratory Medicine