Effect of a Vibratory Stimulus on Mitigating Nociception-specific Responses to Skin Puncture in Neonates

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Summary – We aim to fill existing knowledge gaps in neonatal pain mitigation by conducting an interventional randomized control trial (RCT) using a novel non-pharmacological approach to decrease the nociceptive response to skin puncture. In addition to validating the effectiveness of our intervention, time-locked electroencephalographic (EEG) methods will provide an objective measure to which behavioral responses will be compared. This project will allow the development of a battery of quantitative and accurate tools for neonatal pain assessment, translate neuroscientific theory of gating into a bedside application in the intensive care unit, and facilitate future investigation by pediatric anesthesiologists into regional alternatives for neonatal pain management. **Because of the established associations between nociceptive experiences in the neonatal period and worse developmental, neurological and behavioral consequences, our current line of rigorous research can be broadly developed, leading to more positive long-term outcomes for hospitalized infants.**

Overall Aim – Optimizing the developmental potential of neonates requires appropriate pain control that depends on accurate pain assessment. For pediatric anesthesiologists, this remains challenging due to the lack of self-reporting of subjective experiences in nonverbal patients. Many current uni- and multidimensional pain assessment tools for neonates fail to consider developmentally important cues and have incomplete psychometric testing[1]. In a research setting, neonatal pain responses can be accurately measured at the cortical level. Our overall goal is to establish founding principles on which to build objective, clinically practical tools that will guide the development of effective pain management in infants. As validation of behavioral components of the pain response is best performed in a non-pharmacological setting, we propose a non-invasive technique using a standardized vibratory stimulus (V) to mitigate the nociception-specific response to heel lance (HL). Our brain-oriented measurement approach uses EEG-based analysis time-locked to painful or vibratory stimuli, and masked coding of facial expression and withdrawal reflex video segments (after reliability training). To prove the efficacy of using a Neonatal Intensive Care Unit (NICU)-safe vibration approach in decreasing pain during common skin punctures, we will leverage our expertise of EEG methods through an RCT in hospitalized infants (n= 134) with the following aims.

Aim 1: Demonstrate that amplitude of cortical nociceptive response (EEG-based) during skin puncture decreases in infants treated with controlled vibration prior to puncture, compared to those who receive standard care.

Aim 2: Establish the feasibility of a novel, evidence-based bedside assessment tool to quantify pain responses in infants with and without the intervention, by comparing scores on the bedside tool to cortical measures.

Hypotheses – Our hypotheses will be tested using prospectively designed experiments with stimulus conditions sequentially administered (see Table).

Conditions	Control group	Treatment group
A - No stimulus	✓	✓
B - V without HL	✓	✓
C - HL without V	✓	
D - HL during V		✓

First, we hypothesize that during the 350-700ms post-stimulus window, there will be a decrease in mean amplitude of the EEG nociceptive response at the group level (treatment vs. control), as measured by the difference in response to HL (C vs. D). There should also be a decrease in video-coded behavioral scores. We will then examine associations between nociceptive amplitude and behavioral responses across the entire cohort, with and without adjustment for post-menstrual age at testing time.

Secondly, we hypothesize that EEG will allow measurement of the effect size of vibration on amplitude of the nociceptive response at the individual level (pairwise, B vs. D); behavioral scores may or may not allow this measurement if a partial reduction of the nociceptive response is below the threshold of bedside scales, but not below that of EEG.

Finally, we hypothesize that there will be significant differences both in the EEG-based and the behavioral responses between baseline and HL (A vs. C). Conversely, responses to non-nociceptive stimulus (A vs. B), will be detectable via EEG response amplitude and frequency, but not by video coding.

Background, Significance, and Relevance –

Among the 4 million infants hospitalized every year in the U.S., nearly one-third are infants with complicating diagnoses increasing their likelihood of exposures to iatrogenic pain. During the first two weeks of life in the NICU, each newborn may undergo an average of 8 to 17 invasive procedures daily[2]. In preterm infants, this may result in 14 painful procedures per day, up to 80% of which are not preceded by specific analgesia[3]. An exposure rate of up to 300 painful events during a NICU stay, or even higher in the youngest preterm neonates[4], can have serious adverse effects. These range from micro- and macro-structural brain changes in the neonatal period, to neurophysiologic alterations of touch and pain processing at discharge, to sensory and behavioral problems in adolescence[5,6]. In addition to pain hypersensitivity and dysesthesias, problems with chronic pain and early uncontrolled pain can alter the responsiveness of the neuroendocrine and immune systems to stress in adulthood[7]. While the American Academy of Pediatrics regularly reaffirms its practice statement that mitigation of pain in neonates is of critical importance[8], there are currently few effective non-pharmacological strategies[9], none intended as a prophylactic intervention at the site of a painful stimulus.

The mainstay of bedside management in the NICU consists of reducing the number of painful procedures and providing extra hands-on behavior-regulation support. The development of novel methodologies to reduce pain is complicated by the difficulties of accurate evaluation of pain in neonates. A national survey of neonatal nurses revealed that only half of them feel adequately trained on the topic of pain[10]. While over three quarters of these nurses reported using pain assessment tools and having confidence in providing interventions, fewer than half reported that pain was well managed and that their pain protocols were research or evidence-based. A perceived fear of side effects of medications and incorrect interpretation of pain signals, as well as lack of trust in pain assessment tools, is in line with other published findings: a considerable number of health professionals do not assess the level of pain based on scales developed for this purpose[3]. Finding an accurate means of pain assessment and its appropriate treatment in neonates is of particular importance to pediatric anesthesiologists who are often called upon to assist in neonatal procedures involving skin breaks, or to mitigate pain experienced during necessary operations. **Therefore, projects aiming to safely and effectively reduce neonatal pain from skin-puncturing procedures can have a broad impact on clinical practice, especially if they are based on rigorous mechanistic principles and accurate measurements of neonatal pain.**

An initial gap in knowledge we plan to address is the lack of feasible, standardized non-pharmacological interventions to mitigate pain during routine skin-breaking procedures in the NICU. Mitigation of neonatal pain during skin-breaking has been achieved using pharmacological means, such as general anesthesia, for complex surgical operations or more recently, regional anesthesia through indwelling catheters. Minor neonatal procedures usually employ systemic analgesic and sedative administration, but needless administration of these agents (from opiates, to benzodiazepines, to sucrose) are associated with multiple concerns including prolonged need for mechanical ventilation, delayed feeding, impaired brain growth, poor socialization skills, and impaired performance in short-term memory tasks[11,12]. Current effective non-pharmacological methods include skin-to-skin care and facilitated tucking, but they are difficult to implement without parental presence or additional personnel resources. A mechanistically-based alternative would be to leverage the “gate-control” properties of the peripheral nervous system by stimulating nerve fibers that conduct non-noxious stimuli[13]. Two proposed mechanisms for gate control of nociception are either the stimulation of inhibitory interneurons by activation of the large Aβ fibers by vibration, or the induction of low frequency (9-20 Hz) “interfering” oscillations by continuous vibration[14,15]. The concept of “vibration anesthesia” has been applied to reduce discomfort in adults undergoing injections[16,17], but has

not been used in the neonatal population. This approach does not simply mask the behavioral signs of pain or act as a sedative, it decreases the amplitude of a signal being transmitted to the somatosensory cortex. **Therefore, delivering a controlled vibration prior to skin-breaking could decrease amplitude of the pain response.**

In order to ensure the feasibility of implementing novel pain mitigation approaches, a second critical gap in knowledge that must be addressed is the lack of quantitative bedside pain measures that have been rigorously validated against brain-based methodologies. The first infant behavioral pain measure was developed 30 years ago and was later incorporated into several multidimensional scales[18]. Many of these scales integrate facial and physiological components in addition to non-pain related measures like gestational age. Most scales have incomplete psychometric testing, conflicting evidence with regards to their accuracy, and little evidence for their use in pain management[1]. Validation using brain-oriented methods has been investigated with EEG providing direct evidence of pain-induced brain activity[19]. **While the clinical practicality of time-locked EEG is still limited, this methodology provides a means to validate more feasible bedside neonatal pain assessment tools within the context of an RCT of a pain-mitigating intervention.**

With advancements in technology and a progressively larger number of neonates undergoing invasive treatments in the critical care setting in lieu of having them done in operating room, the role of pediatric anesthesiologists in optimizing pain assessment and management in the youngest of patients has grown beyond the perioperative period. Suggestions that general anesthesia may induce toxic biochemical and morphological changes in the brain has become a widespread concern[20]. This advocates for a trend toward more conservative therapies such as using regional or local anesthetic techniques or non-pharmacological interventions when feasible. As fewer patients may become subject to sedating protocols, it becomes increasingly important to establish clinically practical tools that allow for reliable pain assessment and effective treatments in neonates who are behaviorally intact. Our proposed project will set the framework on which to develop such tools and therapies, which we will adapt accordingly to a variety of clinical situations, whether it be perioperatively, in the critical care setting, or during follow-up for chronic outpatient conditions.

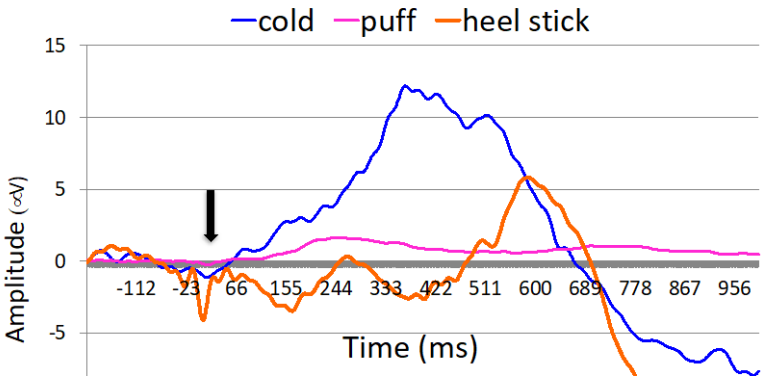
Therefore, the development of novel approaches to pain mitigation for skin breaking procedures in hospitalized infants must integrate mechanistic knowledge of nociceptive processing with brain-based measurement methodologies. This will allow the development of more practical, effective and accurate bedside tools. Our current study leverages our anesthesiology team's extensive experience with pain management in the NICU, our laboratory's expertise in EEG and behavioral video-coding and robust preliminary data to develop a bedside pain-mitigation approach, and independently, a validated measurement of its effectiveness.

Preliminary Results –

Our preliminary data as a whole demonstrate the feasibility of our proposed study as well confirm the functionality of our customized vibratory device. The primary weakness noted in the review of our initial proposal pertained to the dependence of a study design on a prototype that was yet to be manufactured. We addressed this concern by having completed construction of the device and with successful acquisition of adult-based data for proof of concept. These pilot data are detailed in the latter part of this section, preceded by previous work that demonstrate our ability to carry out the current study in terms of the using a brain-oriented approach.

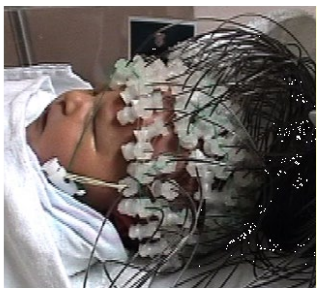
We have shown that our team can conduct accurate and specific measurement of pain and touch using high-density time-locked EEG (**Figure 1**) in cohorts of hospitalized neonates[21,22]. **Figure 2** demonstrates this in a recent cohort of term infants. This published work is the basis for our power analysis in the current proposal. Our team can rapidly and non-invasively acquire large high-quality datasets of brain-based sensory measures, and analyze them with innovative spatiotemporal and spectro-temporal computational tools. This approach allows us to obtain quantitative information with high temporal resolution on neural activation responses, without requiring active participation or directed attention in awake or resting infants.

Figure 1 – Cortical Responses to Somatosensory Stimuli in 33 term infants



We have conducted a rigorous systematic review of all currently published pain scales, behavioral and physiological signs that were compared to measures based on near-infrared spectroscopy or EEG[23]. The findings of this work served in the development of a panel of only the components most highly associated with pain presence and intensity. The withdrawal reflex and changes in facial expression are most strongly associated with nociception-specific brain activity while physiological signs, such heart rate and oxygen saturation, have little to no association with this type of response. We have also developed a system for objective video-coding of behavioral responses in infants with reliability >90% using a highly manualized approach[24].

Figure 2 NICU infant undergoing EEG testing



The device that will provide the intervention in our proposed study has been constructed and trialed successfully in a healthy adult male volunteer. The device has been tested to provide a vibration frequency of 178 Hz, with a free-hanging 0.24 mm range of motion and a power output of less than 5 watts. The internal electronic customizations of the device were tested and work well in terms of labelling the EEG data to allow for appropriate time-locking with the vibratory and/or skin puncturing stimulus. While lancet-based blood sampling in adults is conventionally done using one of the upper extremity digits, we emulated a heel lance more closely by performing skin puncture on the hypothenar eminence. For the purposes of this pilot run, we analyzed data from a single male adult in fronto-central regions under the various conditions (A through D) listed in our study design and specific aims section.

The results illustrate how four stimuli conditions can have different responses that are consistent with the neuroscience of nociceptive processing and the gate-control theory. Because they represent findings in a single subject, the background oscillations smoothed out in the grand averaged figure above (**Figure 1**) can still be noted and measured. We found distinctive waveform patterns after stimulation that varied in terms of mean amplitude and mode frequency. In **Figure 3**, the mean amplitude of these evoked responses over the fronto-central locations can be quantified in the time window of 350-700 ms. In **Figure 4**, the mode frequency for four distinct conditions are identified. (A) Baseline (white bars), mean amplitude of three averaged segments and mode of background frequency is very low. (B) After vibration (gray bars), mean amplitude of three averaged segments increases and a regular 13 Hz frequency is detected. (C) After heel lance without prior vibration (black bars), mean amplitude is elevated and irregular high-frequency oscillations occur. (D) After heel lance with concurrent vibration (striped bars), mean amplitude of the response is visibly lower than compared to heel lance alone, and high frequency oscillations are no longer apparent.

Figure 3

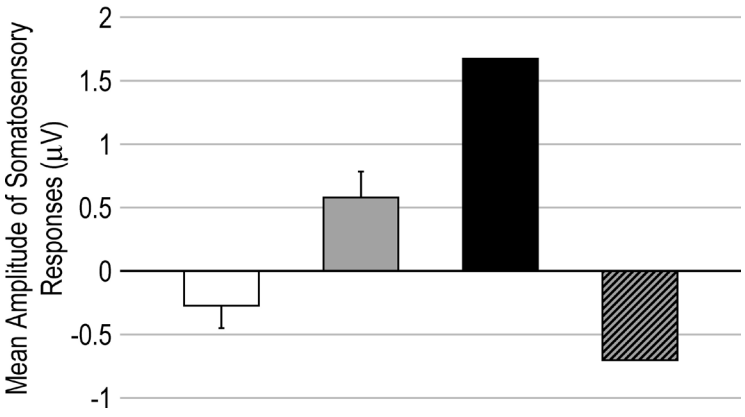
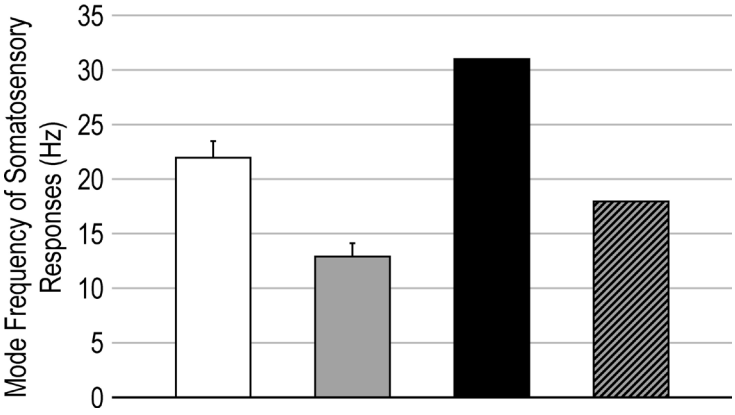


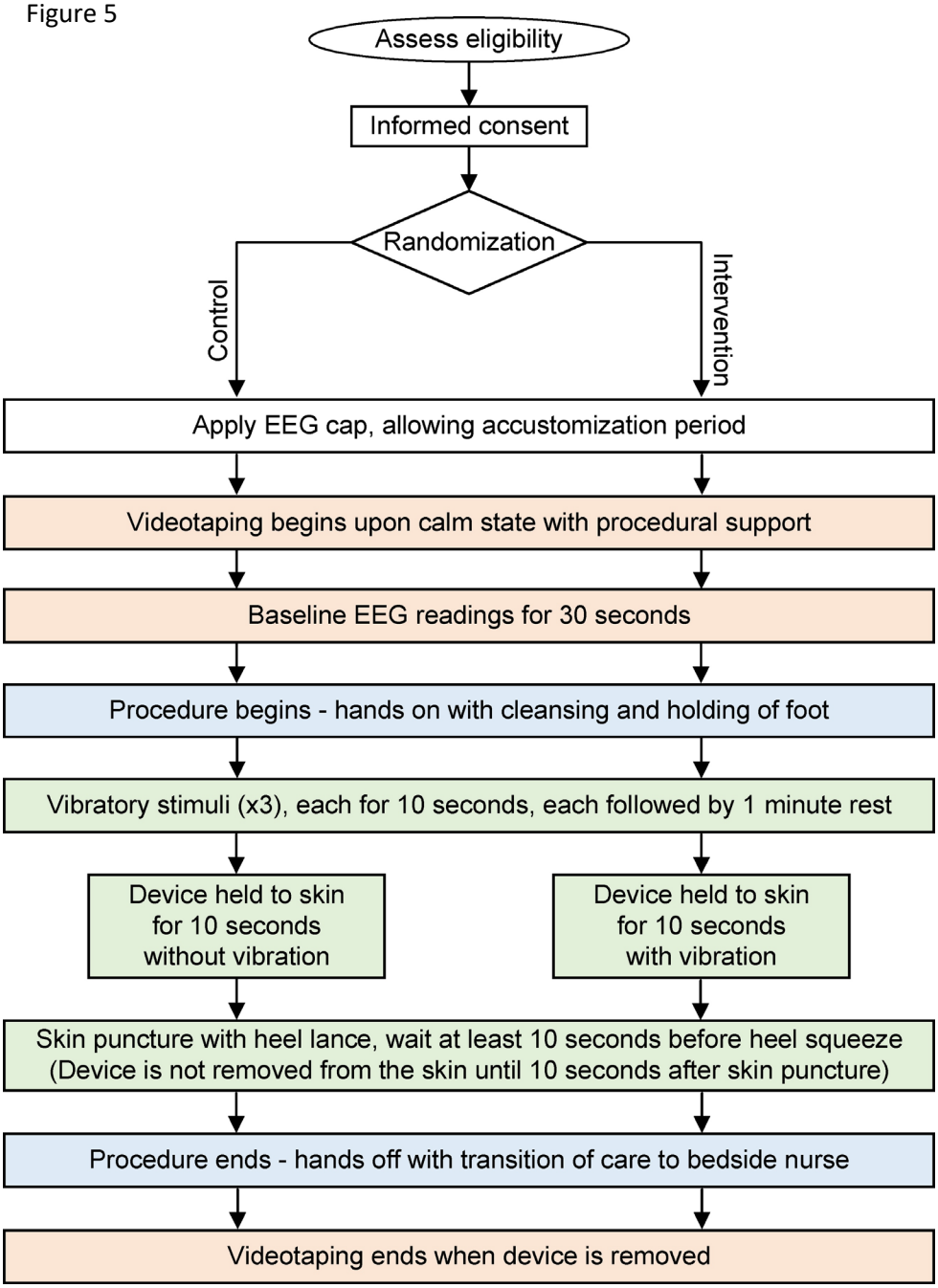
Figure 4



Experimental Plan, Methods and Statistical Plan – We will conduct an RCT in term and preterm infants that are to undergo heel-lancing as part of their routine clinical care. We contracted a group of biomedical engineers (Actuated Medical LLC) to construct a prototype that can provide a non-noxious vibratory stimulus in addition to a standard heel-lance. The function of this device has been time-locked with EEG signal acquisition and video of facial expression along with limb withdrawal. Allocation concealment and blind-to-assignment conditions for EEG and video examiners will minimize bias. The process flow is depicted in **Figure 5** with each component of the protocol described in more detail below.

Process Flow

Figure 5



Study Population

Inclusion criteria: The study population will be comprised of hospitalized infants in the NICU at Nationwide Children's Hospital (NCH). Because the specific pain response is not mature until 36 weeks post-menstrual age (PMA)[25], only infants 36-52 weeks will be included. Included infants must be medically stable and due to have a heel lance as part of their routine medical care.

Exclusion criteria:

- Patient is over 3 months corrected age
- Infants who receive sucrose (SweetEase) or other analgesic or sedative prior to their procedure
- Infants with congenital brain abnormalities

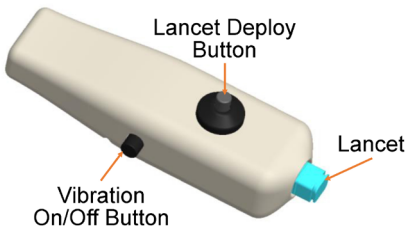
Withdrawal criteria: Infants can be withdrawn from the study at any time at parent's request or if the medical team determines that study participation is not in the patient's best interest.

Recruitment and Informed Consent

Recruitment: The research coordinator will identify prospective participants from the electronic census of infants admitted to the NCH NICU. Informed consent will be obtained from parents using Internal Review Board (IRB) approved documents. Parents will be consented in their native language with the use of translated forms and translators when required. Parents will be given information about the study as well as explanations of risks and benefits. Parents will be given a signed copy of the informed consent; a second signed copy will be placed in a locked cabinet in the Principal Investigator's locked office. No waivers of informed consent will be sought.

Intervention

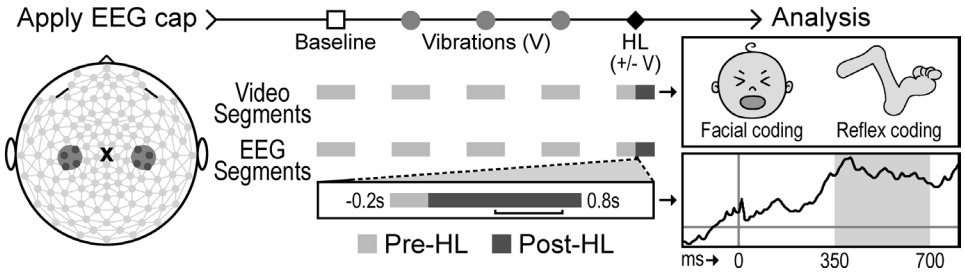
Figure 6 - Prototype Vibratory Device



After a resting baseline measurement, each subject will undergo three vibration sequences (V) to determine an averaged response. The vibration sequence will be provided by a prototype device that connects directly to EEG equipment for synchronization purposes. Output signals occur 1-2 milliseconds after manual button pushes, one that serves to start/stop the vibration, and the other that deploys the lancet (**Figure 6**). The device's power output is under 5 watts and provides a vibration of 178 Hz over a free-hanging distance of 0.24 mm. The Unistik 3™ Gentle lancets are single-use, disposable, 30 gauge needles that penetrate to a depth of 1.5 mm. Respective control and experimental infants will be either treated or not treated with this vibration immediately preceding the heel lance (HL). The V and HL will be time-locked to set video and EEG segments for analysis. Similarly, synchronized video segments will be recorded and coded for facial and reflex components. A schematic of this is outlined in **Figure 7**.

Fidelity of intervention monitoring will be assessed by an independent reviewer not involved in analysis or intervention support using a checklist developed prior to study start. The

Figure 7 - Outline of protocol for time-locked EEG-based experiments.



reviewer will verify a random sample of 10% of unsegmented videos throughout the course of the study and note any deviations on the checklist. If any deviations from the intervention protocol are observed, the reviewer will notify the research coordinator to correct the deviation before the next procedure.

Facial Expression Coding and Limb Withdrawal

Facial expression will be scored following the Neonatal Facial Coding System (NFCS)[26]. Components of this scale include the following: brow bulge, eye squeeze, naso-labial furrow, open lips, horizontal mouth stretch, vertical mouth stretch, lip purse, taut tongue, chin-quiver, tongue protrusion. Each is assigned one point for its presence then summed for a total score. Coding will be based on time-locked video segments that have been processed for the purposes of blinding examiners to the condition. Each video segment will be evaluated by two independent researchers not participating in the intervention and masked to group and to session sequence. Video coders on the research team will undergo reliability training to achieve >90% inter-rater reliability in each domain of assessment on a subset of 20 random video segments. For study scoring purposes, a third masked and reliability-trained coder will also review video segments that have more than a 15% score discrepancy between the first two reviewers over the entire assessment. The third reviewer's score will then be averaged with the other two scores to minimize discrepancies.

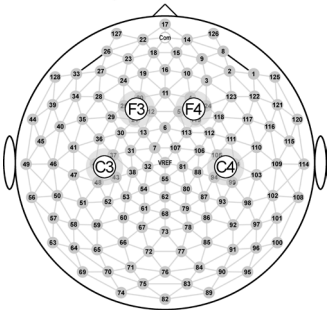
Limb withdrawal will be assessed in a similarly blinded, randomized fashion and using a third coder as needed to address any discrepancies. Limb movement will be assessed based on the following: no movement, ipsilateral withdrawal, contralateral withdrawal or bilateral movement.

Electroencephalography procedure

Recording: A high-density array of 128 electrodes embedded in soft sponges (Electrical Geodesics, Inc. (EGI); Eugene, Oregon, USA) soaked in warm saline and applied to the infant's head will be used to record event related potentials (ERPs) as previously described[27,28], with a sampling rate of 1000 Hz, filters set to 0.1-400 Hz. As per published protocols, the midline Cz electrode will be used as the reference[27]. Infants will be tested in a quiet room in drowsy or quiet alert states. No sucrose or other oral solutions will be used during the procedure and no infants will be tested in skin-to-skin hold, but all infants will receive containment support as per unit protocol. Recording of brainwaves will be controlled by Net Station V.4.3. The vibratory device and heel lance have been electronically engineered to label the EEG stream for time-locking purposes to analyze equivalent time windows for the vibratory stimuli and heel lance.

Processing and analysis: ERP data will be filtered using a 0.3–40 Hz bandpass filter and segmented[29,30], using manually verified algorithms in the NetStation software to exclude segments contaminated by motor or ocular artifacts. Post processing of the data will include NetStation tools for bad channel replacement, montage referencing and baseline correction. Four scalp regions of interest (F3, F4, C3, C4) will be selected for analysis, defined using clusters of electrodes identified in published studies of somatosensory stimuli (**Figure 8**)[31]. Averaged mean amplitude data will be extracted in pre-specified time windows corresponding to nociceptive specific activity (350-700 ms).

Figure 8 - Distribution of electrodes and scalp locations of interest



Statistical Plan

Sample size and power: Based on our previous data[21], setting α at 0.05 and setting power at 0.80, it will take a sample size of 48 patients per group to demonstrate a medium effect size (Cohen's d = 0.57). This translates into a reduction of mean amplitude of the pain response from 5.5 to 3.5 μvolts from our intervention. Factoring in previous rates of artifact-free cortical responses and data loss during heel lance, we plan to enroll a total of 134 subjects into the study to account for a possible attrition rate of 28 percent.

Analysis: Results will be summarized by intervention group and by stimulus segment using means with standard deviations or medians with interquartile range for continuous variables and frequencies with percentages for categorical variables. Paired t-tests will be used to compare the within-patient change in EEG response and facial response scores, while McNemar tests will be used to compare change in extremity response and change in each individual subcomponent of the facial response score (A vs. B). A similar analysis will compare baseline to heel lance responses among the control group subjects (A vs. C), as well as vibration to heel lance with vibration among the intervention group subjects (B vs. D).

For conditions of skin puncture with or without a vibratory stimulus (between control and intervention groups), a two-sample t-test will be used to compare EEG and total facial response scores after heel lance by intervention arm, while chi-square or Fisher's exact tests will be used to compare extremity response and individual subcomponents of the facial response tool. For the secondary analysis of these conditions, EEG response will be treated as the dependent variable and PMA, intervention group, facial response score, and extremity response will be considered as independent variables. Interactions between PMA and intervention group, facial response score, and extremity response will also be tested for this secondary analysis, in order to determine whether the association between each variable and EEG response varies with post-menstrual age; if a significant interaction is found, results will be summarized within PMA strata to aid interpretation.

As a final analysis spanning all four experiments, we will use linear and binomial mixed effects models to evaluate change in response across each stimulus segment among all patients, change in response within each intervention arm, and to compare response in each stimulus segment by intervention arm. All analyses will be conducted using SAS 9.4 with two-sided p-values<0.05 considered statistically significant.

Dealing with potential problems:

- Attrition and sample size: should the attrition due to motion artifact be higher than expected, we can easily increase our recruitment and still remain within the projected timeline. The NCH NICU has 140 beds with over 3,000 annual admissions, with infants having multiple skin breaking procedures during hospital stays ranging from several days to several months.
- Fidelity of intervention and of behavioral coding will be monitored rigorously as described above.
- Diffusion effects between patients are unlikely as only one vibration device exists, for research purposes.
- Differential treatment effect considerations: We do not assume that our treatment will affect all participants equally. Our analysis plan includes secondary exploratory analyses determining the differences in effect as a function of selected variables measured prior to randomization.
- Random assignment of infants to one of two groups, masking of all personnel analyzing data to study group and statistical control of key pre-randomization covariates will minimize selection bias.
- Intent-to-treat analysis is not possible in this design as mechanistic inferences on brain responses can only be made based on time-locking to the actual stimulus.

Approvals for Human Subjects, Potential Problems and Pitfalls, and Risk Mitigation –

Our study has been approved by the NCH IRB. The ERP protocols in our study have also been approved previously for other studies that are actively taking place at NCH. Each section of our protocol is in alignment with the Office of Human Research Protections Category 1 (45 CFR 46.404; 21 CFR 50.51) requirements, the custom device is exempt under 21 CFR 812.2(c), and the FDA-approved heel lancets will be used in accordance with labelling. All heel sticks will be those required for routine clinical care and none of the blood will be used for research purposes.

Informed consent will be obtained from parents using IRB approved documents. An experienced research coordinator will initiate the informed consent process according to the Good Clinical Practice guidelines. Parents will be given information about the study as well as explanations of risks and benefits. The equipment and protocol pose minimal risk to participants, and are reasonable in relation to the anticipated benefits to research participants and others.

ERP paradigm minimal risk rationale: The EGI system used to record the brain responses is electrically isolated from the participant, eliminating the risk of any current flowing to the participant under all conditions, including a ground fault. In the event of a worse-case equipment failure where the participant would be grounded, and current paths are reversed to move towards the participant, no current beyond 0.2 micro-amps would pass to the participant. This level is approximately 250 times lower than the current industrial standards. The electrodes used for this study do not require skin abrasion for proper contact and therefore minimize infection risk. Risk of infection is further reduced through specific electrode care procedures that include rinsing all electrodes with water immediately after testing and then soaking the electrode net in a cold sterilizing solution to eliminate any contaminants that otherwise might pass from participant to participant. Following soaking, the electrodes are again rinsed and then air dried.

The vibratory device is made of construction materials that allow for sanitizing the equipment between patients, and the internal circuitry that will allow for time-locking the function of the device with the EEG data in the EGI system is also electrically isolated from the patients and carry minimal risk.

Video of the subjects will be taken before, during, and after the procedure. The study coordinator and other NCH study personnel who are blinded to the infant's identity will be the only people to view the videos, and all video material will be destroyed after six years from study completion. The video material will be kept in a folder within a secure, NCH-managed Dropbox account and the only people who will have access to this folder are the personnel involved in the study.

Risk minimization includes adherence to the procedural guidelines to maintain standard of care, following unit protocols and infection control procedures, and utilization of RedCap database for data storage. Study data will be stored using REDCap, which is a secure, web-based application designed to support data capture for research studies by building and managing online surveys and databases. Study video material will be stored using an NCH-managed Dropbox account, and the only people who will have access to the study folder will be the personnel involved in the study. Infants will be continuously monitored, not only by study personnel, but also by the infant's bedside nurse during all steps of the protocol, to mitigate any possible risk. Study personnel will be the only people to view the videos, and all video material will be destroyed after six years from study completion.

We do not anticipate withdrawal due to safety or toxicity concerns. Should the participant become medically unstable and participation not be in the patient's best interest, he or she will be withdrawn from the study.

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