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Title: Using sensor-fusion and machine-learning algorithms to assess acute pain in nonverbal infants: a study protocol

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ABSTRACT 271 words out of 300

Introduction:

Objective pain assessment in non-verbal populations is clinically challenging due to their inability to express their pain via self-report. Repetitive exposures to acute or prolonged pain lead to clinical instability, with long-term behavioral and cognitive sequelae in newborn infants. Strong analgesics are also associated with medical complications, potential neurotoxicity and altered brain development. Pain scoring performed by the bedside nurses not only increases nursing workload, but also provides subjective, observer-dependent assessments, rather than objective data for infant pain management. Multimodal pain assessment, using sensor fusion and machine learning algorithms, can provide a patient-centered, context-dependent, observer-independent, and objective pain measure.

Methods and analysis:

In newborns undergoing painful procedures, we use facial electromyography (EMG) to record facial muscle activity associated with infant pain, electrocardiography (ECG) to examine heart rate (HR) changes and HR variability (HRV), electrodermal activity (skin conductance) to measure catecholamine-induced palmar sweating, changes in oxygen saturations and skin perfusion, and electroencephalography (EEG) using active electrodes to assess brain activity in real-time. This multimodal approach has the potential to improve the accuracy of pain assessment in non-verbal infants and allow continuous pain monitoring at the bedside. The feasibility of this approach will be evaluated in an observational prospective study of clinically required painful procedures in 60 preterm and term newborns, and infants aged 6 months or less.

Ethics and dissemination:

The Institutional Review Board of the Stanford University approved the protocol. Study findings will be published in peer-reviewed journals, presented at scientific meetings, taught via webinars, podcasts, video tutorials, and listed on academic/scientific websites. Future studies will refine this approach using the minimum number of sensors required to assess neonatal/infant pain.

Registration number: ClinicalTrials.gov Identifier: NCT03330496

An Article Summary:

'Strengths and limitations of this study'

- An innovative and objective approach for continuous pain monitoring in infants including term and preterm neonates is described.
- To discriminate between noxious and non-noxious events, we used sensor fusion and machine-learning algorithms.
- Multimodal assessments may be more sensitive and specific for identifying pain and quantifying its intensity than the currently used subjective assessments from pain scales.
- Accurate, objective pain assessments will help to reduce infant pain and suffering, enhance recovery, avoid untreated pain vs. analgesic overuse, and allow evaluation of newer analgesics or other therapies in randomized clinical trials.
- Multiple sensors are used for the first step of this study, but recording artifacts may require data corrections and sensor variability may generate a need for recruiting more patients.

INTRODUCTION

Being non-verbal, hospitalized infants are particularly vulnerable to inadequate pain management. Repeated exposures to pain in newborns can lead to short and long-term neurodevelopmental consequences including behavioral and cognitive sequelae [1][2–5]. Conversely, the safety and efficacy of some analgesics in neonates and their negative consequences on the neonatal brain have raised concerns [6][7][8][9]. Objectively assessing the pain responses in infants is thus necessary to assess the efficacy of analgesics in infants in order to avoid over treatment but also undertreatment and the consequences of repetitive pain exposure. Composite pain scales including behavioral and physiological measures are assumed to be the most accurate surrogate measures of infant pain and are currently recommended for the clinical practice [10]. However, they provide a one-time measurement and their use can be challenging for the bedside staff leading to low interrater reliability, with over or under estimation of pain in neonates [11][12]. Depending on the context, behaviors and physiological responses may mirror non-noxious stimuli, leading to misinterpretation and a lack of specificity of subjective pain scales [13][14][15].

Pain from clinically required invasive procedures leads to well-described neurophysiological responses in term and preterm infants [15]. These responses imply that the central, peripheral, and autonomic nervous systems can be monitored using various behavioral and physiologic modalities [16][17,18][19]. Since pain is a complex process, multimodal measurement may improve the accuracy of pain assessment, also suggested by studies reporting the presence of pain-evoked potentials in some neonates showing no facial expressions of pain or others pointing out different profiles of pain responses [13][20].

Thus, developing new methods to assess the responses to pain in infants deserves a high priority. Multimodal measurements that provide an objective assessment of real-time and continuous pain monitoring at the bedside will avoid the subjective bias and limitations associated with clinical pain scales, especially when behavioral assessment is limited by the medical conditions [21].

Our study protocol was designed to develop a multimodal pain assessment system, using sensor fusion and novel machine learning algorithms to provide an objective measure of pain in infants that is patient-centered, context-dependent, and observer-independent.

METHODS AND ANALYSIS

Study design

We designed a prospective observational study enrolling subjects from the Lucile Packard Children's Hospital at Stanford.

Study population

We will collect data from 15 study subjects in each of the following age groups: 1) Preterm infants (34-37 weeks of corrected gestational age, postnatal age 3-30 days); 2) Term newborns (37-42 weeks of corrected gestational age, less than 1 month of age); 3) Infants from 1-3 months age; 4) Older infants from 3-6 months age.

Eligibility criteria

After obtaining parental consent, we will include all infants less than 6 months of corrected chronological age requiring an acute painful procedure for routine clinical care in the participating units at Lucile Packard Children's Hospital.

We will exclude newborns with birth trauma, intrapartum asphyxia (5-minute Apgar Score <4 or cord pH < 7.01), fetal growth restriction (birth weight < 5th percentile for gestation), congenital anomalies or metabolic disorders, or any kind of brain injury; if their mothers had a history of heavy smoking or drug abuse (alcohol, cocaine, ketamine, and heroin/other opiates) or psychiatric drugs used during this pregnancy; infants requiring positive pressure ventilation using a face mask (BiPAP) or endotracheal tube; those receiving continuous infusions of opioid drugs (morphine, fentanyl, and others) and nerve blocks or neuraxial analgesia affecting the site of the invasive procedure in the 24 hours prior to study entry; infants with facial anomalies (cleft lip), injuries or other pathologies affecting the facial area; and infants breastfed to alleviate pain during the painful procedure.

Objectives/Outcomes

Primary Objective:

Our primary objective is to identify the specific signals and patterns from each sensor that correlate with the pain stimulus.

Primary Outcome:

We will extract pain-related information using non-invasive multimodal sensors. Specific features of the physiological/ behavioral indicators of infant pain will require unique processing algorithms. We will record pain signals using facial electromyography (EMG), electrocardiography (ECG), electrodermal activity (skin conductance), oxygen saturation (SpO2), and electroencephalography (EEG) in real-time. Dedicated algorithms for each sensor will extract pain-related information such as facial grimacing or heart rate variability. The reliability and validity of these algorithms will be tested prospectively on data from preterm and term neonates, and 1-6 month-old infants experiencing acute pain during invasive procedures.

Secondary Objectives:

We aim to identify if multiple sensors will provide overlapping information, which a sensor fusion framework can integrate to identify “pain” and “no pain” related features. These features will be used to train machine learning algorithms that will finally provide reliable, objective assessments of pain intensity in real-time.

We will also study if the pain intensity measured by the sensor fusion framework will show clinical validity, inter-rater reliability, as well as responsiveness to pain relief using analgesic drugs or non- pharmacological therapies.

Secondary outcomes:

We will develop a sensor fusion framework designed to integrate data from different sensor modalities. No single sensor is capable of measuring neonatal pain. Rather, skilled clinicians draw upon multiple sources of information to estimate pain. A machine learning algorithm will be developed to test if the sensor fusion framework can (i) can "calibrate" itself to the unique physiology of each newborn, (ii) handle missing (e.g. sensor failure) or unreliable data (e.g. movement artifact), and (iii) determine specific features from each modality to reach asymptotic levels of sensitivity and specificity. We hypothesize that this automated sensor fusion approach will be able to quantify neonatal pain intensity with greater specificity and sensitivity than the pain scales clinically used at the bedside.

To assess the reliability and validity of the pain intensity measured by this sensor fusion framework, we will compare the objective pain measure with the pain scores assessed by skilled research staff. Variations in the objective pain scores before and after the clinical use of analgesic therapies will also be assessed to explore whether this device can also identify pain relief [22].

Sample size calculations

We assume an α-error =0.05, 1–β error =0.8, and a mean:SD ratio of 2:1 for clinical and sensor-based pain scores (based on prior studies of clinical assessments of procedural pain in infants) [23,24]. Based on these assumptions, to detect a slope that corresponds to a 0.5-unit change in the outcome (e.g., sensor pain score) per 1-unit change in the predictor (e.g., clinical pain score) we will require a minimum of 40 infants in the *training dataset*. For a binary predictor with 25% or 50% prevalence (e.g., mild vs. moderate pain, or male vs. female neonate), with this sample size, we will be able to detect a 0.7 or 0.8-unit change in the outcome, respectively. Given the number of sensors used for the first step of this study, some artifacts may occur requiring data corrections or greater variability, with a need for recruiting more patients. We plan to recruit 60 patients in this study

Interventions/Experimental design

After applying inclusion/exclusion criteria and parental consent, infants' medical data will be recorded including: date of birth, perinatal/medical history, birth weight, gestational age, Apgar scores, congenital anomalies, metabolic defects, other diagnoses, previous surgeries, recent labs, prior imaging, major physical findings, number and types of painful procedures, and all medications used in the 24 hours immediately preceding the study. We will document the type of procedure, time of day, its location and duration, number of attempts and behavioral state of the infant before and after the procedure.

Prior to a clinically-indicated procedure, we will attach skin conductance (SC) leads to measure galvanic skin responses either on a hand palm or foot sole, and electromyography (EMG) to record facial muscle activity from cheek and forehead, and directly obtain recordings from clinical monitors (electrocardiography (ECG) and oxygen saturation (SpO2)). In addition, the infant will wear a cap with Electroencephalography (EEG) leads. For study procedures, research staff will clinically assess pain using the Neonatal Pain & Sedation Scale (N-PASS) before and after the procedure, the Premature Infant Pain Profile-Revised (PIPP-R) and the Neonatal Facial Coding System (NFCS) during the procedure. For older patients, the FLACC (Face, Legs, Activity, Cry, Consolability) scale or Visual Analogue Scale (VAS) will be scored to assess pain during the procedure [25,26][27][28]. Physiological recordings as well as audio recording will start 10-30 minutes before a planned procedure and continue for up to 20-30 minutes after the procedure. At the completion of recording, all the above sensors will be disconnected from each patient and study procedures will be terminated. The entire study will last approximately 30-60 minutes; however the study may be stopped earlier if any infant shows signs of distress or if a bedside nurse or parent has any concerns.

All sensors will be time-locked with an event-marker, to record the exact times of noxious and non-noxious events.

Data analysis

A. Signal-Filtering and Information Extraction from Sensors

For each sensing modality, we will develop a software algorithm to process signals recorded by sensors and extract the clinically relevant information related to pain. Proof-of-concept algorithms will be tested using the collected dataset. The feasibility of each sensing modality will be based on: *i*) sensitivity and specificity of detecting clinically relevant pain-related changes, and *ii*) robustness of sensor readings in the presence of non-pain related disturbances (e.g., movement artifact).

1. Pain Behaviors: Newborn behaviors (e.g., facial expressions, body movements) are accepted as the most sensitive and valid indicators of pain [29][13]. Facial expressions like brow bulge, eye squeeze, nasolabial furrow, and horizontal mouth stretch were verified as the most valid and discriminative components of neonatal pain scales [30,31][32]. Using facial EMG in real-time, we will detect the presence of muscle activity in neonatal pain-associated facial regions [33]. Given the multiple layers of facial muscles, facial EMGs record signals from a facial region as opposed to any specific muscle [33]. Previous studies of startle and blinking in infants used miniature silver (Ag/AgCl) periorbital surface electrodes for recording EMGs [34–36]. We will focus on infant forehead and cheek areas to detect EMG activity associated with brow bulge, eye squeeze and nasolabial furrow [33]. We will iteratively refine our algorithms by using multi-modality sensing and developing robust feature extraction and classification frameworks that address the challenges specific to neonatal/infant pain detection. We will exclude mechanically ventilated infants due to the challenges associated with identifying facial features (occluded by securing tape, ventilator tubing or devices) and their need for ongoing sedation/analgesia.

2. Skin Conductance: Acute pain stimulates the sympathetic post-ganglionic cholinergic neurons [37], leading to diaphoresis, palmar sweating and increased skin conductance [38]. Eliminating painful stimuli results in sweat reabsorption and decreased conductivity. The amplitude of changes in palmar skin conductance reflect increased sympathetic nervous system activity, which tracks with pain intensity [39–42]. Skin conductance can change with body temperature [43,44], but not with the ambient temperatures [44]. Specifically, the number of fluctuations of skin conductance per second (NFSC) was correlated with pain intensity in children [45], and was more sensitive that pain scores in preterm and term neonates [18,40–42,46]. We will use skin conductance using the BrainAmp system® (Brain Products GmbH, Gilching, Germany),

3. Electrocardiography (ECG): Heart rate (HR) changes are components of many pain assessment scales and recent studies have established correlations between HR variability (HRV) and pain [47,48]. A number of linear time-domain (HR mean, standard deviation) and frequency-domain (power spectral density) metrics and non-linear metrics (sample entropy, approximate entropy, etc.) can detect painful stimuli [47,48]. We will record the infant's ECG before, during, and after an acute pain event to extract the linear and nonlinear metrics (listed above) from the ECG signal for further analyses.

4. Electroencephalography (EEG): EEG studies to assess neonatal pain have met with variable results [49–51]. Opioid analgesia in adults leads to slowing of the EEG, whereas painful stimuli activate brain regions identified by neuroimaging studies [52] such as the primary sensory cortex (S1) [53]. EEG amplitudes and frequencies decreased when analgesics were given to newborns in pain [20,54–56]. Using the BrainAmp EEG system® (Brain Products GmbH, Gilching, Germany), we will apply 32 active EEG electrodes using the infant-sized ActiCap® (Brain Products GmbH, Gilching, Germany). Although Hartley et al. have selectively used the vertex (Cz) lead for neonatal pain studies [20,54], we believe that infant pain processing is widely distributed across many brain regions and the current evidence is not sufficiently strong enough to exclude information from other EEG leads. The BrainAmp is similar to other EEG monitors, however, it uses 32 active electrodes allowing for placement of the ActiCap on the infant's head with minimal preparation. Each active electrode amplifies the signal recorded from the skin and records also indicates the impedance of each electrode at the start of the recording to improve the quality of recorded signals. Over the past 10 years, this device has been used for research purposes in all age groups including infants and newborns. No side effects have been reported from its use in newborns and small infants.

5. Pulse Oximetry (SpO2): Changes in SpO_2 occur frequently following acute pain and, therefore, have been included in the Premature Infant Pain Profile (PIPP) and other pain scales [57–59]. Newer generation monitors (Masimo, Irvine, CA) use multi-wavelength technology to provide more reliable SpO2 and pulse rate signals, with parallel signal processing engines and adaptive filters to separate the arterial from venous signals, patient motion, or skin perfusion [60]. Changes in skin blood flow were also used as physiological markers for neonatal pain or morphine analgesia [61,62]. We will test the utility of the SpO2 and peripheral perfusion index provided by pulse oximetry monitors as possible signals for neonatal pain.

Statistical approach

A. Sensor Fusion

We have previously investigated using machine learning to detect pain in neonates using facial expressions recorded by a camera [63]. We will develop a sensor fusion framework to detect pain in non-verbal infants based on machine learning to detect pain using multi-modal sensor data. Feasibility of this new framework will be assessed based on its sensitivity and specificity to detect pain events in infants and further refined into a prototype for validation in future studies.

A "calibration" period will be used to establish a baseline for these multiple sensor modalities by monitoring neonates who are not in pain. The clinical staff at the bedside will identify the pain state of each neonate using validated pain scales and record the timing of pain-inducing clinically-indicated procedures such as a heel stick. Our sensor fusion framework will classify the neonatal/infant responses to infer pain intensity based on observed changes from baseline. A probabilistic relationship between pain intensity and sensor measurements can be established, where the unknown parameters of the statistical relationships are identified by a training dataset. The training dataset will also be used to measure the importance of each feature, which can then be used to identify the optimal set of sensors [61].

Pain intensity scores computed by our sensor fusion framework will be compared with pain scores measured concurrently by skilled research staff. All sensors (facial EMG, EEG, ECG, SpO2, and SC) will be time-locked with an event recorder to mark “pain” vs. “no-pain” states. To make the best use of our data, the sensor fusion framework will use standard cross-validation methods to establish the generalizability of this framework.

B. Validation and Correlation with Pain Intensity

We will compare clinical pain scores from nursing assessments with scores from the sensor fusion framework. First, we will examine clinical pain scores to verify agreement with the pain scores assessed by the research staff. Internal consistency will be evaluated by Cronbach’s α, with values > 0.8 to show good internal consistency. Second, we will conduct multivariable linear regressions or generalized estimating equations (GEE) [64,65] to understand the agreement between the device pain scores and the clinical pain scores, as well as the contribution of each modality to the device pain scores. We will examine if these associations vary after adjustment for covariates such as pain medications, age, sex, duration or invasiveness of the procedure. A co-variance matrix will examine the degree of correlation between individual sensor inputs, types of procedures, clinician pain scores, and analgesic therapies used during the procedure. Finally, due to the limited understanding of factors contributing to pain in newborns, linear regression or GEE models will examine the association of the sensor fusion pain scores reported by the device with the demographic and clinical variables of neonates and infants.

Content validity depends on the sensors and sensor variables that we have chosen for the sensor fusion framework. Concurrent validity will depend on the pain scores of skilled research staff using validated pain scoring methods. Construct validity will rest on: *i*) the range of objective pain scores from procedures causing mild, moderate, or severe pain; *ii*) changes in pain scores with analgesic drugs or non-pharmacological therapies; and *iii*) variation in pain scores over time consistent with the expected, natural course of acute procedural pain.

ETHICS AND DISSEMINATION

The Institutional Review Board of the Stanford University approved the protocol. This observational study is registered at www.ClinicalTrials.gov (NCT03330496) and it does not involve any intervention other than those clinically required. Data collection includes physiological recordings and medical information. Other than the facial EMG, skin conductance leads (SC), and 32-channel BrainAmp Standard EEG monitor, all other sensors are used routinely as the standard of care. All sensors are considered non-invasive and safe. We will use standard electrodes which are routinely used for recording vital signs in the hospital (ECG, SpO2). All recordings will use sticky pads attached to the skin. Other than a potential for mild skin irritation from the adhesives used, there are no significant risks associated with these devices, or other study-related procedures.

Since the study subjects are aged less than 6 months, the parent's consent will be obtained for their child as a research subject. The primary risks to study subjects result from potential loss of confidentiality from the information collected and from the medical record and monitoring devices. As described in the consent form, the right to privacy during the consent process, data collection and study procedures, and protection of personal data will be given the utmost importance and strict safeguards will be maintained to protect data confidentiality.

Study findings will be published in peer-reviewed journals and presented at national and international scientific conferences. Practical use of this methodology will be taught at conference workshops, or via webinars, podcasts, video tutorials.

Future studies will test the validity of this approach to pain assessments in larger populations of newborns, older infants and also extend these studies to smaller preterm neonates. Future applications may also include patient populations incapable of expressing pain (children with disability, adults with dementia, or mechanically ventilated patients).

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Using sensor-fusion and machine-learning algorithms to assess acute pain in nonverbal infants: a study protocol

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Introduction:

Objective pain assessment in non-verbal populations is clinically challenging due to their inability to express their pain via self-report. Repetitive exposures to acute or prolonged pain lead to clinical instability, with long-term behavioral and cognitive sequelae in newborn infants. Strong analgesics are also associated with medical complications, potential neurotoxicity and altered brain development. Pain scores performed by bedside nurses provide subjective, observer-dependent assessments rather than objective data for infant pain management; the required observations are labour-intensive, difficult to perform by a nurse who is concurrently performing the procedure, and increase the nursing workload. Multimodal pain assessment, using sensor fusion and machine learning algorithms, can provide a patient-centered, context-dependent, observer-independent, and objective pain measure.

Methods and analysis:

In newborns undergoing painful procedures, we use facial electromyography (EMG) to record facial muscle activity related infant pain, electrocardiography (ECG) to examine heart rate (HR) changes and HR variability (HRV), electrodermal activity (skin conductance) to measure catecholamine-induced palmar sweating, changes in oxygen saturations and skin perfusion, and electroencephalography (EEG) using active electrodes to assess brain activity in real-time. This multimodal approach has the potential to improve the accuracy of pain assessment in non-verbal infants and may even allow continuous pain monitoring at the bedside. The feasibility of this approach will be evaluated in an observational prospective study of clinically required painful procedures in 60 preterm and term newborns, and infants aged 6 months or less.

Ethics and dissemination:

The Institutional Review Board of the Stanford University approved the protocol. Study findings will be published in peer-reviewed journals, presented at scientific meetings, taught via webinars, podcasts, video tutorials, and listed on academic/scientific websites. Future studies will validate and refine this approach using the minimum number of sensors required to assess neonatal/infant pain.

Registration number: ClinicalTrials.gov Identifier: NCT03330496

An Article Summary:

'Strengths and limitations of this study'

- An innovative and objective approach for continuous pain monitoring in infants including term and preterm neonates is described.
- To discriminate between noxious and non-noxious events, we use sensor fusion and machine-learning algorithms.
- Multimodal assessments may be more sensitive and specific for identifying pain and quantifying its intensity than the subjective assessments currently used in pain scales.
- Accurate, objective pain assessments may help reduce infant pain and suffering, enhance recovery, avoid untreated pain vs. analgesic overuse, and allow evaluation of newer analgesics or other therapies in randomized clinical trials.
- Multiple sensors are used for the first step of this study, but recording artifacts may require data corrections and sensor variability may generate a need for recruiting more patients.

INTRODUCTION

Being non-verbal, hospitalized infants are particularly vulnerable to inadequate pain management. Repetitive pain in newborns leads to short- and long-term neurodevelopmental consequences including behavioral and cognitive sequelae [1][2–5]. Conversely, the safety and efficacy of some analgesics in neonates and their negative consequences on the neonatal brain have raised concerns [6][7][8][9]. Objectively assessing the pain responses in infants is thus necessary to assess the efficacy of analgesics in infants in order to avoid over treatment but also undertreatment and the consequences of repetitive pain exposure.

Composite pain scales including behavioral and physiological measures are the most widely used surrogate measures of infant pain and are currently recommended for the clinical practice [10]. However, they provide a one-time measurement and their use can be challenging for the bedside staff leading to low interrater reliability, with over- or underestimation of infant pain [11][12]. Depending on the context, behaviors or physiological responses may mirror non-noxious stimuli, leading to misinterpretation and a lack of specificity in subjective pain scales [13][14][15].

Pain from clinically required invasive procedures leads to well-described neurophysiological responses in term and preterm infants [15]. These responses imply that the central, peripheral, and autonomic nervous systems can be monitored using various behavioral and physiologic modalities [16][17,18][19]. Since pain is a complex process, multimodal measurement may improve the accuracy of pain assessment, also suggested by studies reporting the presence of pain-evoked potentials in some neonates showing no facial expressions of pain or others pointing out different profiles of pain responses [13][20].

Thus, developing new methods to assess the responses to pain in infants deserves a high priority. Multimodal measurements that provide an objective estimate of real-time and continuous pain

monitoring at the bedside will avoid the subjective bias and limitations associated with clinical pain scales, especially when behavioral assessment is limited by the medical conditions [21]. Our study protocol was designed to develop a multimodal pain assessment system, using sensor fusion and novel machine learning algorithms to provide an objective estimate of acute pain intensity in infants that is patient-centered, context-dependent, and observer-independent.

METHODS AND ANALYSIS

Study design

We designed a prospective observational study enrolling subjects from Lucile Packard Children's Hospital at Stanford. The study started on October 30th, 2017 and will be completed on November 30th, 2025 (www.ClinicalTrials.gov, NCT03330496).

Study population

We will collect data from 15 study subjects in each of the following age groups: 1) Preterm infants (34-37 weeks of corrected gestational age, postnatal age 3-30 days); 2) Term newborns (37-42 weeks of corrected gestational age, less than 1 month of age); 3) Infants from 1-3 months age; 4) Older infants from 3-6 months age.

Eligibility criteria

After obtaining parental consent, we will include all infants less than 6 months of corrected chronological age requiring an acute painful procedure for routine clinical care in Lucile Packard Children's Hospital.

We will exclude newborns with birth trauma, intrapartum asphyxia (5-minute Apgar Score <4 or cord pH < 7.01), fetal growth restriction (birth weight < 5th percentile for gestation), congenital

anomalies or metabolic disorders, or any kind of brain injury; if their mothers had a history of heavy smoking or drug abuse (alcohol, cocaine, ketamine, and heroin/other opiates) or psychiatric drugs used during this pregnancy; infants requiring positive pressure ventilation using a face mask (BiPAP) or endotracheal tube; those receiving continuous infusions of opioid drugs (morphine, fentanyl, and others) and nerve blocks or neuraxial analgesia affecting the site of the invasive procedure in the 24 hours prior to study entry; infants with facial anomalies (cleft lip), injuries or other pathologies affecting the facial area; and infants breastfed to alleviate pain during the painful procedure.

Objectives/Outcomes

Primary Objective:

Our primary objective is to identify the specific signals and patterns from each sensor that correlate with the pain stimulus. This pilot study is designed to exclusively assess acute pain responses during routine, clinically-required skin-breaking procedures – it measures the intensity of acute pain from the physiological responses of each subject.

Primary Outcome:

We will extract pain-related information using non-invasive multimodal sensors. Specific features of the physiological/behavioral indicators of infant pain will require unique processing algorithms. We will record pain signals using facial electromyography (EMG), electrocardiography (ECG), electrodermal activity (skin conductance), oxygen saturation ($SpO2$), and electroencephalography (EEG) in real-time. Dedicated algorithms for each sensor will extract pain-related information such as facial grimacing or heart rate variability. The reliability and validity of these algorithms will be tested prospectively on data from preterm and term neonates, and 1-6 month-old infants experiencing acute pain during invasive procedures.

Secondary Objectives:

We aim to identify if multiple sensors will provide overlapping information, which a sensor fusion framework can integrate to identify “pain” and “no pain” related features. These features will be used to train machine learning algorithms that will finally provide reliable, objective assessments of pain intensity in real-time.

We will also study if the pain intensity measured by the sensor fusion framework will show clinical validity, inter-rater reliability, as well as responsiveness to pain relief using analgesic drugs or non- pharmacological therapies. However, no interventions are planned in this study.

Secondary outcomes:

We will develop a sensor fusion framework designed to integrate data from different sensor modalities. No single sensor is capable of measuring neonatal pain. Rather, skilled clinicians draw upon multiple sources of information to estimate pain. A machine learning algorithm will be developed to test if the sensor fusion framework (i) can “calibrate” itself to the unique physiology of each newborn, (ii) handle missing (e.g. sensor failure) or unreliable data (e.g. movement artifact), and (iii) determine specific features from each modality to reach asymptotic levels of sensitivity and specificity. We hypothesize that this automated sensor fusion approach will be able to estimate neonatal pain intensity with greater specificity and sensitivity than the pain scales clinically used at the bedside.

To assess the reliability and validity of the pain intensity measured by the sensor fusion framework, we will compare the objective pain measure with the pain scores assessed by skilled research staff. Variations in the objective pain scores before and after the clinical use of analgesic therapies will also be assessed to explore whether this device can also identify pain relief [22].

Sample size calculations

We assume an α-error =0.05, 1–β error =0.8, and a mean:SD ratio of 2:1 for clinical and sensor-based pain scores (based on prior studies of clinical assessments of procedural pain in infants) [23,24]. Based on these assumptions, to detect a slope that corresponds to a 0.5-unit change in the outcome (e.g., sensor pain score) per 1-unit change in the predictor (e.g., clinical pain score) we will require a minimum of 40 infants in the *training dataset*. For a binary predictor with 25% or 50% prevalence (e.g., mild vs. moderate pain, or male vs. female neonate), with this sample size, we will be able to detect a 0.7 or 0.8-unit change in the outcome, respectively. Given the number of sensors used for the first step of this study, some artifacts may occur requiring data corrections or greater variability, with a need for recruiting more patients. We plan to recruit 60 patients in this study.

Interventions/Experimental design

After applying inclusion/exclusion criteria and parental consent, infants' medical data will be recorded including: date of birth, perinatal/medical history, birth weight, gestational age, Apgar scores, congenital anomalies, metabolic defects, other diagnoses, previous surgeries, recent labs, prior imaging, major physical findings, number and types of painful procedures, and all medications used in the 24 hours immediately preceding the study. We will document the type of procedure, time of day, its location and duration, number of attempts and behavioral state of the infant before and after the procedure.

Prior to a clinically-indicated procedure such as a heel stick, subcutaneous or intramuscular injections (vaccine, drug shot), we will attach skin conductance (SC) leads to measure galvanic skin responses either on a hand palm or foot sole, and electromyography (EMG) to record facial muscle activity from cheek and forehead, and directly obtain recordings from clinical monitors (electrocardiography (ECG) and oxygen saturation (SpO2)). In addition, the infant will wear a cap with Electroencephalography (EEG) leads. For study procedures, research staff will clinically assess pain using the Neonatal Pain & Sedation Scale (N-PASS) before and after the procedure, the Premature Infant Pain Profile-Revised (PIPP-R) and the Neonatal Facial Coding System (NFCS) during the procedure. For older patients, the FLACC (Face, Legs, Activity, Cry, Consolability) scale or Visual Analogue Scale (VAS) will be scored to assess pain during the procedure [25,26][27][28]. Physiological and audio recordings will start 10-30 minutes before a planned procedure and continue for up to 20-30 minutes after the procedure.

All sensors will be monitored and displayed on the same laptop. We will use the Brain Vision software to display and record EEG, EMG and skin conductance responses and the MediCollector software for ECG and SpO2. The recording time of the 2 softwares will be synchronised based on the laptop digital clock. The event marker will be triggered by the researcher using a dedicated function of the Brain Vision software to time-lock and record times of noxious and non-noxious events for all sensors (Brain Vision and MediCollector).

At the completion of recording, all the above sensors will be disconnected from each patient and study procedures will be terminated. The entire study will last approximately 30-60 minutes; however the study may be stopped earlier if any infant shows signs of distress or if a bedside nurse or parent has any concerns.

Data analysis

A. Signal-Filtering and Information Extraction from Sensors

For each sensing modality, we will develop a software algorithm to process signals recorded by sensors and extract the clinically relevant information related to pain. Proof-of-concept algorithms will be tested using the collected dataset. The feasibility of each sensing modality will be based on: *i*) sensitivity and specificity of detecting clinically relevant pain-related changes, and *ii*) robustness of sensor readings in the presence of non-pain related disturbances (e.g., movement artifact).

In order to address artifacts due to movement or suboptimal electrode-skin contact, we will initially use filtering techniques (e.g., to remove power line interference). In addition, we will identify channels exhibiting artifacts by considering the range of signal values, where signals showing extreme deviation from average values or channels showing virtually zero activity will be excluded. Specifically for EEG analysis, we will identify and remove EMG-related artifacts using well-established techniques such as filtering and independent component analysis.

1. Pain Behaviors: Newborn behaviors (e.g., facial expressions, body movements) are accepted as the most sensitive and valid indicators of pain [29][13]. Facial expressions like brow bulge, eye squeeze, nasolabial furrow, and horizontal mouth stretch were verified as the most valid and discriminative components of neonatal pain scales [30,31][32]. Using facial EMG in real-time, we will detect the presence of muscle activity in neonatal pain-associated facial movements [33]. Given the multiple overlapping layers of facial muscles, facial EMGs record signals from a facial region as opposed to any specific muscle [33]. Previous studies of startle and blinking in infants used miniature silver (Ag/AgCl) periorbital surface electrodes for recording EMGs [34–36]. We will focus on infant forehead and cheek areas to detect EMG activity associated with brow bulge, eye squeeze and nasolabial furrow [33]. We will iteratively refine our algorithms by using multi-modality sensing and developing robust feature extraction and classification frameworks that address the challenges specific to neonatal/infant pain detection. We will exclude mechanically ventilated infants due to the challenges associated with identifying facial features (occluded by securing tape, ventilator tubing or devices) and their need for ongoing sedation/analgesia.

2. Skin Conductance: Acute pain stimulates the sympathetic post-ganglionic cholinergic neurons [37], leading to diaphoresis, palmar sweating and increased skin conductance [38]. Eliminating painful stimuli results in sweat reabsorption and decreased conductivity. The amplitude of changes in palmar skin conductance reflect increased sympathetic nervous system activity, which tracks with pain intensity [39–42]. Skin conductance can change with body temperature [43,44], but not with the ambient temperatures [44]. Specifically, the number of fluctuations of skin conductance per second (NFSC) was correlated with pain intensity in children [45], and was more sensitive that pain scores in preterm and term neonates [18,40–42,46]. We will use skin conductance using the BrainAmp system® (Brain Products GmbH, Gilching, Germany),

3. Electrocardiography (ECG): Heart rate (HR) changes are components of many pain assessment scales and recent studies have established correlations between HR variability (HRV) and pain [47,48]. A number of linear time-domain (HR mean, standard deviation) and frequency-domain (power spectral density) metrics and non-linear metrics (sample entropy, approximate entropy, etc.) can detect painful stimuli [47,48]. We will record the infant's ECG before, during, and after an acute pain event to extract the linear and nonlinear metrics (listed above) from the ECG signal for further analyses.

4. Electroencephalography (EEG): EEG studies to assess neonatal pain have met with variable results [49–51]. Opioid analgesia in adults leads to slowing of the EEG, whereas painful stimuli activate brain regions identified by neuroimaging studies [52] such as the primary sensory cortex (S1) [53]. EEG amplitudes and frequencies decreased when analgesics were given to newborns in pain [20,54–56]. Using the BrainAmp EEG system® (Brain Products GmbH, Gilching, Germany), we will apply 32 active EEG electrodes using the infant-sized ActiCap® (Brain Products GmbH, Gilching, Germany). Although Hartley et al. have selectively used the vertex (Cz) lead for neonatal pain studies [20,54], we believe that infant pain processing is widely distributed across many brain regions and the current evidence is not sufficiently strong enough to exclude information from other EEG leads. The BrainAmp is similar to other EEG monitors, however, it uses 32 active electrodes allowing for placement of the ActiCap on the infant's head with minimal preparation. Each active electrode amplifies the signal recorded from the skin and records also indicates the impedance of each electrode at the start of the recording to improve the quality of recorded signals. Over the past 10 years, this device has been used for research purposes in all age groups including infants and newborns. No side effects were reported from its use in newborns and small infants.

5. Pulse Oximetry (SpO2): Changes in SpO_2 occur frequently following acute pain and, therefore, have been included in the Premature Infant Pain Profile (PIPP) and other pain scales [57–59]. Newer generation monitors (Masimo, Irvine, CA) use multi-wavelength technology to provide more reliable SpO2 and pulse rate signals, with parallel signal processing engines and adaptive filters to separate the arterial from venous signals, patient motion, or skin perfusion [60]. Changes in skin blood flow were also used as physiological markers for neonatal pain or morphine analgesia [61,62]. We will test the utility of the SpO2 and peripheral perfusion index provided by pulse oximetry monitors as possible signals for neonatal pain.

Statistical approach

A. Sensor Fusion

We have previously investigated using machine learning to detect pain in neonates using facial expressions recorded by a camera [63]. We will develop a sensor fusion framework to detect pain in non-verbal infants based on machine learning to detect pain using multi-modal sensor data. Feasibility of this new framework will be assessed based on its sensitivity and specificity to detect pain events in infants and further refined into a prototype for validation in future studies.

A "calibration" period will be used to establish a baseline for these multiple sensor modalities by monitoring neonates who are not in pain. The clinical staff at the bedside will identify the pain state of each neonate/infant using validated pain scales (N-PASS, NFCS and PIPP-R; FLACC, VAS) and record the timing of pain-inducing clinically-indicated procedures. Our sensor fusion framework will classify the neonatal/infant responses to infer pain intensity based on observed changes from baseline. A probabilistic relationship between pain intensity and sensor measurements can be established, where the unknown parameters of the statistical relationships are identified by a training dataset. The training dataset will also be used to estimate the importance of each feature, which can then be used to identify the optimal set of sensors [61].

Pain intensity scores computed by our sensor fusion framework will be compared with pain scores measured concurrently by skilled research staff. All sensors (facial EMG, EEG, ECG, SpO2, and SC) will be connected with an event recorder to mark "pain" vs. "no-pain" states. To make the best use of our data, the sensor fusion framework will use standard cross-validation methods to establish the generalizability of this framework.

Data from patients will be divided to a training set and a test set. The training set is used for model training and optimization of model parameters. A leave-one-patient-out cross validation technique will be used, where the machine learning classifier is trained on data from all but one patient and the performance of the classifier is assessed on the remaining patient. Once the appropriate machine learning classifier and its associated parameters are selected using the training set and the associated cross validation procedure, the performance of the machine learning classifier will be assessed on the test set.

B. Validation and Correlation with Pain Intensity

We will compare clinical pain scores from nursing assessments with scores from the sensor fusion framework. First, we will examine clinical pain scores to verify agreement with the pain scores assessed by the research staff. Internal consistency will be evaluated by Cronbach's α, with values > 0.8 to show good internal consistency. Second, we will conduct multivariable linear regressions or generalized estimating equations (GEE) [64,65] to understand the agreement between the device pain scores and the clinical pain scores, as well as the contribution of each modality to the device pain scores. We will examine if these associations vary after adjustment for covariates such as pain medications, age, sex, duration or invasiveness of the procedure. A co-variance matrix will examine the degree of correlation between individual sensor inputs, types of procedures, clinician pain scores, and analgesic therapies used during the procedure. Finally, due to the limited understanding of factors contributing to pain in newborns, linear regression or GEE models will examine the association of the sensor fusion pain scores reported by the device with the demographic and clinical variables of neonates and infants.

Content validity depends on the sensors and sensor variables that we have chosen for the sensor fusion framework. Concurrent validity will depend on the pain scores of skilled research staff using validated pain scoring methods. Construct validity will rest on: *i*) the range of objective pain scores from procedures causing mild, moderate, or severe pain; *ii*) changes in pain scores with analgesic drugs or non-pharmacological therapies; and *iii*) variation in pain scores over time consistent with the expected, natural course of acute procedural pain.

ETHICS AND DISSEMINATION

The Institutional Review Board of the Stanford University approved the protocol. This observational study is registered at www.ClinicalTrials.gov (NCT03330496) and it does not involve any intervention other than those clinically required. Data collection includes physiological recordings and medical information. Other than the facial EMG, skin conductance leads (SC), and 32-channel BrainAmp EEG data acquisition system, all other sensors are used routinely as the standard of care. All sensors are non-invasive and safe. We will use standard electrodes which are routinely used for recording vital signs in the hospital (ECG, SpO2). All recordings will use sticky pads attached to the skin. Other than a potential for mild skin irritation from the adhesives used, there are no significant risks associated with these devices, or other study-related procedures.

Since all study subjects are aged less than 6 months, the parent's consent will be obtained for their child as a research subject. The primary risks to study subjects result from potential loss of confidentiality from the information collected and from the medical record and monitoring devices. As described in the consent form, the right to privacy during the consent process, data collection and study procedures, and protection of personal data will be given the utmost importance and strict safeguards will be maintained to protect data confidentiality. All physiological data will be deidentified by the team at Stanford prior to transfer for further analysis by the team at Autonomous Healthcare.

Study findings will be published in peer-reviewed journals and presented at national and international scientific conferences. Practical use of this methodology will be taught at conference workshops, or via webinars, podcasts, video tutorials.

Future studies will test the validity of this approach to pain assessments in larger populations of newborns, older infants and also extend these studies to smaller preterm neonates. Future applications may also include patient populations incapable of expressing pain (children with disability, adults with dementia, or mechanically ventilated patients).

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Using sensor-fusion and machine-learning algorithms to assess acute pain in nonverbal infants: a study protocol

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Introduction:

Objective pain assessment in non-verbal populations is clinically challenging due to their inability to express their pain via self-report. Repetitive exposures to acute or prolonged pain lead to clinical instability, with long-term behavioral and cognitive sequelae in newborn infants. Strong analgesics are also associated with medical complications, potential neurotoxicity and altered brain development. Pain scores performed by bedside nurses provide subjective, observer-dependent assessments rather than objective data for infant pain management; the required observations are labour-intensive, difficult to perform by a nurse who is concurrently performing the procedure, and increase the nursing workload. Multimodal pain assessment, using sensor fusion and machine learning algorithms, can provide a patient-centered, context-dependent, observer-independent, and objective pain measure.

Methods and analysis:

In newborns undergoing painful procedures, we use facial electromyography (EMG) to record facial muscle activity related infant pain, electrocardiography (ECG) to examine heart rate (HR) changes and HR variability (HRV), electrodermal activity (skin conductance) to measure catecholamine-induced palmar sweating, changes in oxygen saturations and skin perfusion, and electroencephalography (EEG) using active electrodes to assess brain activity in real-time. This multimodal approach has the potential to improve the accuracy of pain assessment in non-verbal infants and may even allow continuous pain monitoring at the bedside. The feasibility of this approach will be evaluated in an observational prospective study of clinically required painful procedures in 60 preterm and term newborns, and infants aged 6 months or less.

Ethics and dissemination:

The Institutional Review Board of the Stanford University approved the protocol. Study findings will be published in peer-reviewed journals, presented at scientific meetings, taught via webinars, podcasts, video tutorials, and listed on academic/scientific websites. Future studies will validate and refine this approach using the minimum number of sensors required to assess neonatal/infant pain.

Registration number: ClinicalTrials.gov Identifier: NCT03330496

An Article Summary:

‘Strengths and limitations of this study’

- An innovative and objective approach for continuous pain monitoring in infants including term and preterm neonates is described.
- To discriminate between noxious and non-noxious events, we use sensor fusion and machine-learning algorithms.
- Multimodal assessments may be more sensitive and specific for identifying pain and quantifying its intensity than the subjective assessments currently used in pain scales.
- Accurate, objective pain assessments may help reduce infant pain and suffering, enhance recovery, avoid untreated pain vs. analgesic overuse, and allow evaluation of newer analgesics or other therapies in randomized clinical trials.
- Multiple sensors are used for the first step of this study, but recording artifacts may require data corrections and sensor variability may generate a need for recruiting more patients.

INTRODUCTION

Being non-verbal, hospitalized infants are particularly vulnerable to inadequate pain management. Repetitive pain in newborns leads to short- and long-term neurodevelopmental consequences including behavioral and cognitive sequelae [1][2–5]. Conversely, the safety and efficacy of some analgesics in neonates and their negative consequences on the neonatal brain have raised concerns [6][7][8][9]. Objectively assessing the pain responses in infants is thus necessary to assess the efficacy of analgesics in infants in order to avoid over treatment but also undertreatment and the consequences of repetitive pain exposure.

Composite pain scales including behavioral and physiological measures are the most widely used surrogate measures of infant pain and are currently recommended for the clinical practice [10]. However, they provide a one-time measurement and their use can be challenging for the bedside staff leading to low interrater reliability, with over- or underestimation of infant pain [11][12]. Depending on the context, behaviors or physiological responses may mirror non-noxious stimuli, leading to misinterpretation and a lack of specificity in subjective pain scales [13][14][15].

Pain from clinically required invasive procedures leads to well-described neurophysiological responses in term and preterm infants [15]. These responses imply that the central, peripheral, and autonomic nervous systems can be monitored using various behavioral and physiologic modalities [16][17,18][19]. Since pain is a complex process, multimodal measurement may improve the accuracy of pain assessment, also suggested by studies reporting the presence of pain-evoked potentials in some neonates showing no facial expressions of pain or others pointing out different profiles of pain responses [13][20].

Thus, developing new methods to assess the responses to pain in infants deserves a high priority. Multimodal measurements that provide an objective estimate of real-time and continuous pain

monitoring at the bedside will avoid the subjective bias and limitations associated with clinical pain scales, especially when behavioral assessment is limited by the medical conditions [21]. Our study protocol was designed to develop a multimodal pain assessment system, using sensor fusion and novel machine learning algorithms to provide an objective estimate of acute pain intensity in infants that is patient-centered, context-dependent, and observer-independent.

METHODS AND ANALYSIS

Study design

We designed a prospective observational study enrolling subjects from Lucile Packard Children's Hospital at Stanford. The study started on October 30th, 2017 and will be completed on November 30th, 2025 (www.ClinicalTrials.gov, NCT03330496).

Study population

We will collect data from 15 study subjects in each of the following age groups: 1) Preterm infants (34-37 weeks of corrected gestational age, postnatal age 3-30 days); 2) Term newborns (37-42 weeks of corrected gestational age, less than 1 month of age); 3) Infants from 1-3 months age; 4) Older infants from 3-6 months age.

Eligibility criteria

After obtaining parental consent, we will include all infants less than 6 months of corrected chronological age requiring an acute painful procedure for routine clinical care in Lucile Packard Children's Hospital.

We will exclude newborns with birth trauma, intrapartum asphyxia (5-minute Apgar Score <4 or cord pH < 7.01), fetal growth restriction (birth weight < 5th percentile for gestation), congenital

anomalies or metabolic disorders, or any kind of brain injury; if their mothers had a history of heavy smoking or drug abuse (alcohol, cocaine, ketamine, and heroin/other opiates) or psychiatric drugs used during this pregnancy; infants requiring positive pressure ventilation using a face mask (BiPAP) or endotracheal tube; those receiving continuous infusions of opioid drugs (morphine, fentanyl, and others) and nerve blocks or neuraxial analgesia affecting the site of the invasive procedure in the 24 hours prior to study entry; infants with facial anomalies (cleft lip), injuries or other pathologies affecting the facial area; and infants breastfed to alleviate pain during the painful procedure.

Objectives/Outcomes

Primary Objective:

Our primary objective is to identify the specific signals and patterns from each sensor that correlate with the pain stimulus. This pilot study is designed to exclusively assess acute pain responses during routine, clinically-required skin-breaking procedures – it measures the intensity of acute pain from the physiological responses of each subject.

Primary Outcome:

We will extract pain-related information using non-invasive multimodal sensors. Specific features of the physiological/behavioral indicators of infant pain will require unique processing algorithms. We will record pain signals using facial electromyography (EMG), electrocardiography (ECG), electrodermal activity (skin conductance), oxygen saturation (SpO_2), and electroencephalography (EEG) in real-time. Dedicated algorithms for each sensor will extract pain-related information such as facial grimacing or heart rate variability. The reliability and validity of these algorithms will be tested prospectively on data from preterm and term neonates, and 1-6 month-old infants experiencing acute pain during invasive procedures.

Secondary Objectives:

We aim to identify if multiple sensors will provide overlapping information, which a sensor fusion framework can integrate to identify “pain” and “no pain” related features. These features will be used to train machine learning algorithms that will finally provide reliable, objective assessments of pain intensity in real-time.

We will also study if the pain intensity measured by the sensor fusion framework will show clinical validity, inter-rater reliability, as well as responsiveness to pain relief using analgesic drugs or non- pharmacological therapies. However, no interventions are planned in this study.

Secondary outcomes:

We will develop a sensor fusion framework designed to integrate data from different sensor modalities. No single sensor is capable of measuring neonatal pain. Rather, skilled clinicians draw upon multiple sources of information to estimate pain. A machine learning algorithm will be developed to test if the sensor fusion framework (i) can “calibrate” itself to the unique physiology of each newborn, (ii) handle missing (e.g. sensor failure) or unreliable data (e.g. movement artifact), and (iii) determine specific features from each modality to reach asymptotic levels of sensitivity and specificity. We hypothesize that this automated sensor fusion approach will be able to estimate neonatal pain intensity with greater specificity and sensitivity than the pain scales clinically used at the bedside.

To assess the reliability and validity of the pain intensity measured by the sensor fusion framework, we will compare the objective pain measure with the pain scores assessed by skilled research staff. Variations in the objective pain scores before and after the clinical use of analgesic therapies will also be assessed to explore whether this device can also identify pain relief [22].

Sample size calculations

We assume an α-error =0.05, 1–β error =0.8, and a mean:SD ratio of 2:1 for clinical and sensor-based pain scores (based on prior studies of clinical assessments of procedural pain in infants) [23,24]. Based on these assumptions, to detect a slope that corresponds to a 0.5-unit change in the outcome (e.g., sensor pain score) per 1-unit change in the predictor (e.g., clinical pain score) we will require a minimum of 40 infants in the *training dataset*. For a binary predictor with 25% or 50% prevalence (e.g., mild vs. moderate pain, or male vs. female neonate), with this sample size, we will be able to detect a 0.7 or 0.8-unit change in the outcome, respectively. Given the number of sensors used for the first step of this study, some artifacts may occur requiring data corrections or greater variability, with a need for recruiting more patients. We plan to recruit 60 patients in this study.

Interventions/Experimental design

After applying inclusion/exclusion criteria and parental consent, infants' medical data will be recorded including: date of birth, perinatal/medical history, birth weight, gestational age, Apgar scores, congenital anomalies, metabolic defects, other diagnoses, previous surgeries, recent labs, prior imaging, major physical findings, number and types of painful procedures, and all medications used in the 24 hours immediately preceding the study. We will document the type of procedure, time of day, its location and duration, number of attempts and behavioral state of the infant before and after the procedure.

Prior to a clinically-indicated procedure such as a heel stick, subcutaneous or intramuscular injections (vaccine, drug shot), we will attach skin conductance (SC) leads to measure galvanic skin responses either on a hand palm or foot sole, and electromyography (EMG) to record facial muscle activity from cheek and forehead, and directly obtain recordings from clinical monitors (electrocardiography (ECG) and oxygen saturation (SpO2)). In addition, the infant will wear a cap with Electroencephalography (EEG) leads. For study procedures, research staff will clinically assess pain using the Neonatal Pain & Sedation Scale (N-PASS) before and after the procedure, the Premature Infant Pain Profile-Revised (PIPP-R) and the Neonatal Facial Coding System (NFCS) during the procedure. For older patients, the FLACC (Face, Legs, Activity, Cry, Consolability) scale or Visual Analogue Scale (VAS) will be scored to assess pain during the procedure [25,26][27][28]. Physiological and audio recordings will start 10-30 minutes before a planned procedure and continue for up to 20-30 minutes after the procedure.

All sensors will be monitored and displayed on the same laptop. We will use the Brain Vision software to display and record EEG, EMG and skin conductance responses and the MediCollector software for ECG and SpO2. The recording time of the 2 softwares will be synchronised based on the laptop digital clock. The event marker will be triggered by the researcher using a dedicated function of the Brain Vision software to time-lock and record times of noxious and non-noxious events for all sensors (Brain Vision and MediCollector).

At the completion of recording, all the above sensors will be disconnected from each patient and study procedures will be terminated. The entire study will last approximately 30-60 minutes; however the study may be stopped earlier if any infant shows signs of distress or if a bedside nurse or parent has any concerns.

Data analysis

A. Signal-Filtering and Information Extraction from Sensors

For each sensing modality, we will develop a software algorithm to process signals recorded by sensors and extract the clinically relevant information related to pain. Proof-of-concept algorithms will be tested using the collected dataset. The feasibility of each sensing modality will be based on: *i*) sensitivity and specificity of detecting clinically relevant pain-related changes, and *ii*) robustness of sensor readings in the presence of non-pain related disturbances (e.g., movement artifact).

In order to address artifacts due to movement or suboptimal electrode-skin contact, we will initially use filtering techniques (e.g., to remove power line interference). In addition, we will identify channels exhibiting artifacts by considering the range of signal values, where signals showing extreme deviation from average values or channels showing virtually zero activity will be excluded. Specifically for EEG analysis, we will identify and remove EMG-related artifacts using well-established techniques such as filtering and independent component analysis.

Windows of different lengths will be used for the analysis. Specifically, for ECG, SpO2, EMG, and skin conductance signals an analysis window of 1-5 minutes will be used to extract appropriate features. The window lengths ranging from 400ms to 5 seconds will be used for EEG signals.

1. Pain Behaviors: Newborn behaviors (e.g., facial expressions, body movements) are accepted as the most sensitive and valid indicators of pain [29][13]. Facial expressions like brow bulge, eye squeeze, nasolabial furrow, and horizontal mouth stretch were verified as the most valid and discriminative components of neonatal pain scales [30,31][32]. Using facial EMG in real-time, we will detect the presence of muscle activity in neonatal pain-associated facial movements [33]. Given the multiple overlapping layers of facial muscles, facial EMGs record signals from a facial region as opposed to any specific muscle [33]. Previous studies of startle and blinking in infants used miniature silver (Ag/AgCl) periorbital surface electrodes for recording EMGs [34–36]. We will focus on infant forehead and cheek areas to detect EMG activity associated with brow bulge, eye squeeze and nasolabial furrow [33]. We will iteratively refine our algorithms by using multi-modality sensing and developing robust feature extraction and classification frameworks that address the challenges specific to neonatal/infant pain detection. We will exclude mechanically ventilated infants due to the challenges associated with identifying facial features (occluded by securing tape, ventilator tubing or devices) and their need for ongoing sedation/analgesia.

2. Skin Conductance: Acute pain stimulates the sympathetic post-ganglionic cholinergic neurons [37], leading to diaphoresis, palmar sweating and increased skin conductance [38]. Eliminating painful stimuli results in sweat reabsorption and decreased conductivity. The amplitude of changes in palmar skin conductance reflect increased sympathetic nervous system activity, which tracks with pain intensity [39–42]. Skin conductance can change with body temperature [43,44], but not with the ambient temperatures [44]. Specifically, the number of fluctuations of skin conductance per second (NFSC) was correlated with pain intensity in children [45], and was more sensitive that pain scores in preterm and term neonates [18,40–42,46]. We will use skin conductance using the BrainAmp system® (Brain Products GmbH, Gilching, Germany),

3. Electrocardiography (ECG): Heart rate (HR) changes are components of many pain assessment scales and recent studies have established correlations between HR variability (HRV) and pain [47,48]. A number of linear time-domain (HR mean, standard deviation) and frequency-domain (power spectral density) metrics and non-linear metrics (sample entropy, approximate entropy, etc.) can detect painful stimuli [47,48]. We will record the infant's ECG before, during, and after an acute pain event to extract the linear and nonlinear metrics (listed above) from the ECG signal for further analyses.

4. Electroencephalography (EEG): EEG studies to assess neonatal pain have met with variable results [49–51]. Opioid analgesia in adults leads to slowing of the EEG, whereas painful stimuli activate brain regions identified by neuroimaging studies [52] such as the primary sensory cortex (S1) [53]. EEG amplitudes and frequencies decreased when analgesics were given to newborns in pain [20,54–56]. Using the BrainAmp EEG system® (Brain Products GmbH, Gilching, Germany), we will apply 32 active EEG electrodes using the infant-sized ActiCap® (Brain Products GmbH, Gilching, Germany). Although Hartley et al. have selectively used the vertex (Cz) lead for neonatal pain studies [20,54], we believe that infant pain processing is widely distributed across many brain regions and the current evidence is not sufficiently strong enough to exclude information from other EEG leads. The BrainAmp is similar to other EEG monitors, however, it uses 32 active electrodes allowing for placement of the ActiCap on the infant's head with minimal preparation. Each active electrode amplifies the signal recorded from the skin and records also indicates the impedance of each electrode at the start of the recording to improve the quality of recorded signals. Over the past 10 years, this device has been used for research purposes in all age groups including infants and newborns. No side effects were reported from its use in newborns and small infants.

In order to analyze EEG signals and extract appropriate features, we will first remove noise and artifacts using standard techniques such as independent component analysis (ICA) and wavelet denoising [57]. After artifacts are removed, we will investigate the correlation between features extracted from EEG data and pain. Specifically, we will use spectral decomposition and extract features such as mean power in different frequency bands (delta, theta, alpha, and beta) as well as asymmetry measures for each homologous pair and functional connectivity measures for further investigation.

5. Pulse Oximetry (SpO2): Changes in SpO_2 occur frequently following acute pain and, therefore, have been included in the Premature Infant Pain Profile (PIPP) and other pain scales [58–60]. Newer generation monitors (Masimo, Irvine, CA) use multi-wavelength technology to provide more reliable SpO2 and pulse rate signals, with parallel signal processing engines and adaptive filters to separate the arterial from venous signals, patient motion, or skin perfusion [61]. Changes in skin blood flow were also used as physiological markers for neonatal pain or morphine analgesia [62,63]. We will test the utility of the SpO2 and peripheral perfusion index provided by pulse oximetry monitors as possible signals for neonatal pain.

Statistical approach

A. Sensor Fusion

We have previously investigated using machine learning to detect pain in neonates using facial expressions recorded by a camera [64]. We will develop a sensor fusion framework to detect pain in non-verbal infants based on machine learning to detect pain using multi-modal sensor data.

Feasibility of this new framework will be assessed based on its sensitivity and specificity to detect pain events in infants and further refined into a prototype for validation in future studies.

A "calibration" period will be used to establish a baseline for these multiple sensor modalities by monitoring neonates who are not in pain. The clinical staff at the bedside will identify the pain state of each neonate/infant using validated pain scales (N-PASS, NFCS and PIPP-R; FLACC, VAS) and record the timing of pain-inducing clinically-indicated procedures. Our sensor fusion framework will classify the neonatal/infant responses to infer pain intensity based on observed changes from baseline. A probabilistic relationship between pain intensity and sensor measurements can be established, where the unknown parameters of the statistical relationships are identified by a training dataset. The training dataset will also be used to estimate the importance of each feature, which can then be used to identify the optimal set of sensors [62].

Gestational age, postnatal age, and days of life and in hospital will be taken into account in the statistical analyses. We will initially focus on recruiting term neonates who are studied within 1 week after birth and have minimal exposures to prior painful events. This will increase the homogeneity of our sample and minimize the variability in physiological responses due to gestational age, postnatal age, days in the hospital, and long-lasting effects of previous painful experiences.

Interventions to manage pain will be allowed including non pharmacological and pharmacological treatments apart from continuous infusions of opioid drugs. This will be considered in the statistical analysis.

Pain intensity scores computed by our sensor fusion framework will be compared with pain scores measured concurrently by skilled research staff. All sensors (facial EMG, EEG, ECG, SpO_2, and SC) will be connected with an event recorder to mark “pain” vs. “no-pain” states. To make the best use of our data, the sensor fusion framework will use standard cross-validation methods to establish the generalizability of this framework.

The extracted features from each modality will be used to train a machine learning algorithm. Specifically, we will train a binary classifier to assign “pain” and “no pain” class labels based on the extracted features. We will specifically investigate using the random forests classifier given their robustness to outliers and its classification performance when a large number of features are used for classification.

Data from patients will be divided to a training set and a test set. The training set is used for model training and optimization of model parameters. A leave-one-patient-out cross validation technique will be used, where the machine learning classifier is trained on data from all but one patient and the performance of the classifier is assessed on the remaining patient. Once the appropriate machine learning classifier and its associated parameters are selected using the training set and the associated cross validation procedure, the performance of the machine learning classifier will be assessed on the test set.

B. Validation and Correlation with Pain Intensity

We will compare clinical pain scores from nursing assessments with scores from the sensor fusion framework. First, we will examine clinical pain scores to verify agreement with the pain scores assessed by the research staff. Internal consistency will be evaluated by Cronbach's α, with values > 0.8 to show good internal consistency. Second, we will conduct multivariable linear regressions or generalized estimating equations (GEE) [65,66] to understand the agreement between the device pain scores and the clinical pain scores, as well as the contribution of each modality to the device pain scores. We will examine if these associations vary after adjustment for covariates such as pain medications, age, sex, duration or invasiveness of the procedure. A co-variance matrix will examine the degree of correlation between individual sensor inputs, types of procedures, clinician pain scores, and analgesic therapies used during the procedure. Finally, due to the limited understanding of factors contributing to pain in newborns, linear regression or GEE models will examine the association of the sensor fusion pain scores reported by the device with the demographic and clinical variables of neonates and infants.

Content validity depends on the sensors and sensor variables that we have chosen for the sensor fusion framework. Concurrent validity will depend on the pain scores of skilled research staff using validated pain scoring methods. Construct validity will rest on: *i*) the range of objective pain scores from procedures causing mild, moderate, or severe pain; *ii*) changes in pain scores with analgesic drugs or non-pharmacological therapies; and *iii*) variation in pain scores over time consistent with the expected, natural course of acute procedural pain.

We will also develop a machine learning algorithm to predict subjective pain. As part of the validation, we will evaluate the machine learning pain assessment algorithm which has been trained on clinical classification of pain based on validated pain scales and compare the results with the results provided by the machine learning pain assessment algorithm which has been trained on data involving objective pain events (e.g., heel stick).

ETHICS AND DISSEMINATION

The Institutional Review Board of the Stanford University approved the protocol. This observational study is registered at www.ClinicalTrials.gov (NCT03330496) and it does not involve any intervention other than those clinically required. Data collection includes physiological recordings and medical information. Other than the facial EMG, skin conductance leads (SC), and 32-channel BrainAmp EEG data acquisition system, all other sensors are used routinely as the standard of care. All sensors are non-invasive and safe. We will use standard electrodes which are routinely used for recording vital signs in the hospital (ECG, SpO2). All recordings will use sticky pads attached to the skin. Other than a potential for mild skin irritation from the adhesives used, there are no significant risks associated with these devices, or other study-related procedures.

Since all study subjects are aged less than 6 months, the parent's consent will be obtained for their child as a research subject. The primary risks to study subjects result from potential loss of confidentiality from the information collected and from the medical record and monitoring devices. As described in the consent form, the right to privacy during the consent process, data collection and study procedures, and protection of personal data will be given the utmost importance and strict safeguards will be maintained to protect data confidentiality. All physiological data will be deidentified by the team at Stanford prior to transfer for further analysis by the team at Autonomous Healthcare.

Study findings will be published in peer-reviewed journals and presented at national and international scientific conferences. Practical use of this methodology will be taught at conference workshops, or via webinars, podcasts, video tutorials.

The sample size calculated for the first step of the study may represent a challenge for machine learning by limiting samples sizes for the training and testing datasets. Therefore, the results provided by our analyses will be confirmed in larger sample sizes within the next steps of the study.

Future studies will test the validity of this approach to pain assessments in larger populations of newborns, older infants and also extend these studies to smaller preterm neonates. Future applications may also include patient populations incapable of expressing pain (children with disability, adults with dementia, or mechanically ventilated patients).

Authors' contributions: JMR, KJS and WMH, BG were responsible for manuscript writing. JMR, KJS, IM, WMH and BG contributed to the concept, protocol development and study design. KJS and BG secured funding for the project. JMR, IM and KJS are responsible for recruitment of study patients. All authors critically revised and approved the manuscript before submission and are accountable for all aspects of the work.

Competing interests statement: JMR reports an international mobility scholarship from Chiesi Pharmaceuticals; WMH and BG have equity ownership in Autonomous Healthcare, Inc.; WMH, BG and KJSA have proprietary interests in the potential devices that may be developed from these studies; Some equipments used in this study were provided by Autonomous Healthcare, Inc.

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Using sensor-fusion and machine-learning algorithms to assess acute pain in nonverbal infants: a study protocol

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ABSTRACT 288 words out of 300

Introduction:

Objective pain assessment in non-verbal populations is clinically challenging due to their inability to express their pain via self-report. Repetitive exposures to acute or prolonged pain lead to clinical instability, with long-term behavioral and cognitive sequelae in newborn infants. Strong analgesics are also associated with medical complications, potential neurotoxicity and altered brain development. Pain scores performed by bedside nurses provide subjective, observer-dependent assessments rather than objective data for infant pain management; the required observations are labour-intensive, difficult to perform by a nurse who is concurrently performing the procedure, and increase the nursing workload. Multimodal pain assessment, using sensor fusion and machine learning algorithms, can provide a patient-centered, context-dependent, observer-independent, and objective pain measure.

Methods and analysis:

In newborns undergoing painful procedures, we use facial electromyography (EMG) to record facial muscle activity related infant pain, electrocardiography (ECG) to examine heart rate (HR) changes and HR variability (HRV), electrodermal activity (skin conductance) to measure catecholamine-induced palmar sweating, changes in oxygen saturations and skin perfusion, and electroencephalography (EEG) using active electrodes to assess brain activity in real-time. This multimodal approach has the potential to improve the accuracy of pain assessment in non-verbal infants and may even allow continuous pain monitoring at the bedside. The feasibility of this approach will be evaluated in an observational prospective study of clinically required painful procedures in 60 preterm and term newborns, and infants aged 6 months or less.

Ethics and dissemination:

The Institutional Review Board of the Stanford University approved the protocol. Study findings will be published in peer-reviewed journals, presented at scientific meetings, taught via webinars, podcasts, video tutorials, and listed on academic/scientific websites. Future studies will validate and refine this approach using the minimum number of sensors required to assess neonatal/infant pain.

Registration number: ClinicalTrials.gov Identifier: NCT03330496

An Article Summary:

‘Strengths and limitations of this study’

- An innovative and objective approach for continuous pain monitoring in infants including term and preterm neonates is described.
- To discriminate between noxious and non-noxious events, we use sensor fusion and machine-learning algorithms.
- Multimodal assessments may be more sensitive and specific for identifying pain and quantifying its intensity than the subjective assessments currently used in pain scales.
- Accurate, objective pain assessments may help reduce infant pain and suffering, enhance recovery, avoid untreated pain vs. analgesic overuse, and allow evaluation of newer analgesics or other therapies in randomized clinical trials.
- Multiple sensors are used for the first step of this study, but recording artifacts may require data corrections and sensor variability may generate a need for recruiting more patients.

INTRODUCTION

Being non-verbal, hospitalized infants are particularly vulnerable to inadequate pain management. Repetitive pain in newborns leads to short- and long-term neurodevelopmental consequences including behavioral and cognitive sequelae [1][2–5]. Conversely, the safety and efficacy of some analgesics in neonates and their negative consequences on the neonatal brain have raised concerns [6][7][8][9]. Objectively assessing the pain responses in infants is thus necessary to assess the efficacy of analgesics in infants in order to avoid over treatment but also undertreatment and the consequences of repetitive pain exposure.

Composite pain scales including behavioral and physiological measures are the most widely used surrogate measures of infant pain and are currently recommended for the clinical practice [10]. However, they provide a one-time measurement and their use can be challenging for the bedside staff leading to low interrater reliability, with over- or underestimation of infant pain [11][12]. Depending on the context, behaviors or physiological responses may mirror non-noxious stimuli, leading to misinterpretation and a lack of specificity in subjective pain scales [13][14][15].

Pain from clinically required invasive procedures leads to well-described neurophysiological responses in term and preterm infants [15]. These responses imply that the central, peripheral, and autonomic nervous systems can be monitored using various behavioral and physiologic modalities [16][17,18][19]. Since pain is a complex process, multimodal measurement may improve the accuracy of pain assessment, also suggested by studies reporting the presence of pain-evoked potentials in some neonates showing no facial expressions of pain or others pointing out different profiles of pain responses [13][20].

Thus, developing new methods to assess the responses to pain in infants deserves a high priority. Multimodal measurements that provide an objective estimate of real-time and continuous pain

monitoring at the bedside will avoid the subjective bias and limitations associated with clinical pain scales, especially when behavioral assessment is limited by the medical conditions [21]. Our study protocol was designed to develop a multimodal pain assessment system, using sensor fusion and novel machine learning algorithms to provide an objective estimate of acute pain intensity in infants that is patient-centered, context-dependent, and observer-independent.

METHODS AND ANALYSIS

Study design

We designed a prospective observational study enrolling subjects from Lucile Packard Children's Hospital at Stanford. The study is registered at www.ClinicalTrials.gov (NCT03330496) and it does not involve any intervention other than those clinically required. The study started on October 30th, 2017 and will be completed on November 30th, 2025.

Study population

We will collect data from 15 study subjects in each of the following age groups: 1) Preterm infants (34-37 weeks of corrected gestational age, postnatal age 3-30 days); 2) Term newborns (37-42 weeks of corrected gestational age, less than 1 month of age); 3) Infants from 1-3 months age; 4) Older infants from 3-6 months age.

Eligibility criteria

After obtaining parental consent, we will include all infants less than 6 months of corrected chronological age requiring an acute painful procedure for routine clinical care in Lucile Packard Children's Hospital.

We will exclude newborns with birth trauma, intrapartum asphyxia (5-minute Apgar Score <4 or cord pH < 7.01), fetal growth restriction (birth weight < 5th percentile for gestation), congenital

anomalies or metabolic disorders, or any kind of brain injury; if their mothers had a history of heavy smoking or drug abuse (alcohol, cocaine, ketamine, and heroin/other opiates) or psychiatric drugs used during this pregnancy; infants requiring positive pressure ventilation using a face mask (BiPAP) or endotracheal tube; those receiving continuous infusions of opioid drugs (morphine, fentanyl, and others) and nerve blocks or neuraxial analgesia affecting the site of the invasive procedure in the 24 hours prior to study entry; infants with facial anomalies (cleft lip), injuries or other pathologies affecting the facial area; and infants breastfed to alleviate pain during the painful procedure.

Objectives/Outcomes

Primary Objective:

Our primary objective is to identify the specific signals and patterns from each sensor that correlate with the pain stimulus. This pilot study is designed to exclusively assess acute pain responses during routine, clinically-required skin-breaking procedures – it measures the intensity of acute pain from the physiological responses of each subject.

Primary Outcome:

We will extract pain-related information using non-invasive multimodal sensors. Specific features of the physiological/behavioral indicators of infant pain will require unique processing algorithms. We will record pain signals using facial electromyography (EMG), electrocardiography (ECG), electrodermal activity (skin conductance), oxygen saturation ($SpO2$), and electroencephalography (EEG) in real-time. Dedicated algorithms for each sensor will extract pain-related information such as facial grimacing or heart rate variability. The reliability and validity of these algorithms will be tested prospectively on data from preterm and term neonates, and 1-6 month-old infants experiencing acute pain during invasive procedures.

Secondary Objectives:

We aim to identify if multiple sensors will provide overlapping information, which a sensor fusion framework can integrate to identify "pain" and "no pain" related features. These features will be used to train machine learning algorithms that will finally provide reliable, objective assessments of pain intensity in real-time.

We will also study if the pain intensity measured by the sensor fusion framework will show clinical validity, inter-rater reliability, as well as responsiveness to pain relief using analgesic drugs or non- pharmacological therapies. However, no interventions are planned in this study.

Secondary outcomes:

We will develop a sensor fusion framework designed to integrate data from different sensor modalities. No single sensor is capable of measuring neonatal pain. Rather, skilled clinicians draw upon multiple sources of information to estimate pain. A machine learning algorithm will be developed to test if the sensor fusion framework (i) can "calibrate" itself to the unique physiology of each newborn, (ii) handle missing (e.g. sensor failure) or unreliable data (e.g. movement artifact), and (iii) determine specific features from each modality to reach asymptotic levels of sensitivity and specificity. We hypothesize that this automated sensor fusion approach will be able to estimate neonatal pain intensity with greater specificity and sensitivity than the pain scales clinically used at the bedside.

To assess the reliability and validity of the pain intensity measured by the sensor fusion framework, we will compare the objective pain measure with the pain scores assessed by skilled research staff. Variations in the objective pain scores before and after the clinical use of analgesic therapies will also be assessed to explore whether this device can also identify pain relief [22].

Sample size calculations

We assume an α-error =0.05, 1–β error =0.8, and a mean:SD ratio of 2:1 for clinical and sensor-based pain scores (based on prior studies of clinical assessments of procedural pain in infants) [23,24]. Based on these assumptions, to detect a slope that corresponds to a 0.5-unit change in the outcome (e.g., sensor pain score) per 1-unit change in the predictor (e.g., clinical pain score) we will require a minimum of 40 infants in the *training dataset*. For a binary predictor with 25% or 50% prevalence (e.g., mild vs. moderate pain, or male vs. female neonate), with this sample size, we will be able to detect a 0.7 or 0.8-unit change in the outcome, respectively. Given the number of sensors used for the first step of this study, some artifacts may occur requiring data corrections or greater variability, with a need for recruiting more patients. We plan to recruit 60 patients in this study.

The sample size calculated for the first step of the study may represent a challenge for machine learning by limiting samples sizes for the training and testing datasets. Therefore, the results provided by our analyses will be confirmed in larger sample sizes within the next steps of the study.

Interventions/Experimental design

After applying inclusion/exclusion criteria and parental consent, infants' medical data will be recorded including: date of birth, perinatal/medical history, birth weight, gestational age, Apgar scores, congenital anomalies, metabolic defects, other diagnoses, previous surgeries, recent labs, prior imaging, major physical findings, number and types of painful procedures, and all medications used in the 24 hours immediately preceding the study. We will document the type of procedure, time of day, its location and duration, number of attempts and behavioral state of the infant before and after the procedure.

Prior to a clinically-indicated procedure such as a heel stick, subcutaneous or intramuscular injections (vaccine, drug shot), we will attach skin conductance (SC) leads to measure galvanic skin responses either on a hand palm or foot sole, and electromyography (EMG) to record facial muscle activity from cheek and forehead, and directly obtain recordings from clinical monitors (electrocardiography (ECG) and oxygen saturation (SpO2)). In addition, the infant will wear a cap with Electroencephalography (EEG) leads. For study procedures, research staff will clinically assess pain using the Neonatal Pain & Sedation Scale (N-PASS) before and after the procedure, the Premature Infant Pain Profile-Revised (PIPP-R) and the Neonatal Facial Coding System (NFCS) during the procedure. For older patients, the FLACC (Face, Legs, Activity, Cry, Consolability) scale or Visual Analogue Scale (VAS) will be scored to assess pain during the procedure [25,26][27][28]. Physiological and audio recordings will start 10-30 minutes before a planned procedure and continue for up to 20-30 minutes after the procedure.

All sensors will be monitored and displayed on the same laptop. We will use the Brain Vision software to display and record EEG, EMG and skin conductance responses and the MediCollector software for ECG and SpO2. The recording time of the 2 softwares will be synchronised based on the laptop digital clock. The event marker will be triggered by the researcher using a dedicated function of the Brain Vision software to time-lock and record times of noxious and non-noxious events for all sensors (Brain Vision and MediCollector).

At the completion of recording, all the above sensors will be disconnected from each patient and study procedures will be terminated. The entire study will last approximately 30-60 minutes;

however the study may be stopped earlier if any infant shows signs of distress or if a bedside nurse or parent has any concerns.

Data collection includes physiological recordings and medical information. Other than the facial EMG, skin conductance leads (SC), and 32-channel BrainAmp EEG data acquisition system, all other sensors are used routinely as the standard of care. All sensors are non-invasive and safe. We will use standard electrodes which are routinely used for recording vital signs in the hospital (ECG, SpO2). All recordings will use sticky pads attached to the skin. Other than a potential for mild skin irritation from the adhesives used, there are no significant risks associated with these devices, or other study-related procedures.

Data analysis

A. Signal-Filtering and Information Extraction from Sensors

For each sensing modality, we will develop a software algorithm to process signals recorded by sensors and extract the clinically relevant information related to pain. Proof-of-concept algorithms will be tested using the collected dataset. The feasibility of each sensing modality will be based on: *i*) sensitivity and specificity of detecting clinically relevant pain-related changes, and *ii*) robustness of sensor readings in the presence of non-pain related disturbances (e.g., movement artifact).

In order to address artifacts due to movement or suboptimal electrode-skin contact, we will initially use filtering techniques (e.g., to remove power line interference). In addition, we will identify channels exhibiting artifacts by considering the range of signal values, where signals showing extreme deviation from average values or channels showing virtually zero activity will be excluded. Specifically for EEG analysis, we will identify and remove EMG-related artifacts using well-established techniques such as filtering and independent component analysis.

Windows of different lengths will be used for the analysis. Specifically, for ECG, SpO2, EMG, and skin conductance signals an analysis window of 1-5 minutes will be used to extract appropriate features. The window lengths ranging from 400ms to 5 seconds will be used for EEG signals.

1. Pain Behaviors: Newborn behaviors (e.g., facial expressions, body movements) are accepted as the most sensitive and valid indicators of pain [29][13]. Facial expressions like brow bulge, eye squeeze, nasolabial furrow, and horizontal mouth stretch were verified as the most valid and discriminative components of neonatal pain scales [30,31][32]. Using facial EMG in real-time, we will detect the presence of muscle activity in neonatal pain-associated facial movements [33]. Given the multiple overlapping layers of facial muscles, facial EMGs record signals from a facial region as opposed to any specific muscle [33]. Previous studies of startle and blinking in infants used miniature silver (Ag/AgCl) periorbital surface electrodes for recording EMGs [34–36]. We will focus on infant forehead and cheek areas to detect EMG activity associated with brow bulge, eye squeeze and nasolabial furrow [33]. We will iteratively refine our algorithms by using multi-modality sensing and developing robust feature extraction and classification frameworks that address the challenges specific to neonatal/infant pain detection. We will exclude mechanically ventilated infants due to the challenges associated with identifying facial features (occluded by securing tape, ventilator tubing or devices) and their need for ongoing sedation/analgesia.

2. Skin Conductance: Acute pain stimulates the sympathetic post-ganglionic cholinergic neurons [37], leading to diaphoresis, palmar sweating and increased skin conductance [38]. Eliminating painful stimuli results in sweat reabsorption and decreased conductivity. The amplitude of changes in palmar skin conductance reflect increased sympathetic nervous system activity, which tracks with pain intensity [39–42]. Skin conductance can change with body temperature [43,44], but not with the ambient temperatures [44]. Specifically, the number of fluctuations of skin conductance per second (NFSC) was correlated with pain intensity in children [45], and was more sensitive that pain scores in preterm and term neonates [18,40–42,46]. We will use skin conductance using the BrainAmp system® (Brain Products GmbH, Gilching, Germany),

3. Electrocardiography (ECG): Heart rate (HR) changes are components of many pain assessment scales and recent studies have established correlations between HR variability (HRV) and pain [47,48]. A number of linear time-domain (HR mean, standard deviation) and frequency-domain (power spectral density) metrics and non-linear metrics (sample entropy, approximate entropy, etc.) can detect painful stimuli [47,48]. We will record the infant's ECG before, during, and after an acute pain event to extract the linear and nonlinear metrics (listed above) from the ECG signal for further analyses.

4. Electroencephalography (EEG): EEG studies to assess neonatal pain have met with variable results [49–51]. Opioid analgesia in adults leads to slowing of the EEG, whereas painful stimuli activate brain regions identified by neuroimaging studies [52] such as the primary sensory cortex (S1) [53]. EEG amplitudes and frequencies decreased when analgesics were given to newborns in pain [20,54–56]. Using the BrainAmp EEG system® (Brain Products GmbH, Gilching, Germany), we will apply 32 active EEG electrodes using the infant-sized ActiCap® (Brain Products GmbH, Gilching, Germany). Although Hartley et al. have selectively used the vertex (Cz) lead for neonatal pain studies [20,54], we believe that infant pain processing is widely distributed across many brain regions and the current evidence is not sufficiently strong enough to exclude information from other EEG leads. The BrainAmp is similar to other EEG monitors, however, it uses 32 active electrodes allowing for placement of the ActiCap on the infant's head with minimal preparation. Each active electrode amplifies the signal recorded from the skin and records also indicates the impedance of each electrode at the start of the recording to improve the quality of recorded signals. Over the past 10 years, this device has been used for research purposes in all age groups including infants and newborns. No side effects were reported from its use in newborns and small infants.

In order to analyze EEG signals and extract appropriate features, we will first remove noise and artifacts using standard techniques such as independent component analysis (ICA) and wavelet denoising [57]. After artifacts are removed, we will investigate the correlation between features extracted from EEG data and pain. Specifically, we will use spectral decomposition and extract features such as mean power in different frequency bands (delta, theta, alpha, and beta) as well as asymmetry measures for each homologous pair and functional connectivity measures for further investigation.

5. Pulse Oximetry (SpO2): Changes in SpO_2 occur frequently following acute pain and, therefore, have been included in the Premature Infant Pain Profile (PIPP) and other pain scales [58–60]. Newer generation monitors (Masimo, Irvine, CA) use multi-wavelength technology to provide more reliable SpO2 and pulse rate signals, with parallel signal processing engines and adaptive filters to separate the arterial from venous signals, patient motion, or skin perfusion [61]. Changes in skin blood flow were also used as physiological markers for neonatal pain or morphine analgesia [62,63]. We will test the utility of the SpO2 and peripheral perfusion index provided by pulse oximetry monitors as possible signals for neonatal pain.

Statistical approach

A. Sensor Fusion

We have previously investigated using machine learning to detect pain in neonates using facial expressions recorded by a camera [64]. We will develop a sensor fusion framework to detect pain in non-verbal infants based on machine learning to detect pain using multi-modal sensor data.

Feasibility of this new framework will be assessed based on its sensitivity and specificity to detect pain events in infants and further refined into a prototype for validation in future studies.

A “calibration” period will be used to establish a baseline for these multiple sensor modalities by monitoring neonates who are not in pain. The clinical staff at the bedside will identify the pain state of each neonate/infant using validated pain scales (N-PASS, NFCS and PIPP-R; FLACC, VAS) and record the timing of pain-inducing clinically-indicated procedures. Our sensor fusion framework will classify the neonatal/infant responses to infer pain intensity based on observed changes from baseline. A probabilistic relationship between pain intensity and sensor measurements can be established, where the unknown parameters of the statistical relationships are identified by a training dataset. The training dataset will also be used to estimate the importance of each feature, which can then be used to identify the optimal set of sensors [62].

Gestational age, postnatal age, and days of life and in hospital will be taken into account in the statistical analyses. We will initially focus on recruiting term neonates who are studied within 1 week after birth and have minimal exposures to prior painful events. This will increase the homogeneity of our sample and minimize the variability in physiological responses due to gestational age, postnatal age, days in the hospital, and long-lasting effects of previous painful experiences.

Interventions to manage pain will be allowed including non pharmacological and pharmacological treatments apart from continuous infusions of opioid drugs. This will be considered in the statistical analysis.

Pain intensity scores computed by our sensor fusion framework will be compared with pain scores measured concurrently by skilled research staff. All sensors (facial EMG, EEG, ECG, SpO_2, and SC) will be connected with an event recorder to mark "pain" vs. "no-pain" states. To make the best use of our data, the sensor fusion framework will use standard cross-validation methods to establish the generalizability of this framework.

The extracted features from each modality will be used to train a machine learning algorithm. Specifically, we will train a binary classifier to assign "pain" and "no pain" class labels based on the extracted features. We will specifically investigate using the random forests classifier given their robustness to outliers and its classification performance when a large number of features are used for classification.

Data from patients will be divided to a training set and a test set. The training set is used for model training and optimization of model parameters. A leave-one-patient-out cross validation technique will be used, where the machine learning classifier is trained on data from all but one patient and the performance of the classifier is assessed on the remaining patient. Once the appropriate machine learning classifier and its associated parameters are selected using the training set and the associated cross validation procedure, the performance of the machine learning classifier will be assessed on the test set.

B. Validation and Correlation with Pain Intensity

We will compare clinical pain scores from nursing assessments with scores from the sensor fusion framework. First, we will examine clinical pain scores to verify agreement with the pain scores assessed by the research staff. Internal consistency will be evaluated by Cronbach's α, with values > 0.8 to show good internal consistency. Second, we will conduct multivariable linear regressions or generalized estimating equations (GEE) [65,66] to understand the agreement between the device pain scores and the clinical pain scores, as well as the contribution of each modality to the device pain scores. We will examine if these associations vary after adjustment for covariates such as pain medications, age, sex, duration or invasiveness of the procedure. A co-variance matrix will examine the degree of correlation between individual sensor inputs, types of procedures, clinician pain scores, and analgesic therapies used during the procedure. Finally, due to the limited understanding of factors contributing to pain in newborns, linear regression or GEE models will examine the association of the sensor fusion pain scores reported by the device with the demographic and clinical variables of neonates and infants.

Content validity depends on the sensors and sensor variables that we have chosen for the sensor fusion framework. Concurrent validity will depend on the pain scores of skilled research staff using validated pain scoring methods. Construct validity will rest on: *i*) the range of objective pain scores from procedures causing mild, moderate, or severe pain; *ii*) changes in pain scores with analgesic drugs or non-pharmacological therapies; and *iii*) variation in pain scores over time consistent with the expected, natural course of acute procedural pain.

We will also develop a machine learning algorithm to predict subjective pain. As part of the validation, we will evaluate the machine learning pain assessment algorithm which has been trained on clinical classification of pain based on validated pain scales and compare the results with the results provided by the machine learning pain assessment algorithm which has been trained on data involving objective pain events (e.g., heel stick).

Future studies will test the validity of this approach to pain assessments in larger populations of newborns, older infants and also extend these studies to smaller preterm neonates. Future applications may also include patient populations incapable of expressing pain (children with disability, adults with dementia, or mechanically ventilated patients).

ETHICS AND DISSEMINATION

The Institutional Review Board of the Stanford University approved the protocol (Protocol #39076). The ethics approval includes anonymity and written consent will be provided by the parents.

Study findings will be published in peer-reviewed journals and presented at national and international scientific conferences. Practical use of this methodology will be taught at conference workshops, or via webinars, podcasts, video tutorials.

Authors' contributions: JMR, KJS and WMH, BG were responsible for manuscript writing. JMR, KJS, IM, WMH and BG contributed to the concept, protocol development and study design. KJS and BG secured funding for the project. JMR, IM and KJS are responsible for recruitment of study patients. All authors critically revised and approved the manuscript before submission and are accountable for all aspects of the work.

Competing interests statement: JMR reports an international mobility scholarship from Chiesi Pharmaceuticals; WMH and BG have equity ownership in Autonomous Healthcare, Inc.; WMH, BG and KJSA have proprietary interests in the potential devices that may be developed from these studies; Some equipments used in this study were provided by Autonomous Healthcare, Inc.

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Patient and Public Involvement: This research was done without patient involvement. Patients were not invited to comment on the study design and were not consulted to develop patient relevant outcomes or interpret the results. Patients were not invited to contribute to the writing or editing of this document for readability or accuracy.

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