THE INFLUENCE OF BREASTFEEDING ON PAIN-RELATED EVENT-RELATED POTENTIALS AND BIO-BEHAVIOURAL INDICATORS OF PROCEDURAL PAIN IN NEWBORNS: A RANDOMIZED CONTROLLED TRIAL

by

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DEDICATION

For Olivia.

The kindest soul, who lived and endured with beauty, strength, and resilience. It is my hope that this work makes a small contribution to helping build a future where no child has to experience suffering.

(2001 – 2019)

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ABSTRACT

Background: Breastfeeding and oral sucrose have strong evidence for reducing infant bio-behavioural responses to pain, however, no studies have compared the effect of these interventions on pain-related electrophysiologic brain activity.

Aims: To examine the influence of breastfeeding on pain-related electroencephalographic activity in newborns during heel lance, compared to 24% oral sucrose. Secondary aims were to compare a) bio-behavioural pain scores (Premature Infant Pain Profile – Revised [PIPP-R]), b) physiologic recovery, c) maternal acceptance, and d) adverse events between groups.

Methods: Healthy full term normally breastfeeding infants ($n = 39$) were randomly assigned to be breastfeeding or to receive 0.24 mL of 24% oral sucrose plus offered non-nutritive sucking two minutes prior to heel lance. Pain-related potential time-locked to heel lance was recorded on neonatal electroencephalogram and isolated using principal component analysis. Secondary outcomes of PIPP-R at 30-, 60-, 90-, and 120-seconds following heel lance and physiologic recovery were measured using continuous video and pulse oximeter recording. Maternal acceptance was measured using a study-specific questionnaire. Occurrence of adverse events were documented throughout study procedures. Data were analyzed per protocol as registered on ClinicalTrials.gov: NCT03272594.

Results: Twenty infants were randomized to the breastfeeding group and 19 infants to the oral sucrose group. Infants who received oral sucrose had an appreciably larger, yet not statistically significantly different ($F[1,15.9] = 0.58$, $p = 0.64$, $SE = 11.79$), amplitude pain-related potential (peak amplitude 8.97 μV) following heel lance compared to breastfeeding infants (peak amplitude 0.29 μV). Mean PIPP-R scores were not statistically significantly different between groups following heel lance, however, they were indicative of low to no pain across groups. Mean time in seconds to physiologic recovery was faster in breastfeeding infants ($M = 17.5$, $SD = 31.1$) compared to oral sucrose ($M = 70.8$, $SD = 144.3$). Mothers reported positive perceptions of the interventions pain-reducing effects and study participation.

Conclusions: Breastfeeding and oral sucrose may differentially modulate pain response in the infant brain. Continued use of these interventions to reduce bio-behavioural pain scores during clinical procedures is warranted. Further research to advance measurement and interpretation of diverse indicators of procedural pain in infants is needed.

LIST OF ABBREVIATIONS AND SYMBOLS USED

BIIP	Behavioral Indicators of Infant Pain
CI	Confidence Interval
CONSORT	Consolidated Standards of Reporting Trials
DAN	Douleur Aiguë du Nouveau-né
EEG	Electroencephalogram
EGI	Electrical Geodesic Incorporated
ERP	Event-Related Potential
EMLA	Eutectic Mixture of Local Anesthetics
fMRI	Functional Magnetic Resonance Imaging
GA	Gestational Age
GRADE	Grading of Recommendations Assessment, Development, and Evaluation
IM	Intramuscular
IQR	Inter-quartile range
ITT	Intention-to-treat
KMC	Kangaroo Mother Care
KG	Kilogram
LGA	Large for Gestational Age
M	Mean
MD	Mean Difference
MFCS	Modified Facial Coding System
mL	Millilitres
NFCS	Neonatal Facial Coding System
NIPS	Neonatal Infant Pain Scale
NIRS	Near Infrared Spectroscopy
NNS	Non-Nutritive Sucking
NMDA	N-methyl-D-aspartate
NN1	Non-Noxious One
NN2	Non-Noxious Two
PC	Principal Component
PCA	Principal Component Analysis
PNA	Postnatal age
PIBBS	Primary Infant Breastfeeding Behaviours Scale
PIPP	Premature Infant Pain Profile
PIPP-R	Premature Infant Pain Profile-Revised
RCT	Randomized Controlled Trial
ROP	Retinopathy of Prematurity
SD	Standard Deviation
SGA	Small for Gestational Age
SSC	Skin-to-Skin Contact
VAS	Visual Analogue Scale
wDPT	Whooping Cough, Diphtheria, Pertussis, and Tetanus
WMD	Weighted Mean Difference

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CHAPTER 1: INTRODUCTION

There is increasing recognition of the problem of newborn pain. National guidelines from the Canadian Paediatric Society (Barrington, Batton, Finley, Wallman, & Canadian Paediatric Society Fetus and Newborn Committee, 2017) and the American Academy of Pediatrics (2016) recommend that all health care facilities caring for newborns implement routine pain assessment and management plans, both as an ethical imperative and to prevent the adverse outcomes associated with early pain exposure in this population (American Academy of Pediatrics, Committee on Fetus and Newborn, & Section on Anesthesiology and Pain Medicine, 2016). However, despite these national recommendations, newborn pain continues to be under assessed and managed (Carbajal et al., 2008). This likely stems from major gaps in the literature regarding optimal pain assessment methods and non-pharmacologic interventions for management of newborn pain. The purpose of this study was to contribute to the literature examining non-pharmacologic interventions for acute pain and novel pain assessment measures in newborns. The following chapter contains essential information regarding the problem of pain in newborns. It will begin by summarizing the epidemiology of pain exposure in both preterm and full term neonates, followed by a discussion of the outcomes associated with this early pain exposure. This will lead into a brief discussion of available interventions for newborn pain, highlighting the limitations of pharmacologic interventions for acute pain management and introducing the non-pharmacologic interventions most relevant to this study (i.e., breastfeeding and 24% oral sucrose). This chapter will then present a brief discussion of available infant pain assessment methods, highlighting the need to advance our understanding of brain-based measures of infant pain, and will conclude with the purpose of the study.

Ubiquitous Exposure to Acute Pain in Newborns

Exposure in preterm and critically ill newborns. All infants experience early pain as part of routine medical care, with this exposure to pain being particularly high in preterm and critically ill infants who are admitted to neonatal intensive care units (NICUs). Data from Canadian (Johnston, Barrington, Taddio, Carbajal, & Filion, 2011) and European (Barker & Rutter, 1995; Carbajal et al., 2008; Roofthooft, Simons, Anand, Tibboel, & van Dijk, 2014; Simons, van Dijk, Anand, Roofthooft, van Lingen, & Tibboel, 2003) studies demonstrate that infants can undergo anywhere from one to 14 procedures per day when hospitalized in the NICU. A recent synthesis of 18 available studies examining pain exposure and analgesic practices conducted by Cruz and colleagues (2016) found that hospitalized neonates were undergoing from seven to 17 painful procedures per day, with the most common procedures being heel lancing and naso- and endo-tracheal suctioning, venepuncture, and insertion of peripheral venous catheters. Studies in this review reported that infants went without analgesia during painful procedures ranging from 42 to 100% of the time, with the majority of studies reporting no pain treatment. These findings are consistent with those of Johnston and colleagues (2011) who conducted a prospective observational study over one week in 14 Canadian NICUs. Of the reported 3,508 tissue damaging ($M = 5.8$, $SD = 15$) and 14,085 non-tissue damaging procedures ($M = 25.6$, $SD = 15$), approximately half were completed with no analgesic intervention (46% of tissue damaging and 57% of non-tissue damaging). Factors in this study that predicted the use of pharmacologic interventions (e.g., opiates) during tissue breaking procedures included being less ill at birth and receiving high frequency ventilator support, whereas parental presence significantly predicted the use of

sweet taste or non-pharmacologic interventions (i.e., non-nutritive sucking, swaddling, rocking, positioning, skin-to-skin contact, breastfeeding).

Exposure in healthy full term born infants. While there is considerable research regarding pain exposure in critically ill and preterm infants, the literature examining the incidence of pain in healthy and full term infants is limited. However, they are a population who are also exposed to pain in early life. For example, all infants will undergo a routine intramuscular injection of Vitamin K to prevent bleeding (Ng, Loewy, & Canadian Paediatric Society Fetus and Newborn Committee, 2018) and a heel lance to collect blood for metabolic testing (Maritime Newborn Screening Service, 2014) and routine total serum bilirubin screening (Barrington, Sankaran, & Canadian Paediatric Society Fetus and Newborn Committee, 2016) within the first days of age. In those infants diagnosed with hyperbilirubinemia, which includes approximately 60% of term newborns, repeated heel lancing may be required to monitor response to treatment (Stevenson, Fanaroff, Maisels, Young, Wong, & Vreman, 2001). Furthermore, full term infants who are at risk of hypoglycemia will undergo repeated heel lancing for blood glucose testing based on recommendations from the Canadian Paediatric Society screening guidelines for infants at risk for low blood glucose (Aziz, Dancey, & Canadian Paediatric Society Fetus and Newborn Committee, 2004). This includes small for gestational age infants (weight at less than the 10th percentile), large for gestational age infants (weight at more than the 90th percentile), and infants born to diabetic mothers, who constitute approximately five percent of live births (Shand, Bell, McElduff, Morris, & Roberts, 2008; Williams, 1997; 2005). Furthermore, children in Canada undergo upwards of 20 intramuscular injections for immunizations (e.g., DTaP-IPV-Hib [Diptheria, tetanus, whooping cough, polio, haemophilus influenza disease], Pneu-C-13

[pneumococcal disease], and MMR-Var [measles, mumps, rubella, varicella]) with the majority occurring from two to 18-months of age (Public Health Agency of Canada, 2013).

Outcomes Associated with Early Exposure to Acute Pain

Studies with both animals and humans have linked pain exposure in infancy with adverse outcomes that are attributable to cardiorespiratory, hormonal, and neurological responses during and following pain exposure. In human infants, physiological elevations in heart rate, blood pressure, and oxygen requirements can lead to fluctuations in intracranial pressure, possibly leading to intraventricular hemorrhage (IVH) and periventricular leukomalacia (Scanlon, 1991; Stevens, Johnston, & Horton, 1993). Pain is associated with increased stress and inflammatory hormone release that impedes regulation of growth and tissue repair (Mörelius, He, & Shoreywhich, 2016; Mörelius, Theodoursson, & Nelson, 2005) and has adverse effects on cognition and memory systems (Holsti, Grunau, Whifield, Oberlander, & Lindh, 2006). Furthermore, there are structural and functional alterations in both the peripheral and central nervous system that may lead to prolonged alteration of pain responses (Anand & Scalzo, 2000; Fitzgerald & Beggs, 2001).

Outcomes of early pain in preterm newborns. In research conducted with largely preterm infant samples, repeated pain exposure is associated with changes in somatosensory processing that continues into childhood, including changes in sensitivity and response to later pain (Grunau et al., 2005; Grunau, Oberlander, Whitfield, Fitzgerald, Morison, & Saul, 2001; Johnston & Stevens, 1996; Hermann, Hohmeister, Demirakca, Zohsel, & Flor, 2006; Walker, Franck, Fitzgerald, Myles, Stocks, & Marlow, 2009; Walker, Melbourne, Reilly, Beckmann, Ourselin, & Marlow, 2018). Pain exposure

in preterm infants has additionally been associated with structural changes in the brain, including reduced maturation of white and subcortical grey matter at term equivalent age (Brummelte et al., 2012); slower thalamic, basal ganglia, and total brain volume growth (Duerden et al., 2018; Schneider et al., 2018); and reduced cortical thickness (Ranger et al., 2013) and cerebellum volume (Ranger et al., 2015) at school age in children born very preterm. Functional connectivity between the thalamus and sensorimotor cortices has also been found to be negatively associated with invasive procedure exposure (Schneider et al., 2018).

The influence of untreated procedural pain on the developing brain is further evidenced by research suggesting long-term motor, cognitive, and behavioural deficits. For example, exposure to pain in the neonatal period has been shown to be associated with poor body and head growth (Vinall, Miller, Chau, Brummelte, Synnes, & Grunau, 2012), reduced visual perceptual abilities at school age (Doesburg et al., 2013), poorer language outcomes at 18-months corrected age (Vinall et al., 2015), greater internalizing behaviours throughout childhood (Ranger, Synnes, Vinall, & Grunau, 2014; Vinall, Miller, Synnes, & Grunau, 2013), and altered development of the hypothalamic-pituitary-adrenal axis (Brummelte et al., 2015; Duerden et al., 2018; Grunau et al., 2010; Grunau, Haley, Whitfield, Weinberg, Yu, & Thiessen, 2007; Grunau, Weinberg, & Whitfield, 2004; Schneider et al., 2018). Such findings underscore the significant adverse immediate and long-term outcomes associated with unmanaged early pain exposure during this period of development.

Outcomes of early pain in full term newborns. While the majority of research examining the influence of untreated pain to date has been conducted with preterm newborns, the available evidence suggests a relationship between pain exposure and

adverse outcomes in full term born infants. Studies examining the influence of early exposure of full term infants to major surgery (Andrews & Fitzgerald, 2002; Peters et al., 2003; Peters, Schouw, Anand, van Dijk, Duivenvoorden, & Tibboel, 2005; Schmelzle-Lubiecki, Campbell, Howard, Franck, & Fitzgerald, 2007), circumcision (Taddio, Goldbach, Ipp, Stevens, & Koren, 1995; Taddio, Katz, Ilersich, & Koren, 1997), burns (Wollgarten-Haamek, Hohmeister, Demirakca, Zohsel, Flor, & Hermann, 2009), and repeated acute procedural pain (Ozawa, Kanda, Hirata, Kusakawa, & Suzuki, 2011; Piira, Champion, Bustus, Donnelly, & Lui, 2007; Taddio, Shah, Atenafu, & Katz, 2009; Taddio, Shah, Gilbert-MacLeod, & Katz, 2002) suggest that this exposure may be associated with heightened pain responses to later painful stimuli.

Treatment of Newborn Acute Pain

In recognizing the adverse consequences of untreated pain in newborns, national guidelines for evidence informed pain assessment and management practices in this population have been developed (American Academy of Pediatrics Committee on Fetus and Newborn, 2016; Barrington, Batton, Finley, Wallman, & Canadian Paediatric Society Fetus and Newborn Committee, 2017). Such guidelines make recommendations regarding the use of pharmacologic and non-pharmacologic interventions for neonates undergoing acute procedures, circumcision, and major surgery.

Pharmacologic treatment of acute pain. While pharmacologic agents such as acetaminophen, opioids, and topical anaesthetics have been studied, there are several limitations associated with use of such drugs during the common acute painful procedures that infants undergo. Although topical anaesthetics have demonstrated effectiveness for reducing circumcision related pain (Brady-Fryer, Wiebe, & Lander, 2004; Lehr & Taddio, 2007; Taddio et al., 1997; Yamada, Stinson, Lamba, Dickson, McGrath, &

Stevens, 2008), research suggests that they are ineffective in reducing the pain associated with heel lance (Larsson, Norman, Bjerring, Egevist, Lagercrantz, & Olsson, 1996; Stevens et al., 1999), venipuncture (Shah, 1998), or insertion of intravenous or intra-arterial lines (Ballantyne, McNair, Ung, Gibbins, & Stevens, 2003; Yamamoto & Boychuck, 1998). Additionally, safe and effective dosing of systemic drugs, specifically opioids, for pain relief in infants is challenging as infant neurodevelopmental stage makes them highly sensitive to drug effects (Nandi, Beacham, Middleton, Koltzenburg, Howard, & Fitzgerald, 2004; Nandi & Fitzgerald, 2005) and they demonstrate slow drug clearance (Allegaert, Simons, Vanhole, & Tibboel, 2007; Bouwmeester, Anderson, Tibboel, & Holford, 2004; Koren, Butt, Chinyanga, Soldin, Tan, & Pape, 1985; Lynn & Slattery, 1987; Zuppa, Mondick, Davis, & Cohen, 2009). While counterintuitive given infant sensitivity to opioid dosing, research demonstrates limited efficacy of opioids and acetaminophen for relief of pain associated with the acute procedures most common in infants (Carbajal, Lenclen, Jugie, Paupe, Barton, & Anand, 2005; Shah, Taddio, & Ohlsson, 1998). It is hypothesized that the lack of analgesic efficacy of these drugs may be a result of low opioid receptor concentrations and affinity (Kinney, Ottoson, & White, 1990; Rahman, Dashwood, Fitzgerald, Aynsley-Green, & Dickenson, 1998) and/or reduced efficacy of the immature liver to produce the analgesic metabolic side products of opioids (e.g., conversion of morphine to morphine-6-glucuronide [Osborne, Thompson, Joel, Trew, Patel, & Slevin, 1992]). As such, research examining alternate non-pharmacologic methods is required in order to effectively manage newborn pain.

Non-pharmacologic and sweet taste interventions. In light of the limitations of pharmacologic interventions in managing acute pain, numerous non-pharmacologic and sweet taste interventions have been tested for use during painful procedures in infants,

and can be grouped into the categories of 1) sensorial saturation (e.g., positioning/swaddling, non-nutritive sucking, rocking, music [Corff, Seideman, Venkataraman, Lutes, & Yates, 1995; Huang, Tung, Kuo, & Ying-Ju, 2004]), 2) nutritive (e.g., oral sweet solutions, provision of expressed breast milk [Shah, Herbozo, Aliwalas, & Shah, 2012; Stevens, Yamada, Ohlsson, Haliburton, & Shorkey, 2013]), and 3) parent-driven interventions (e.g., skin-to-skin contact, direct breastfeeding, maternal odour, maternal voice [Campbell-Yeo, Fernandes, & Johnston, 2011; Johnston et al., 2017]). For the purpose of this study, breastfeeding and 24% oral sucrose are the interventions of interest.

Sucrose. Oral sweet solutions are the most studied and recommended treatment for acute pain management in newborns to date. Studies using sucrose, the most studied sweet taste, began in the late 1980's (Blass et al., 1987; Blass & Hoffmeyer, 1991) and have been meta-analyzed (Stevens, Yamada, Ohlsson, Haliburten, & Shorkey, 2016). Summative data from seventy-four randomized controlled trials (n = 7,049 neonates) demonstrates that 24% oral sucrose solution reduces composite bio-behavioural pain scores during acute needle and heel lance procedures (Stevens et al., 2016). While the mechanisms underlying the effectiveness of sucrose as a pain-relieving intervention are not fully understood, it is hypothesized that oral sweet taste potentially activates endogenous opioid release that attenuates nociception at the level of the dorsal horn (Gibbons & Stevens, 2001). Despite the overwhelming evidence for the efficacy of sucrose in reducing bio-behavioural pain response during single procedures, questions related to the optimal dose, sustained effect, potential for long-term adverse outcomes, and concern regarding the sedative versus analgesic effect of sucrose remain. Of particular interest, data using alternate assessment measures brings the analgesic effect of

this intervention into question. Slater and colleagues (2010a) enrolled 59 newborn infants in a double blind, randomized controlled trial and found that while 24% oral sucrose reduced bio-behavioural pain response measured using the Premature Infant Pain Profile (PIPP), it did not significantly reduce the amplitude of a pain-related potential in the infant brain measured using electroencephalogram. The authors of this study concluded that the reduction of bio-behavioural pain scores should not be interpreted as pain relief and that further investigation is necessary (Slater et al., 2010a). The potential limitations of sucrose with respect to analgesic efficacy and prevention of long-term adverse effects is supported by Taddio's (2009) work where receiving sucrose for every painful procedure following birth did not protect against the hyperalgesia that developed in those infants who were highly exposed to pain. Such findings bring into question the analgesic abilities of sucrose, generating a need for research examining the effectiveness of alternate interventions in reducing pain measured using multi-modal pain indicators in newborns.

Breastfeeding. The most recent Cochrane systematic review of twenty studies (n = 2,071 neonates) supports the effectiveness of direct breastfeeding as a pain-relieving intervention for full term neonates. Of the ten studies included in this systematic review which examined direct breastfeeding (as opposed to provision of expressed breast milk), behavioural pain scores were significantly lower in full term infants who were breastfeeding during heel lance or venipuncture when compared to those who were positioned in their mothers' arms, received a placebo, or received no intervention (Shah, Herbozo, Aliwalas, & Shah, 2012). However, direct breastfeeding and sucrose were found to have the same level of effectiveness in reducing behavioural pain scores. In comparison to direct breastfeeding, there was considerable variability across the ten

studies examining provision of expressed breast milk for pain relief. While expressed breast milk was found to reduce heart rate, duration of crying, and behavioural pain response measured using the Neonatal Facial Coding System (NFCS) when compared to placebo, sucrose was found to be more effective than expressed breast milk in reducing duration of crying and heart rate (Shah et al., 2012).

There are several potential mechanisms by which breastfeeding or provision of breast milk acts as an analgesic in infants. One hypothesized mechanism is that the close and comforting presence of an infant's mother results in the release of oxytocin, which has been found to have a pain reducing effect in animal studies (Matthiesen, Ransjo-Avidson, Nissen, Uvnas-Moberg, 2001; Nelson & Panksepp, 1998). Both full term and preterm born infants have olfactory memory and demonstrate a preference for their own mother's amniotic fluid and breast milk. This recognition has been found to reduce crying during prolonged maternal-infant separation as well as pain response during heel lance (Marlier, Schaal, & Soussignan, 1998; Rattaz, Goubet, & Bullinger, 2005; Schaal, Marlier, & Soussignan, 1998; Varendi, Christensson, Porter, & Winberg, 1998). Release of endogenous opioids in response to the sweetness of breast milk has also been identified as a potential analgesic mechanism, however, breast milk contains only seven percent lactose (in contrast to the 24% oral sucrose found to be effective in the aforementioned systematic review and meta-analysis), and thus may not be as effective when expressed and administered without maternal contact and smell. Additionally, breast milk contains trypotophan, which is a precursor to melatonin (Heine, 1999). Melatonin has been found to promote the release of beta-endorphins, which provides an additional potential analgesic mechanism. Sucking on the nipple may additionally have analgesic properties that result from the stimulation of oro-tactile and mechanoreceptors that may or may not

be mediated by endogenous opioid pathways (Anseloni et al., 2004; Carbajal et al., 2004). The Neuromatrix Theory of Pain, which is the theoretical framework guiding this work, proposes that the experience of painful events is influenced by multiple cognitive, sensory, and emotion related factors which modulate the nociceptive pain response traveling through ascending pain pathways (Melzack, 2005; 1999). The Neuromatrix Theory of Pain would thus suggest that it is the synergistic effect of the multiple hypothesized mechanisms encompassed within breastfeeding that contributes to its effectiveness as an analgesic intervention.

Infant Pain Assessment

The majority of the infant pain assessment tools that have been developed to date encompass either behavioural responses, physiological responses, or combine both into a composite measure (Lee & Stevens, 2014; Ranger, Johnston, & Anand, 2007). Behavioural responses to pain have been well characterized in infants, and include facial actions (e.g., brow bulge, eye squeeze, naso-labial furrow), body movements (e.g., flexion of fingers and toes), and qualities of infant cry (e.g., onset, pitch, duration). Research suggests that behavioural responses, particularly facial actions, are the most sensitive pain indicators in infants (Craig, Whitfield, Grunau, Linton, & Hadjistavropoulos, 1993) as they are more likely to selectively respond to pain when compared to physiological responses, such as heart rate and oxygen saturation, which fluctuate in response to both pain and non-pain related events such as hunger or stress (Johnston, Stevens, Franck, Jack, Stremler, & Platt, 1999; Stevens, Johnston, & Horton, 1993). Thus, numerous uni-dimensional behavioural measures of pain have been developed and validated. However, such measures do not consider the influence of contextual factors on pain responding. For example, postnatal age and gestational age at birth (Craig et al., 1993, Johnston,

Stevens, Yang, & Horton, 1995; Johnston & Stevens, 1996; Johnston et al., 1999), neuro-behavioural state of the infant at the time of procedure (Stevens & Johnston, 1994; Stevens, Johnston, & Horton, 1994; Johnston et al., 1999), numbers of past painful procedures (Johnston & Stevens, 1996), and proximity between repeated painful procedures and handling (Grunau, Holsti, & Peters, 2006; Holsti, Grunau, Oberlander, & Whitfield, 2005; Holsti, Grunau, Whifield, Oberlander, & Lindh, 2006) are factors that have been documented to influence behavioural pain response.

Composite multi-dimensional pain measures that encompass behavioural, physiological, and contextual factors (e.g., gestational age, behavioural state) have been developed in an attempt to address the limitations of uni-dimensional measures. Such bio-behavioural tools have been extensively psychometrically validated and are now considered the most representative measure of infant acute pain responding (Lee & Stevens, 2014). However, these tools continue to have recognized limitations. Behavioural and physiological responses may be non-specific to pain and are often poorly correlated, making the results difficult to interpret (Barr, 1998; Stevens, Johnston, & Horton, 1994; Johnston et al., 1995; Pillai Riddell, Fitzgerald, Slater, Stevens, Johnston, & Campbell-Yeo, 2016). Thus, continued exploration of potential sensitive and specific pain indicators is a current focus of the infant pain literature and could advance the measurement and understanding of infant pain responding.

Utilizing neurophysiologic imaging methods to measure brain response to painful procedures is an exciting new area of research that holds promise to advance our understanding and measurement of infant nociception and pain (Hartley & Slater, 2014). To date, studies utilizing near infrared spectroscopy (Bartocci et al., 2006; Beken et al., 2014; Bembich et al., 2013; 2018; Bucher et al., 1995; Olsson et al., 2015; Ozawa et al.,

2011; Ranger et al., 2013; Rioualen et al., 2017; Slater et al., 2006; 2008), functional magnetic resonance imaging (Goksan et al., 2015; Williams et al., 2015), and electroencephalography (Fabrizi et al., 2011; Hartley et al., 2015; 2016; Jones et al., 2017; Maimon et al., 2013; Maitre et al., 2018; Norman et al., 2008; Slater et al., 2010a; Slater, Fabrizi, Worley, Meek, Boyd, & Fitzgerald, 2010b; Slater et al., 2010c; Verriotis, Fabrizi, Lee, Ledwidge, Meek, & Fitzgerald, 2015; Verriotis, Fabrizi, Lee, Cooper, Fitzgerald, & Meek, 2016; Verriotis et al., 2018) have demonstrated that, like adults (Rainville, 2002), infants demonstrate cortical responses to painful clinical and experimental stimuli. By time-locking a neonatal electroencephalogram (EEG) recording to a medically required heel lance, Slater and colleagues (2010a-c) have used principal component analysis (PCA) to demonstrate the presence of a distinct positive event-related potential (ERP) occurring 560-milliseconds post painful procedure. This pain-related brain potential has been identified in preterm (Fabrizi et al., 2011; Hartley et al., 2016; Slater et al., 2010b; Verriotis et al., 2017) and full term neonates (Jones et al., 2017; Slater et al., 2010a; 2010c; Verriotis et al., 2016) and in infants at one month and 12-months of age (Verriotis, Fabrizi, Lee, Ledwidge, Meek, & Fitzgerald, 2015). Furthermore, the amplitude of this potential has been found to be positively associated with stimulus intensity (Hartley et al., 2015) and stress (Jones et al., 2017), reproducible in individual infants undergoing repeated procedures (Verriotis et al., 2015), and independent of infant sleep state (Slater et al., 2010a). Interestingly, studies examining the effectiveness of pain-relieving interventions in reducing nociceptive response in the brain have found dissociation between electrophysiologic and behavioural response to pain in infants receiving sucrose (Slater et al., 2010a) and general anaesthesia (Hartley et al., 2014). Assessment of electroencephalogram ERPs is thus a method to advance

understanding of pain-related response in the infant brain, as well as a potential important indicator to consider when studying the efficacy of pain-relieving interventions in this population.

Conclusion

Full term infants routinely undergo acute painful procedures and this exposure may result in hypersensitivity during later painful events. While breastfeeding shows great promise as an intervention to reduce bio-behavioural indicators of pain, no studies to date have examined the effect of breastfeeding on pain-related potentials on neonatal electroencephalogram. Given evidence questioning the effectiveness of 24% oral sucrose in reducing the amplitude of previously characterized pain-related potentials in the infant brain, there is an urgent need to determine optimal interventions to reduce pain response in the infant brain during acute procedures. An important step towards reaching this goal is to advance understanding of optimal ways to measure infant pain by better understanding the relationship between existing bio-behavioural indicators and neurophysiologic brain-based measures of infant pain. This study addressed this significant knowledge gap by examining to what extent breastfeeding impacts on pain-related electroencephalographic response in the infant brain during an acute painful procedure in comparison to 24% oral sucrose. The next chapter will provide an in-depth discussion of the literature pertaining to newborn pain exposure, management, and measurement.

CHAPTER 2: LITERATURE REVIEW

This study examined the influence of breastfeeding on pain-related electroencephalographic brain response in newborns during a heel lance procedure in comparison to 24% oral sucrose solution. Thus, this literature review on breastfeeding and 24% oral sucrose as pain-reducing interventions is divided into four main sections. First, evidence for outcomes associated with untreated early pain exposure in healthy full term newborns will be discussed, providing a rational for management of acute pain in this population. This will lead into a discussion of the empirical evidence for pain-relieving interventions in newborns, specifically breastfeeding and 24% oral sucrose, as they are the most relevant to this study. The following section will describe issues of pain assessment in newborns, highlighting current evidence related to the utilization of behavioural, physiological, and brain-based methods of measuring infant pain. Next the theoretical rational and conceptual framework that underpinned study hypotheses will be presented, followed by a discussion of the potential underlying mechanisms of breastfeeding as a pain reducing intervention and potential confounding variables that were considered a priori.

Rationale for Pain Management in Full Term Newborns

As noted in the previous chapter, preterm and critically ill newborns are highly exposed to pain, and there is extensive evidence reporting on the adverse structural, functional, and behavioural outcomes associated with pain exposure in this population. However, even the healthiest of infants undergo routine painful procedures as part of universal medical care. In addition to causing unnecessary suffering to the smallest and most vulnerable of our population, full term newborns exposed to early pain are vulnerable to adverse neurologic consequences, with studies demonstrating that exposure

to major surgery (Andrews & Fitzgerald, 2002; Peters et al., 2003; Peters, Schouw, Anand, van Dijk, Duivenvoorden, & Tibboel, 2005; Schmelzle-Lubiecki, Campbell, Howard, Franck, & Fitzgerald, 2007), circumcision (Taddio, Goldbach, Ipp, Stevens, & Koren, 1995; Taddio, Katz, Ilersich, & Koren, 1997), burns (Wollgarten-Haamek, Hohmeister, Demirakca, Zohsel, Flor, & Hermann, 2009), and acute procedural pain (Ozawa, Kanda, Hirata, Kusakawa, & Suzuki, 2011; Piira, Champion, Bustus, Donnelly, & Lui, 2007; Taddio, Shah, Atenafu, & Katz, 2009; Taddio, Shah, Gilbert-MacLeod, & Katz, 2002) may be associated with heightened pain responses to later painful stimuli. The influence of untreated acute painful procedures is of particular importance, as all infants will undergo at least two acute tissue breaking painful procedures (e.g., a heel lance to collect blood for routine metabolic screening and an intramuscular injection of Vitamin K to prevent blood clotting disorders) in the first days of age.

Taddio and colleagues (2002) were the first to examine if infants who underwent repeated heel lancing for blood collection learned to anticipate pain and experienced more pain during subsequent heel lancing. Utilizing a prospective cohort design, they sampled a group of healthy full term infants who were pain-naïve ($n = 21$) to compare to a group of infants who were born to diabetic mothers and thus underwent repeated heel lancing to collect blood for glucose monitoring ($n = 21$). Both groups of infants underwent a venipuncture to collect blood for metabolic screening after the first 24-hours of age, and their pain response to this procedure was assessed using facial response (i.e., brow bulge, eye squeeze, naso-labial furrow), visual analog scale (VAS) scoring, and percentage of time crying. Infants of diabetic mothers had significantly higher facial response scores during skin cleansing prior to the procedure when compared to those infants who were pain-naïve, suggesting that they may have anticipated the painful procedure (Taddio et

al., 2002). Furthermore, infants of diabetic mothers demonstrated significantly higher facial pain response, VAS scores, and percentage of time crying during the venipuncture. This statistical significance was maintained after adjusting for the elevated pain response during skin cleansing in the high pain exposure group for both facial response and VAS scores, but not for crying time percentage. While this study was the first to report the development of pain hypersensitivity following acute pain in full term newborns, it had a moderate risk of bias scoring (Sterne, Higgins, & Reeves, 2014) with concerns related to the potential confounding influence of medical condition (i.e., being an infant of diabetic mother may have influenced pain hypersensitivity as opposed to prior pain exposure).

Taddio and colleagues (2009) went on to produce similar findings in a randomized controlled trial examining pain response to venipuncture in a similar sample of infants (i.e., one group of infants who were pain-naïve and one group who underwent repeated heel lancing for blood glucose testing) who were randomly assigned to receive 24% oral sucrose or a placebo during every painful procedure they underwent following birth. This randomized trial, which had a low risk of bias scoring (Higgins et al., 2011), attempted to address the limitations of their prior observational study. Similar to their previous findings, infants in the high pain exposure group had higher Premature Infant Pain Profile (PIPP) scores and higher VAS pain scores compared to their pain-naïve counterparts. Further, an interesting finding reported by these authors was that while 24% oral sucrose reduced PIPP, VAS scores, and cry duration during venipuncture, there was no statistically significant interaction between procedure exposure level and treatment allocation for PIPP ($p = 0.742$), VAS scores ($p = 0.547$), or cry duration ($p = 0.344$). The authors concluded that as 24% oral sucrose is a relatively weak analgesic, with a reported modest (16%) reduction in overall infant pain response across multiple painful procedures

(Taddio et al., 2008), it did not prevent the development of hyperalgesia and thus should not be given as the sole analgesic during acute procedures (Taddio, Shah, Atenafu, & Katz, 2009).

A more recent study conducted by Gökulu and colleagues (2016) compared pain responses to heel lance between 20 full term large for gestational age (LGA) infants to 40 full term appropriate for gestational age controls. Large for gestational age infants had undergone significantly more heel lances (median [LGA] = 10, median [control] = 1; $p < 0.001$) and venous blood draws (median [LGA] = 1, median [control] = 0; $p < 0.001$) than control infants. Consistent with previous studies, LGA infants who had higher prior pain exposure demonstrated statistically significantly longer crying time, higher Neonatal Infant Pain Scale (NIPS) scores, and lower oxygen saturations compared to the control infants when undergoing a heel lance procedure. These findings supplement the existing literature reporting on the development of pain hypersensitivity in full term newborns, providing further support that adverse behavioural and physiological outcomes associated with early pain exposure are not limited to preterm infants.

While there are relatively few studies examining the influence of early exposure to acute pain on later responding to painful procedures in the full term infant population, the findings reported by Taddio (2002; 2009) and Gökulu (2016) are consistent with research conducted in animal models and the theoretical literature proposing underlying mechanisms of early life stress and pain exposure on later pain hypersensitivity. Characteristics of the full term infant peripheral and central nervous system may make them particularly vulnerable to neurobiological and behavioural changes secondary to aversive experiences (Hofer, 1994; Rosenblum & Andrews, 1994). For example, full term infant sensitivity to peripheral nociceptor changes as a result of neurotrophic factors

has been reported in both animal and human studies. Neurotrophic factors are growth factor proteins that are released following tissue damage and are involved in the production of inflammatory pain responses. Neurotrophic factors have been implicated in the development of pain hypersensitivity in infants as newborns demonstrate up-regulation of neurotropic factors in the skin up to four times that seen in adults following pain exposure (Alvares, Robinson, & Fitzgerald, 1999; Constantinou, Reynolds, Woolf, Safieh-Garabedian, & Fitzgerald, 1994). Infants additionally demonstrate high sensitivity to levels of neurotrophic factors in the skin that promote the development and sensitivity of nociceptive-specific sensory neurons (Caroll, Lewin, Koltzenburg, Toyka, & Thoenen, 1998; Koltzenberg, Janiq, & Stucky,1999; Lewin & Mendell, 1993). This hyper-innervation of peripheral nociceptive neurons may be greatest when the tissue injury occurs early in life, as tissue damage in infancy results in hyper-innervation that extends into adulthood, whereas the resulting hyper-innervation dissipates within a matter of weeks when the injury occurs in adulthood (Reynolds & Fitzgerald, 1995; Fitzgerald & Beggs, 2001).

Full term newborns are additionally vulnerable to central nervous system alterations secondary to early pain exposure. For example, sensory neurons in the dorsal horn of the full term infant spinal cord demonstrate large receptor fields over the first two weeks of age and peak levels of N-methyl-D-aspartate (NMDA) receptors (Anand & Scalzo, 2000; Fitzgerald, 1985; Fitzgerald & Beggs, 2001; Fitzgerald & Jennings, 1999). These characteristics of the immature dorsal horn contribute to pain hyper-sensitization following early pain exposure as they contribute to increased baseline neuronal excitability and thus a lower sensory threshold to pain (Fitzgerald, 1985; Fitzgerald & Beggs, 2001; Fitzgerald & Jennings, 1999). Infants additionally have immature

descending inhibition pathways in the dorsal horn compared to adults (Fizgerald, 1991; Fitzgerald & Koltzenburg, 1986). The immaturity of such an important endogenous analgesic system, in combination with increased nociceptive neuron excitability, suggests that infants' sensory responses to pain and vulnerability to hyper-sensitization may be greater than in adults, resulting in more adverse outcomes when repeated pain exposure occurs in early life (Chu, Chan, Tsou, Lin, Hsieh, & Tao, 2007; Fitzgerald & Beggs, 2001; Hatfield, 2014).

This section highlighted the empirical and theoretical evidence reporting on the outcomes associated with early pain exposure in full term newborns. While there are fewer studies in full term infants compared to those studies reporting on those born preterm, there is sufficient evidence to suggest that minimal exposure to repeated acute painful procedures may be associated with the development of pain hypersensitivity in the full term newborn population. The behavioural and physiological hyper-sensitization to acute pain demonstrated in those primary studies of human infants is aligned with the theoretical and animal literature proposing potential underlying peripheral and central nervous system mechanisms of pain hypersensitivity to which full term infants are particularly vulnerable. As such, it is imperative that exposure to clinically unnecessary painful procedures is minimized and that the pain associated with necessary procedures is managed with effective pain-reducing interventions. The following sections of this literature review will outline the current evidence regarding 24% oral sucrose and breastfeeding as pain-relieving interventions, which are two interventions commonly used for acute pain management in this population and are of particular relevance to this study. It is important to note that there is substantial diversity in the methods used to assess infant pain in the literature examining the efficacy of pain reducing interventions.

Throughout the following sections of this literature review it will be noted if the assessment measures reported are physiological (e.g., changes in heart rate, oxygen saturation), neurophysiological (e.g., electroencephalographic activity), behavioural (e.g., cry duration, facial action scales), or multi-dimensional (e.g., combinations of behaviour, physiology, and contextual factors) to enhance interpretability of reported findings.

Sweet-Taste Analgesia: 24% Oral Sucrose

Administration of oral sucrose is the most studied pain-relieving intervention in neonates, with seventy-four randomized controlled trials (n = 7,049 neonates) being included in a recent Cochrane systematic review and meta-analysis examining its efficacy for reducing the pain associated with acute procedures (Stevens et al., 2016). While the mechanisms underlying the pain reducing effect of sucrose are not yet fully elucidated, studies examining the analgesic and stress reducing effects first began in infant rat models in the late 1980's and found that the effects of sucrose occurred rapidly, were short lasting, and were blocked by systemic opioid receptor antagonists, suggesting that the effects are mediated by endogenous opioid release (Blass, Shide, & Weller, 1989; de Freitas, Kübler, Elias-Filho, & Coimbra, 2012; Ren, Blass, Zhou, & Dubner, 1997; Shide & Blass, 1989; Stevens et al., 2016). More recent studies in human infants support the hypothesis that oral sweet taste stimulates the release of endogenous opioids, producing a calming and potentially analgesic effect (Blass & Hoffmeyer, 1991; Blass & Shah, 1995; Stevens et al., 2016). For example, in a study examining full term infants born to mothers who used methadone in pregnancy, sucrose did not provide a calming effect (Blass, Ciaramataro, & Barr, 1994). As methadone would competitively block opioid receptors, the finding that sucrose was less effective in this population supports the hypothesis that the effects of sucrose are opioid mediated (Blass, Ciaramataro, & Barr, 1994). The

greatest analgesic effect of sucrose is observed when administered approximately two-minutes prior to a painful procedure, which is thought to coincide with the timing of endogenous opioid release (Blass, Ciaramataro, & Barr, 1994; Johnston, Stremler, Horton, & Friedman, 1999)

While endogenous opioid-mediated analgesia is one proposed mechanism by which sucrose solution reduces procedural pain, there are studies which suggest that opioid pathways may not be the sole means of its efficacy. Full term infants who received the opioid antagonist Naloxone prior to sucrose administration still demonstrated reduced pain responses (Gradin & Schollin, 2005). Further, in a small sample of preterm newborns sucrose was not found to increase levels of beta-endorphins, an opioid agonist often used to evaluate the efficacy of opioid analgesics (Taddio, Shah, Shah, & Katz, 2003). As these findings suggest analgesic mechanisms that are not opioid mediated, it is likely that alternate mechanisms are contributing to the calming and pain-reducing effects of sucrose.

One such potential mechanism is dopamine (Holsti & Grunau, 2010). Dopamine plays a primary role in the descending modulation of acute pain, as it is released in sufficient quantity to stimulate post-synaptic receptors which results in a rapid response to stimuli (Wood, 2006). In a rat model, stimulation of the foot with a mild shock stimulus resulted in an increase in extracellular dopamine in the shell of the nucleus accumbens (Kalivas & Duffy, 1995). Further studies in humans demonstrate a role of dopamine in the supra-spinal modulation of pain (Holsti & Grunau, 2010; Magnusson & Fisher, 2000; Pertovaara et al., 2004). Acetylcholine additionally has a role in pain modulation. Through muscarinic receptors in the spinal cord and nicotinic receptors in the thalamic peri-aqueductal gray area, increases in acetylcholine have been found to reduce excitatory

amino acid release (i.e., glutamate) and increase inhibitory amino acids such as gamma-aminobutyric acid (Jones & Dunlop, 2007; Li, Chen, Pan, & Levey, 2002; Li & Eisenach, 2002).

There is a growing body of evidence that demonstrates common links between the central processing of sweet taste and the neural systems involved in pain processing. For example, studies in animals demonstrate that sugar stimulates dopamine release in the nucleaus accumbens (Colantuoni et al., 2001; Hajnal & Norgren, 2002; Hajnal, Smith, & Norgren, 2004) with this effect being associated with the potency of the sweet taste solution, as dopamine release is proportional to sucrose concentration as opposed to the solution volume (Hajnal, Smith, Norgren, 2004). This effect is demonstrated in human studies where sweeter and more concentrated solutions (e.g., 24% oral sucrose; 20 to 30% glucose [Bueno et al., 2013]) produce more robust pain-reducing effects than fructose, lactose, or 12% sucrose which have lower concentrations of sweet taste (Blass, Ciaramataro, & Barr, 1994; Stevens, Taddio, Ohlsson, & Einarson, 1997). Excessive intake of sugar has been found to not only increase mu-1 opioid receptors, but also DRD1 dopamine receptors (Colantuoni et al., 2001). With respect to acetylcholine-based sweet taste analgesic responses, rats who received increasing volumes of 10% sucrose solution over a three-week period were found to have significantly increased levels of both dopamine and acetylcholine immediately following each dose, as well as significant increases in the post-dose acetylcholine response from baseline over the course of the three week experimental period (Rada, Avena, & Hoebel, 2005). Taken together, the findings of these animal and human studies suggest that in addition to potential endogenous opioid-mediated mechanisms, both dopamine and acetylcholine likely contribute to the analgesic effects of sweet taste (Holsti & Grunau, 2010).

In addition to the discussed mechanisms postulated to act largely on receptor sites in the mid- and forebrain, there is suggestion that the pain-reducing effects elicited by oral sucrose administration may occur at the level of the brainstem. Anseloni and colleagues (2005), completed mid-collicular transections in rat pups (postnatal days 10 – 12) who subsequently received intra-oral sucrose prior to experimental exposure to mechanical pain stimuli. Control animals completed the same procedures, however, did not undergo craniotomy and were left neurologically intact. The magnitude of hindpaw withdrawal response to the mechanical von Frey hair pain stimuli was similar between rat pups who underwent transections and those who were intact, suggesting that the analgesia induced by oral sucrose administration does not require forebrain involvement and can be modulated at the level of the brainstem (Anseloni, Ren, Dubner, & Ennis, 2005).

Despite the lack of consensus regarding the mechanisms of sweet taste analgesia, it is evident from the literature that sucrose is effective in reducing behavioural and composite bio-behavioural pain scores in response to single acute procedures in infants. To date, a total of 74 randomized controlled trials have been published examining the effectiveness of sucrose in reducing acute pain response in preterm or term born neonates in comparison to placebo or other pain-reducing interventions (Stevens et al., 2016). It is important to note that while a large number of trials have been conducted in this area, few studies were able to be combined in meta-analyses in the most recent Cochrane review due to marked heterogeneity across studies (Stevens et al., 2016). Of these 74 studies, which included a total of 7,049 newborns, the majority recruited strictly full term born infants (38 studies), whereas 31 studies recruited preterm born infants and five included both preterm and term born infants (Stevens et al., 2016). To date, heel lance is the most studied with 38 studies reporting on this painful procedure. The remaining studies

examined the influence of sucrose on pain response to venipuncture ($n = 9$), retinopathy of prematurity examination ($n = 7$), intramuscular injection ($n = 4$), circumcision ($n = 4$), subcutaneous injection ($n = 2$), naso- or oro-gastric tube insertion ($n = 3$), bladder catheterization ($n = 1$), stress during echocardiography ($n = 1$), arterial puncture ($n = 1$), and multiple painful procedures in one study ($n = 4$).

Given the use of sucrose as a control intervention during heel lance procedure in this dissertation study, the following section is organized to discuss the evidence reporting on the effectiveness of sucrose in reducing pain associated with this painful procedure specifically. Further, as sweet taste concentration has been proposed to be associated with the effectiveness of oral sucrose, this section will subsequently be organized based on the sucrose concentration studied as well as the comparison intervention. While several studies compare the analgesic effects of sucrose and breastfeeding, these studies are discussed in the breastfeeding section of this literature review in the interest of clarity.

Sucrose (12 – 12.5% concentration) compared to water. To date, studies examining the efficacy of 12 – 12.5% sucrose in reducing pain response during heel lance have measured pain response based on crying time and changes in heart rate. One study conducted with full term infants ($n = 42$) found that this concentration of sucrose significantly reduced total crying time in comparison to those infants receiving water (Greenberg, 2002). In contrast, a trial examining heart rate response in full term newborns found no significant difference in percentage change in heart rate 60-seconds following heel lance between the sucrose and water intervention groups (Haouri, Wood, Griffiths, & Levene, 1995).

Sucrose (20 – 33% concentration) compared to water. When examining the effectiveness of 20 – 33% sucrose alone during heel lance on behavioural and bio-behavioural pain measures, there is inconsistent evidence for its effectiveness across individual studies. Four studies reported on Premature Infant Pain Profile (PIPP) score, a composite pain score that encompasses behavioural and physiologic responses as well as contextual factors (Stevens, Johnston, Petryshen, & Taddio, 1996; Stevens, Johnston, Taddio, Gibbins, & Yamado, 2010). The PIPP can be scored from 0 – 21 with a score of six or greater being indicative of pain and a two-point reduction being considered clinically significant (Stevens et al., 1996). Compared to water, 20 – 33% sucrose was not found to be effective in reducing PIPP scores for preterm born infants at 30-seconds and 60-seconds following heel lance procedure (Johnston, Stremler, Horton, & Friedman, 1999). While this concentration of sucrose was found to significantly reduce PIPP scores 30-seconds following heel lance in a sample of 44 full term born infants (Slater et al., 2010), this effect was not found during heel lance in a sample of 107 full term newborns of diabetic mothers undergoing their first heel lance (Taddio et al., 2008). One study utilizing the Neonatal Infant Pain Scale (NIPS), a uni-dimensional behavioural pain scale based on facial expression, body movements, cry, and breathing patterns (Lawrence, Alcock, McGrath, Kay, MacMurray, Dulberg, 1993), found that sucrose significantly reduced NIPS scores during heel lance in a sample of 56 full term infants (Tutag Lehr, Cortez, Grever, Cepeda, Thomas, & Aranda, 2015); however, it was not found to reduce behavioural responses measured as Douleur Aiguë du Nouveau-né (DAN) scores 30-seconds following heel lance (Mathai, Natrajan, & Rajalakshmi, 2006). Further, while this concentration of sucrose alone did not reduce duration of first cry in response to heel lance in full term or preterm born infants, it was found to significantly reduce full term

infant total crying time following heel lance in two studies (Isik, Ozek, Bilgen, & Cebeci, 2000; Mathai et al., 2006).

While the studies reporting the effect of 20 – 33% sucrose on behaviourally based infant pain indicators are inconsistent, they do demonstrate some positive benefit. In contrast, studies reporting physiologic outcomes demonstrate the limited efficacy of sucrose independently to reduce pain response. In studies that examined heart rate response in full term infants, sucrose was not significantly associated with a decrease in heart rate beats per minute during the heel lance procedure (Guala et al., 2001; Tutag Lehr et al., 2015), or with a significant reduction in percent change in heart rate in the 60-seconds following heel lance. Further, Tutag Lehr and colleagues (2015) found no differences in oxygen saturation, respiratory rate, or skin blood flow in perfusion units between those infants receiving sucrose and water during heel lance. Finally, one study reporting pain-related brain activity in 44 full term newborns found no significant difference between the sucrose and water groups (Slater et al., 2010a).

Sucrose (50% concentration) compared to water. While fewer studies have reported on outcomes associated with administration of 50% oral sucrose during heel lance, findings are consistent with the evidence generated by studies using 20 to 30% sucrose. Specifically, meta-analysis of two studies involving 80 infants demonstrated a significantly reduced behavioural response and duration of first cry in those infants receiving 50% oral sucrose compared to water (weighted mean difference [*WMD*] = 63.20, 95% confidence interval [CI] [-79.20, -47.19], [Stevens et al., 2016; Haouari et al., 1995; Ogawa et al., 2005]). In contrast, the one study reporting on the influence of 50% sucrose on the physiologic pain indicator of percent change in heart rate over the first

minute after heel lance, found no significant difference between the sucrose and water groups.

Sucrose (24%) plus non-nutritive sucking (NNS) compared to water plus NNS. The strongest evidence for the pain-reducing influence of sucrose is for the effect of 24% oral sucrose combined with non-nutritive sucking (which is the active sucking of a pacifier during the procedure) on PIPP scores following a heel lance. To date, three studies have been conducted comparing this intervention to non-nutritive sucking combined with water in preterm infants (Stevens et al., 1999; Asmerom et al., 2013) and both term and preterm infants (Gibbons, 2002). When combined in meta-analysis (n = 278), a significantly lower PIPP score was observed in the combined sucrose and non-nutritive sucking group compared to the water and non-nutritive sucking group at 30-seconds following heel lance (*WMD* = -1.70, 95% CI [-2.13, -1.26], [Stevens et al., 2016]). Two of these studies additionally compared the effect of these interventions on PIPP score at 60-seconds following heel lance (Gibbons et al., 2002; Stevens et al., 1999) and found a significant effect favouring sucrose and non-nutritive sucking (*WMD* = -2.14, 95% CI [-3.34, -0.94], [Stevens et al., 2016]).

With respect to the evidence quality, Stevens and colleagues (2016) completed a Grading of Recommendations Assessment, Development, and Evaluation (GRADE) assessment for the effect of 24% oral sucrose plus non-nutritive sucking on PIPP scores at 30- and 60-seconds following heel lance and reported high quality evidence. The primary purpose of completing a GRADE evidence quality assessment is to evaluate if an interventions effect on an outcome is likely to change based on future research. The GRADE assessment evaluates studies based on four criteria: 1) Risk of bias in trial methods, 2) inconsistency (i.e., heterogeneity across multiple clinical trials), 3)

imprecision (i.e., the size of the confidence interval around the mean effect), and 4) risk of publication bias (Guyatt, Oxman, Kunz, Vist, Falck-Ytter, & Schünemann, 2008). The GRADE assessment that there is high quality evidence for the effect of the combination of 24% oral sucrose and non-nutritive sucking suggests that future studies testing this intervention would continue to demonstrate similar findings. Two studies comparing 24% sucrose with non-nutritive sucking to water and non-nutritive sucking reported no significant effect on crying time following heel lance, however, there was low quality evidence for this effect (Stevens et al., 2016).

Sucrose alone compared to sucrose combined with adjuvant interventions. In their multi-center randomized controlled trial, Leng and colleagues (2015) enrolled 615 newborns to undergo either a shallow or deep heel lance procedure in one of four intervention conditions: 1) 24% sucrose, 2) 24% sucrose plus non-nutritive sucking, 3) oral sucrose combined with swaddling, or 4) oral sucrose combined with both non-nutritive sucking and swaddling. This study, which was scored as being of moderate quality (Stevens et al., 2016), demonstrated a synergistic effect of sucrose and adjuvant interventions, with the combination of sucrose, non-nutritive sucking, and swaddling producing the most analgesic effects as measured by the revised Neonatal Facial Coding Scale (*MD* = 0.43, 95% CI [0.23 – 0.63]). In addition to this finding, Leng et al. (2015) also reported that infants who received the combination of sucrose with non-nutritive sucking or the combination of sucrose, non-nutritive sucking and swaddling had significantly lower increases in heart rate and significantly more stable oxygen saturations that those infants receiving sucrose alone during heel lance (Leng et al., 2015). Those infants who received the combined interventions of sucrose and swaddling did not

demonstrate significantly different physiologic responses (i.e., change in heart rate, oxygen saturation) when compared to sucrose alone (Leng et al., 2015).

In addition to the effect of sucrose combined with non-nutritive sucking on single procedures, there is some evidence that the combination of 20% oral sucrose and non-nutritive sucking during repeated procedures provide synergistic pain-reducing efficacy. Gao and colleagues randomly assigned preterm neonates to receive 1) non-nutritive sucking ($n = 22$), 2) 0.2mL/kg of 20% oral sucrose ($n = 21$), 3) 0.2mL/kg 20% oral sucrose plus non-nutritive sucking ($n = 22$), or 4) no treatment ($n = 21$) during three repeated heel lances during neonatal intensive care hospitalization. Those infants who received 20% oral sucrose combined with non-nutritive sucking had significantly lower PIPP scores, heart rate, and percentage time crying, and significantly higher oxygen saturations during heel lance and procedure recovery compared to all other treatment arms (Gao et al., 2018).

Adverse effects of sucrose. Although studies testing sucrose for procedural pain inconsistently report immediate adverse events associated with this intervention, those reported demonstrate the number of minor adverse occurrences to be low and without evidence of major adverse events (Stevens et al., 2016). Minor adverse events included oxygen desaturation, bradycardia, and minor episodes of choking or spitting up that were all self-resolving with no need for healthcare provider intervention. Additionally, studies reported no differences in the frequency of minor adverse events between the sucrose condition and control conditions. There is no evidence for an association between sucrose administration and major adverse such as hyperglycemia (Taddio et al., 2008), necrotizing enterocolitis, or feeding intolerance (Potana, Dongara, Nimbalkar, Patek, Nimbalkar, Phatak, 2015). Thus, there is currently no evidence to suggest that short term

adverse events are a concern when using sucrose for procedural pain management (Stevens et al., 2016).

Very few studies have examined long-term adverse outcomes associated with repeated administration of sucrose, however, the American Academy of Pediatrics has cautioned the judicious use and tracking of sucrose administration for infant pain management due to limited knowledge on the appropriate dose, mechanisms of action, and long-term effects (American Academy of Pediatrics, Committee on Fetus and Newborn, & Section on Anesthesiology and Pain Medicine, 2016; Ranger, Tremblay, Chau, Holsti, Grunau, & Goldowitz, 2019). To date, only one secondary analysis of data from a clinical study has reported on long-term outcomes of repeated use of sucrose. In a sample of 107 preterm infants born less than 31 weeks, Johnston and colleagues (2002; 2007) reported that infants who received greater than ten doses of sucrose per day were prone to poorer attention and motor development outcomes in early life. One preclinical study (Tremblay et al., 2017) using a mouse model of pain and sucrose administration designed to mimic preterm neonatal exposure in neonatal intensive care randomly assigned 106 mice to one of two treatments (sterile water, 24% oral sucrose) and one of three exposures (ten times daily handling, touch, or needle prick). Irrespective of the type of exposure, mice who received repeated doses of 24% oral sucrose had smaller brain volumes in the corpus callosum, stria terminalis, fimbria, hippocampus, and cerebellum (Tremblay et al., 2017). Using a similar model, these authors went on to report that preterm born mice who received repeated 24% oral sucrose during handling in the neonatal period had poorer short-term memory in adulthood compared to mice who received water during handling (Ranger, Tremblay, Chau, Holsti, Grunau, & Goldowitz, 2019). Interestingly, when exposed to pain, there was no significant difference in

memory between the mice exposed to 24% sucrose and those exposed to water. This provides the first evidence that sucrose may not protect memory outcomes following neonatal pain, and that early repetitive sucrose exposure in the absence of pain may result in poorer memory outcomes (Ranger et al., 2019). However, as these studies have examined outcomes associated with repeated sucrose dosing in prematurity as opposed to the full term infant population who may have a lower exposure, further research and clinical outcome studies are warranted (Stevens et al., 2016). In particular, studies that monitor and control for the potential confounding influence of key factors (e.g., maternal exposure, impact of early sweet taste exposure on weight gain and overall growth) are needed to strengthen the clinical implications drawn from such studies.

Summary of evidence for sucrose administration during heel lance. The findings of the literature to date demonstrate positive effects for sucrose in reducing responses to pain, measured using behavioural and bio-behavioural pain scores, with less evidence for an effect on physiologic responses to pain. This effect is particularly evidenced in 24% sucrose concentration when paired with adjuvant interventions, most notably non-nutritive sucking.

Breastfeeding Analgesia

Both direct breastfeeding and provision of expressed maternal breast milk have been studied extensively as analgesic interventions for newborn acute pain. In their most recent Cochrane systematic review and meta-analysis, Shah and colleagues (2012) reported on twenty studies for a total sample of 2,071 healthy full term neonates who directly breastfed (10 studies, $n = 1,075$) or received expressed breast milk (10 studies, $n = 996$) during acute painful procedures such as venipuncture and heel lance. Of the ten studies included in this systematic review examining direct breastfeeding (Carbajal,

Veerapen, Couderc, Jugie, & Ville, 2003; Efe & Savaşer, 2007; Codipietro, Ceccarelli, & Ponzone, 2008; Gradin et al., 2004; Gray, Miller, Philipp, & Blass, 2002; Phillips, Chantry, & Gallagher, 2005; Leite, Linhares, Lander, Castral, dos Santos, & Scochi, 2009; Okan, Ozdil, Bulbul, Yapici, & Nuhoglu, 2010; Shendurnikar & Gandhi, 2005; Weissman, Aranovitch, Blazer, & Zimmer, 2009), those examining the influence of breastfeeding on physiological and uni-dimensional behavioural measures of infant pain demonstrated consistent findings. Specifically, breastfeeding neonates demonstrated significantly lower heart rates, proportion crying time, duration of first cry, and total crying time compared to infants who were swaddled, held by their mothers, or received oral sucrose, a pacifier, placebo, or no intervention during the procedure (Shah et al., 2012).

With respect to validated behavioural and bio-behavioural measures of infant pain, Premature Infant Pain Profile (PIPP) scores were significantly lower in infants who were directly breastfeeding during heel lance or venipuncture when compared to those who were positioned in their mothers' arms or received oral sucrose or a placebo (Shah et al., 2012). Similarly, Douleur Aiguë du Nouveau-né (DAN) scores were significantly lower in those infants who were breastfeeding during painful procedures compared to those infants who were held in their mothers' arms or received a placebo. However, there was no significant difference in DAN scores between breastfeeding infants and those receiving oral glucose. Similarly, while Neonatal Infant Pain Scale (NIPS) scores were lower for breastfeeding infants compared to infants with no intervention, there was no significant statistical difference in NIPS scores between breastfeeding infants and those who received oral sucrose. Finally, while Neonatal Facial Coding System (NFCS) scores were lower in the breastfeeding group when compared to oral glucose, pacifier use,

maternal holding, or no intervention, breastfeeding was not statistically significantly more effective than provision of formula (Shah et al., 2012). A moderate level of evidence quality based on the GRADE criteria was reported for all of the studies examining the influence of direct breastfeeding.

In comparison to direct breastfeeding, there was considerable variability across the ten studies examining provision of expressed breast milk for pain relief. Expressed breast milk reduced heart rate, duration of crying, and behavioural pain response measured using the NFCS when compared to placebo. In contrast, oral sucrose in 12.5%, 20%, and 25% concentrations; pacifier use, rocking, and no intervention were more effective than breast milk in reducing duration of crying and heart rate (Shah et al., 2012). Furthermore, expressed breast milk was not effective in reducing NIPS, NFCS, and DAN scores (Shah et al., 2012). Taken together the authors of this review reported that direct breastfeeding is clearly superior when compared to the provision of expressed breast milk for procedural pain relief in the full term infant population. However, direct breastfeeding and sweet taste demonstrate similar levels of effectiveness with respect to reducing pain responses measured using behavioural and bio-behavioural measures.

Since the publication of the most recent Cochrane review of the evidence for breastfeeding and breast milk feeding for pain, the number of studies reporting on the use of direct breastfeeding for acute pain management in full term newborns has increased to 24 studies (n = 2,658 infants, Appendix A). Furthermore, there has been a substantial emergence of studies examining the use of breastfeeding and expressed breast milk feeding for procedural pain management in preterm newborns. The following section will provide a description of the findings of the most recent studies examining the use of

breast milk and direct breastfeeding for pain management in both preterm and full term infants.

There is inconsistent evidence for the analgesic efficacy of direct breastfeeding or providing expressed breast milk to preterm infants. Provision of expressed breast milk was not as effective as sweet taste in most studies (Bueno, Stevens, de Camargo, Toma, Krebs, & Kimrua, 2012; Simonse, Mulder, & Beek, 2012; Skogsdal, Eriksson, & Schollin, 1997; Ou-Yang, Chen, Chen, Chung, Chen, & Huang, 2013), however, it had the same effect as sucrose in two studies (Collados-Gómez et al., 2018; Rodriques, Nesargi, Fernandes, Shashidhar, Rao, & Bhat, 2017) and was found to reduce risk of moderate to severe pain following heel lance when combined with non-nutritive sucking and facilitated tucking (Peng et al., 2018). Two studies examined the analgesic effect of breastfeeding or breast milk on pain associated with retinopathy of prematurity (ROP) examinations. Rosali and colleagues (2015) combined expressed breast milk with standard pain care (anesthetic drops, swaddling, and nesting) and found that the addition of breast milk significantly reduced pain scores when compared to standard care alone (Rosali, Nesargi, Mathew, Vasu, Rao, & Bhat, 2015). Similarly, Taplak and Erdem (2017) found that infants who received breast milk or sucrose prior to the ROP examination had lower PIPP scores during the exam, and breast milk fed infants had improved physiologic recovery following the procedure. In the one study found reporting on direct breastfeeding in preterm infants born from 30 – 36 weeks gestational age, there was no significant difference between breastfeeding and non-nutritive sucking on pain response measured using the Behavioral Indicators of Infant Pain (BIIP) scale and heart rate during heel lance (Holsti, Oberlander, & Brant, 2011). However, more mature

breastfeeding behaviours, as measured by the Primary Infant Breastfeeding Behaviours Scale (PIBBS), were associated with lower BIIP scores.

In contrast, the majority of additional studies examining direct breastfeeding in full term newborns report consistent and positive effects for reducing the pain associated with heel lancing and intramuscular injections. Two studies compare direct breastfeeding to topical anesthetics during intramuscular injection (Boroumandfar, Khodaei, Abdeyazdan, & Maroufi, 2013; Gupta, Upadhyay, Agarwal, Goswami, Kumar, & Sreenivas, 2013), four additional studies compare breastfeeding to sweet taste interventions (Bembich, Cont, Causin, Paviotti, Marzari, & Demarini, 2018; Bembich, Davanzo, Brovedani, Clarici, Massaccesi, & Demarini, 2013; Goswani, Upadhyay, Gupta, Chaudhry, Chawla, Sceenivas, 2013; Rioualen, Durier, Hervé, Misery, Sizun, & Roué, 2018), and two compare breastfeeding to skin-to-skin contact (Fallah, Naserzadeh, Ferdosian, & Binesh, 2016; Marin Gabriel et al., 2013). Finally, three new studies compare breastfeeding to simple holding (Erkul & Efe, 2017; Modarres, Jazayeri, Rahnama, & Montazeri, 2013; Obeidat & Shuriquie, 2015; Thomas, Shetty, & Bagali, 2011), one to music therapy (Zhu et al., 2015), and one to infant massage (Zargham-Boroujeni, Elsagh, & Mohammadizadeh, 2017). As the findings of these studies have not previously been synthesized, the following section of this literature review will discuss the results of these studies to supplement the existing knowledge for the effect of breastfeeding during painful procedures in full term newborns.

Breastfeeding versus massage. One study randomly assigned 75 full-term infants to either breastfeeding ($n = 25$), infant massage ($n = 25$), or no treatment ($n = 25$) during venipuncture (Zargham-Boroujeni, Elsagh, & Mohammadizadeh, 2017). Infants who were in the massage group had a significantly lower mean NIPS score in the first 30-

seconds following the venipuncture compared to breastfed infants or those who received no treatment. However, the authors noted that the procedure was completed two-minutes after breastfeeding or two-minutes after three-minutes of massage. Therefore, it is unclear if the infants were actively breastfeeding or receiving the massage intervention at the time of the procedure to allow for optimal pain-reducing efficacy.

Breastfeeding versus music therapy. A single randomized controlled trial of 288 healthy full term newborns compared the influence of music therapy (i.e., listening to classical music a minimum of five-minutes prior to procedure), breastfeeding (initiated five-minutes prior to procedure with no maternal skin-to-skin contact), or the combination of these interventions on Neonatal Infant Pain Scale (NIPS) scores, cry duration, and latency to cry following a heel lance (Zhu et al., 2015). The authors reported that infants who were breastfeeding, both with or without music, demonstrated significantly lower NIPS scores that those who received music therapy alone (Zhu et al., 2015). Furthermore, infants who were breastfeeding had significantly longer latency to first cry and shorter duration of first cry compared to those receiving music therapy. Thus, the findings of this study suggest that when compared to breastfeeding, music therapy is an ineffective intervention for the reduction of procedural pain in full term newborns.

Breastfeeding versus topical anesthetics. In a sample of 144 full term born infants undergoing intramuscular injection for immunization at either two months, four months, or six months of age, infants who were breastfeeding during the procedure demonstrated a significantly higher frequency of painless injections as measured using the Neonatal Infant Pain Scale (NIPS) when compared to those infants who received a vapocoolant spray at the site of injection or no intervention (Boroumandfar et al., 2013). A similar finding was demonstrated by Gupta and colleagues (2013) who assigned 90

healthy full term infants less than three months of age to receive 1) EMLA (*E*utectic *M*ixture of *L*ocal *A*nesthetics) plus breastfeeding, 2) EMLA plus water, or 3) placebo cream plus water before a wDPT immunization. Those infants who received the combined intervention of EMLA and breastfeeding demonstrated significantly lower Modified Facial Coding System (MFCS) scores and shorter duration of cry than those infants receiving EMLA alone or placebo, suggesting synergistic effects of breastfeeding and topical anesthetics during immunization (Gupta et al., 2013).

Breastfeeding versus sweet taste interventions. Recent studies comparing breastfeeding to sweet taste analgesia demonstrate findings consistent with those previously synthesized in the literature. For example, duration of cry, latency of cry, and MFCS scores were consistent between those infants who were breastfed during intramuscular injection and those who received 25% dextrose solution (Goswani et al., 2013). However, both of these interventions (i.e., breastfeeding or receiving 25% dextrose two minutes prior to injection) were found to significantly reduce duration of cry and latency of onset of cry compared to placebo (Goswani et al., 2013). Furthermore, a randomized controlled trial including 30 healthy full term newborns demonstrated that Neonatal Infant Pain Scale (NIPS) was significantly reduced in those infants who were breastfed two minutes before heel lance compared to those receiving 20% oral glucose two minutes prior to procedure (Bembich et al., 2013). In this trial, cortical activation measured using Near Infrared Spectroscopy (NIRS) was reported in addition to the NIPS score. Infants who were breastfeeding during the procedure demonstrated significant increases in oxygenated hemoglobin over the left superior sensorimotor cortex, left somatosensory cortex, right superior sensorimotor cortex, right posterior-superior frontal cortex, and the right posterior parietal cortex (Bembich et al., 2013). Bembich and

colleagues (2018) went on to report similar findings in a subsequent randomized controlled trial where 80 healthy full term neonates were randomly assigned to one of four interventions during a heel lance: 1) 20% oral glucose alone, 2) expressed breast milk alone, 3) 20% oral glucose combined with maternal holding, or 4) breastfeeding. Similar to their previous work, infants who were breastfeeding during heel lance demonstrated the most robust cortical response with bilateral diffuse activation across regions of the sensorimotor cortex, whereas, they had the lowest NIPS scores (Bembich et al., 2018). Infants who received maternal care (either direct breastfeeding or maternal holding combined with 20% glucose) had the lowest NIPS scores compared to glucose or breastmilk alone.

While examination of multi-modal behavioural and neurophysiological responses to pain in the context of breastfeeding is highly interesting and novel, a limitation of this work is that hemodynamic activation of the sensorimotor cortices would be anticipated during breastfeeding due to the sensory and motor stimulation associated with close maternal contact and oral movements. Thus, the influences of breastfeeding on pain-related activity isolated from the confounding activity in this intervention group is difficult to discern without an established pain-related response over and above the response elicited by breastfeeding. For example, Rioualen and colleagues (2018) reported that infants who were randomized to breastfeeding during venipuncture, compared to infants who were held and received sucrose, had higher pain behaviour and exhibited similar 'pain-specific cortical activity' (Rioualen et al., 2018, p. 653). However, in this paper there was no significant difference in the reported NIRS response (total hemoglobin concentration) between the painful venipuncture and a baseline period where the infants were in their assigned intervention group. Thus, the specificity of the

pain measure in this study design warrants evaluation and the current evidence would suggest that breastfeeding is as effective or potentially a more effective intervention than sweet taste for full term infants undergoing acute procedures.

Breastfeeding versus simple holding and direct skin-to-skin contact. Those studies comparing breastfeeding to being held without skin-to-skin contact during painful procedures all demonstrate that breastfeeding is significantly more analgesic (Erkul & Efe, 2017; Modarres et al., 2013; Obeidat et al., 2015; Thomas et al., 2011). Pain associated with intramuscular injection measured using the DAN (Modarres et al., 2013) and the NIPS (Erkul & Efe, 2017; Thomas et al., 2011) was found to be significantly lower when infants were actively breastfeeding two to five minutes prior to and during immunization when compared to simply being held in their mothers' lap during the procedure. Furthermore, in a sample of 128 healthy full term newborns undergoing a heel lance for routine metabolic screening, initiating breastfeeding two minutes prior to lance and sustaining feeding throughout the procedure was found to significantly reduce PIPP scores compared to maternal holding (Obeidat et al., 2015).

In studies that compared breastfeeding to direct maternal-infant skin-to-skin contact (SSC), breastfeeding continues to appear superior for pain management in full term newborns. Marin Gabriel and colleagues (2013) first compared these interventions in a randomized controlled trial of 136 healthy full term newborns who were randomly assigned to one of four intervention conditions: 1) Maternal SSC and breastfeeding (five minutes prior to procedure), 2) maternal SSC (initiated five minutes prior to procedure) and 24% oral sucrose (administered two minutes prior to procedure), 3) 24% oral sucrose alone, or 4) maternal SSC alone. Median NIPS scores and the percentage of time that the neonates were scored as having moderate to severe pain were significantly lower in the

maternal SSC and breastfeeding group compared to the other groups. Furthermore, both the maternal SSC and breastfeeding and maternal SSC and sucrose groups demonstrated a significantly lower percentage of crying compared to the SSC alone group (Marin Gabriel et al., 2013), suggesting a potential additive effect of combining these interventions. Breastfeeding was further demonstrated to be significantly more effective in reducing pain response measured using the NIPS and cry duration than maternal SSC or swaddling initiated ten minutes prior to immunization in healthy full term infants (Fallah et al., 2016). While it is unclear if infants were held in SSC during breastfeeding in this study (Fallah et al., 2016), it is likely that the combination of maternal SSC and breastfeeding provide the greatest benefit based on the hypothesized underlying mechanisms of breastfeeding as a pain-reducing intervention. These factors, along with the theoretical framework that was used to guide this work, will be discussed in a subsequent section of this literature review.

Adverse effects of breastfeeding. Unlike the administration of sucrose during pain, which has reported potential minor adverse effects, no studies to date have reported on adverse effects associated with breastfeeding during painful procedures (Marin Gabriel et al., 2013; Shah et al., 2012). With the exception of the rare risk of transmission of microorganisms from mother to infant (Shah et al., 2012), breastfeeding has only been associated with optimizing newborn outcomes, including improved immunological function (Kramer & Kakuma, 2012), improved developmental outcomes (Kramer et al., 2008), reduction in obesity risk (Ip et al., 2007; Owen et al., 2005), and reduced risk of diabetes (Das, 2007; Ip et al., 2007; Rosenbauer et al., 2008), celiac disease (Akobeng et al., 2006), and inflammatory bowel disease (Barclay et al., 2009). Thus, given that breastfeeding has demonstrated efficacy that is equal to or greater than sucrose in

reducing behavioural and physiological responses to pain in full term infants, there is no evidence to suggest that it should not be utilized as a pain-reducing intervention in this population.

Infant Pain Assessment

As is evidenced from the previous sections of this literature review, there is a great deal of variability in the measures used to assess infant pain responses and the efficacy of pain-relieving interventions. Assessing pain is a critical prerequisite to effective pain management, as it is required to initiate appropriate pain-relieving interventions and evaluate their effectiveness. Given this, accurate and feasible pain assessment measures need to be included in health care practice policies to support clinicians in providing quality care to infants during painful events (Lee & Stevens, 2014). Despite the development and clinical application of approximately four dozen pain assessment tools over the last thirty years (Lee & Stevens, 2014; Meesters, Dilles, Simons, & van Dijk, 2019), there continues to be no gold standard for measuring infant acute pain. This is evidenced by the considerable diversity of pain assessment tools and indicators used in research and clinical practice and needs to be considered when interpreting study findings to inform clinical care and future research. The following section of this literature review will highlight the notable strengths and limitations of physiological, behavioural, and neurophysiologic pain assessment methods providing rationale for the outcome measures examined in this study.

Physiologic indicators. The majority of the infant pain assessment tools that have been developed to date encompass either physiological responses, behavioural responses, or combine both into a composite measure (Lee & Stevens, 2014; Ranger, Johnston, & Anand, 2007). Physiological responses that are commonly examined as an approach to

pain measurement include charges in heart rate, blood pressure, oxygen saturation, cortisol, and skin conductance. As heart rate and oxygen saturation monitoring is prevalent in the hospital setting, these physiologic indicators are commonly evaluated as sole indicators or in combination with behavioural indicators in a composite measure (Lee & Stevens, 2014; Waxman, Pillai Riddell, Tablon, Schmidt, Pinhasov, 2016). Cardiac indices in particular have been extensively studied with diverse measures being reported in the published literature to quantify heart rate response to a painful event, including mean heart rate, minimum and maximum heart rate, heart rate change, and heart rate variability (Waxman et al., 2016). It is well established that heart rate typically increases following painful procedures and that heart rate reactivity and recovery is a commonly used proxy of autonomic nervous system control following a painful event (Oberlander, Grunau, Pitfield, Whitfield, & Saul, 1999; Waxman et al., 2016). However, while commonly used to quantify infant responses to painful procedures, such physiologic measures are not without limitations. In particular, physiologic measures such as heart rate do not selectively respond to pain but rather vary with other physiologic threats and contextual factors such as stress (Johnston et al., 1999; Stevens, Johnston, & Horton, 1993), gestational and postnatal age (Chatow, Davidson, Reichman, & Akselrod, 1995; Oberlander, Grunau, Whitfield, Fitzgerald, Pitfield, & Saul, 1999; Waxman, Pillai Riddell, Tablon, Schmidt, & Pinhasov, 2016), and proximity of prior exposure to painful or tactile stimulation (Holsti, Grunau, Whifield, Oberlander, & Lindh, 2006). Furthermore, quantification of such indicators often requires equipment and statistical analyses to meaningfully interpret responses, limiting their application outside of the context of research in hospital settings. Thus, while a valuable indicator of potential pain

reactivity and regulation, there is ongoing study of potential measures that have greater specificity and accessibility.

Behavioural indicators. Behavioural responses to pain have been well characterized in infants, and include facial actions (e.g., brow bulge, eye squeeze, naso-labial furrow), body movements (e.g., flexion of fingers and toes), and qualities of infant cry (e.g., onset, pitch, duration). Research suggests that behavioural responses, particularly facial actions, are the most sensitive pain indicators in infants (Craig, et al., 1993). Specifically, they are more likely to selectively respond to pain when compared to physiological responses, such as heart rate and oxygen saturation, which fluctuate in response to both pain and non-pain related events such as hunger or stress (Johnston et al., 1999; Stevens, Johnston, & Horton, 1993). Facial actions have been found to account for the greatest amount of variability in acute pain response when included in an exploratory factor analysis of 19 behavioural and physiological infant pain indicators (Stevens et al., 2007).

Numerous uni-dimensional behavioural pain measures have been developed and validated, such as the widely utilized Neonatal Facial Coding System (Grunau & Craig, 1987), Douleur Aiguë du Nouveau-né (Carbajal et al., 1997), and Modified Behavioural Pain Scale (Taddio et al., 1995). However, a limitation of such measures is that they do not take into consideration contextual factors. It is now well recognized that contextual factors, such as previous pain exposure and gestational age, differentially influence facets of infant pain responding. For example, Johnston and colleagues (1999) reported an absence of behavioural and physiological pain response to a normally painful heel lance procedure in 20% of infants. The authors of this study found that newborns who were younger, asleep during the procedure, and had undergone a painful event more recently

were less likely to mount responses to pain (Johnston et al., 1999). There are now multiple studies that suggest that gestational age (e.g., Johnston et al., 1999; Johnston, Stevens, Yang, & Horton, 1995) and exposure to previous pain (e.g., Johnston & Stevens, 1996; Taddio, Shah, Atenafu, & Katz, 2009; Taddio, Shah, Gilbert-MacLeod, & Katz, 2002) influence infant pain responses to subsequent procedures.

Bio-behavioural indicators. Composite multi-dimensional pain measures that encompass behavioural, physiological, and contextual factors (e.g., gestational age, behavioural state) have been developed in an attempt to address the limitations of uni-dimensional measures. Such bio-behavioural tools, including the Premature Infant Pain Profile – Revised (Stevens et., 2014) and Neonatal Infant Pain Scale (Lawrence et al., 1993), have been extensively psychometrically validated and are now considered the most representative measure of infant acute pain (Lee & Stevens, 2014). However, these tools continue to have recognized limitations. Behavioural and physiological responses are often dissociated and poorly correlated, making the results difficult to interpret (Barr, 1998; Stevens, Johnston, & Horton, 1993; Johnston et al., 1995). Furthermore, the responses measured using such tools may continue to be non-specific to pain. Thus, continued exploration of indicators that are sensitive and specific to pain, that can be used in conjunction with or to refine the assessment tools developed to date, is needed to advance the measurement and understanding of infant pain responding and the effectiveness of analgesic interventions.

Neurophysiologic indicators. Use of neurophysiologic imaging methods is a novel and emerging trend in the field of infant acute pain assessment. A growing number of studies are using non-invasive neuroimaging techniques, such as near infrared spectroscopy (NIRS), functional magnetic resonance imaging (fMRI), and

electroencephalography (EEG) to examine responses to acute painful procedures in the infant brain (Fitzgerald, 2015; Hartley & Slater, 2014; Holsti, Grunau, & Shany, 2011; Ranger, Johnston, & Anand, 2007). The findings of studies utilizing each of these outcome measures will be synthesized in the following sections to provide rational for the selected neurophysiologic outcome measure that was used in this study.

Functional magnetic resonance imaging (fMRI). Functional magnetic resonance imaging captures cortical hemodynamic changes, through measurement of the magnetic change of hemoglobin between oxygenated and deoxygenated states, with the most common method being a blood oxygen level dependent (BOLD) form of imaging (Logothetis, Pauls, Augath, Tinath, & Oeltermann, 2001; Logothetis & Pfeuffer, 2004; Newman, 2013; Ogawa, Menon, Kim, & Ugurbil, 1998; Ogawa et al., 1993). The use of fMRI to assess pain in newborns is an emerging trend, with only two studies being published to date (Goksan et al., 2015; Williams et al., 2015). Both of these studies aimed to characterize the BOLD response in full term newborns in response to experimental stimuli such as Von Frey hair filaments (Williams et al., 2015) and PinPrick nociceptive stimulators (Goksan et al., 2015; Williams et al., 2015). Both studies found that infants demonstrated unique patterns of activation over the cerebral cortex in response to painful stimulation. In Goksan and colleagues' work (2015), which compared patterns of activation between infants and adults when exposed to experimental pain stimuli, the BOLD response to experimental stimuli was similar between infants and adults, with infants demonstrating activation in all but two of the 20 brain regions activated in adults (Goksan et al., 2015). Interestingly, the two areas that were not activated in infants in this study were the amygdala and the orbitofrontal cortex – areas which may contribute to the interpretation and contextualization of the painful stimuli

(Goksan et al., 2015; Kahnt, Heinzle, Park, & Haynes, 2010; Simons, Moulton, Linnman, Carpino, Becerra, & Borsook, 2014). Both studies reported bilateral and diffuse patterns of activation (Goksan et al., 2015; Williams et al., 2015), which was unique when compared to adults and was attributed to immaturity of cortico-cortical and interhemispheric pathways in infants (Goksan et al., 2015; Kostović & Jovanov-Milošević, 2006). Taken together, the literature to date supports that the brain regions responsible for encoding sensory pain responses are active in infants, and that the patterns of brain activation exhibited following pain exposure are consistent with those patterns seen in adults (Goksan et al., 2015).

While fMRI appears to be a promising outcome measure when quantifying patterns of brain activation following experimental painful stimuli, it is limited with respect to feasibility to assess pain associated with the common acute clinical procedures that infants undergo. Scanners are expensive to purchase and maintain, are not easily portable, require long data collection periods, and require that subjects remain still to minimize artifacts and provide reliable and interpretable data (Crosson et al., 2010). These issues limit the feasibility of obtaining fMRI data to assess pain response to clinically required procedures and may require that infant participants be sedated during scans, which can reduce the intensity of the pain-related activations observed (Williams et al., 2015). Thus, while this outcome provides valuable information regarding the neuroanatomical localization of areas of activation following pain exposure in the infant brain, alternate neurophysiologic outcome measures are needed to feasibly assess acute pain responding in clinical care contexts.

Near infrared spectroscopy (NIRS). Studies using NIRS, which utilizes near infrared light omitting and absorbing optodes to measure subtle changes in brain tissue

oxygenation (Owen-Reece, Smith, Elwell, & Goldstone, 1999; Ranger, Johnston, Limperopoulos, Rennick, & Plessis, 2011), have reported inconsistent findings when attempting to measure infant pain responses with this technology. Specifically, of those studies that have utilized this measure to simply characterize infant cortical hemodynamic responses during clinical procedures such as venipuncture (Bartocci, Bergqvist, Langercrantz, & Anand, 2006; Ozawa, Kanda, Hirata, Kusakawa, & Suzuki, 2011), heel lance (Slater, Cantarella, Gallella, Worley, Boyd, Meek, & Fitzgerald, 2006; Slater, Cantarella, Franck, Meek, & Fitzgerald, 2008), and chest tube removal (Ranger, Johnston, Rennick, Limperopoulos, Heldt, & du Plessis, 2013), all identified a significant hemodynamic brain-based pain response over the somatosensory cortex. However, all of the studies reported on different outcomes associated with the NIRS signal (e.g., changes in oxyhemoglobin, deoxyhemoglobin, total hemoglobin) making results difficult to interpret and compare across studies.

Studies utilizing NIRS as an outcome measure when testing analgesic interventions also demonstrate inconsistent findings. For example, while the use of 30% glucose was found to result in a significant increase in cerebral blood flow during venipuncture compared to sterile water in a sample of full term infants in one study (Beken et al., 2014), another demonstrated that cerebral blood flow does not appear to be altered by administration of 50% sucrose prior to heel lance (Bucher, Moser, Siebenthal, Keel, Wolf, & Gabriel, 1995). Breastfeeding infants have been found to have greater oxyhemoglobin responses in the somatosensory and sensorimotor cortices than those receiving glucose (Bembich et al., 2013; Bembich et al., 2018), whereas maternal-infant skin-to-skin contact was found to result in significantly reduced changes in oxyhemoglobin in comparison to sucrose administration (Olsson et al., 2015). Across all

of the intervention-based studies, there is complete dissociation between the NIRS response and bio-behavioural response to pain (e.g., increased NIRS activation was not associated with a corresponding increase in bio-behavioural pain score). For example, as previously noted, Bembich and colleagues (2013; 2018) have compared pain responses utilizing both NIRS and a behavioural pain scale (i.e., NIPS) between infants who were breastfeeding and those who received oral glucose. They found that while breastfeeding significantly reduced NIPS scores when compared to glucose (2013; 2018) and expressed breast milk (2018), activation over the somatosensory and motor cortices were greater in breastfeeding infants (2013; 2018). However, a major limitation of NIRS as an indirect hemodynamic measure of neuronal activation is that it is particularly vulnerable to the influence of factors that confound pain-related activation (Ranger et al., 2011; Wolfberg & du Plessis, 2006). The authors of these studies did not isolate pain-related NIRS activity from the baseline somatosensory and motor activity that would be anticipated to be associated with the breastfeeding intervention. It is therefore likely that the heightened hemodynamic brain response found in breastfeeding infants is a result of the sensory and motor activity associated with the intervention itself rather than a heightening of pain response. Thus, further research in brain-based assessment of newborn pain should utilize experimental designs and indicators that allow for the isolation of pain-related activity.

Electroencephalography (EEG). Electroencephalography is being used with increasing frequency to assess infant pain responses, with the majority of the studies identified in the literature reporting on event-related potentials associated with clinically required acute procedures (Benoit, Martin-Misener, Newman, Latimer, Campbell-Yeo, 2017). Event-related potentials (ERPs) are scalp recorded electrical neural signals that

occur at a particular time and are functionally related to an experimental stimulus (Luck, 2013). By time-locking an EEG recording to clinically required heel lancing (Fabrizi et al., 2011; 2016; Hartley et al., 2015; 2016; Jones et al., 2017; Maitre et al., 2018; Slater et al., 2010a; 2010b; 2010c; Verriotis et al., 2016; 2018) or venipuncture (Verriotis et al., 2015), several labs have isolated a 'nociceptive-specific' ERP using analysis procedures of Principal Component Analysis (PCA) or peak-to-peak amplitude detection. Principal component analysis is a statistical technique to reduce the data from the stimulus epochs into principal component (PC) waveforms representing systematic variation in the amplitude of the EEG signal over time. In contrast, peak-to-peak amplitude detection involves the manual identification of the difference between the maximum positive and maximum negative amplitudes of an EEG.

Regardless of analysis procedure, a pain-related potential was identified in all studies examining ERPs. Several studies demonstrated that this ERP was visible in individual infants in response to a single painful event, found that the ERP was maximal at the central electrode sites of Cz and CPz, and was characterized as a brief positive potential that peaked at approximately 500-milliseconds following the painful stimulus (Slater et al., 2010b; 2010c). Initially characterized in healthy full term infants (Slater et al., 2010a; 2010c), it has been further demonstrated in preterm born infants (Slater et al., 2010b), has been reported to emerge as a discriminant nociceptive sensory response at approximately 33 – 35 weeks' gestational age (Fabrizi et al., 2011; Green et al., 2019), corresponds to the emergence of pain-related facial expressions (Green et al., 2019), and increases in amplitude with increasing gestational age (Hartley et al., 2016). Further, it was reported to be significantly larger in preterm born infants when compared to full term born infants at the same post-menstrual age (Slater et al., 2010c). Such brain activity has

been recorded in infants up to one year old, with recent research suggesting that infants demonstrate an event-related complex in response to immunization pain at one to two months of age, and that this complex is significantly greater in infants at 12-months of age (Verriotis et al., 2015). The potential has further been described as being related to stimulus intensity (Hartley et al., 2015) and stress (Jones et al., 2017), reproducible in individual infants undergoing repeated procedures (Verriotis et al., 2015), and independent of infant sleep state (Slater et al., 2010c).

Studies examining the effectiveness of pain-relieving interventions in reducing nociceptive electrophysiologic responses in the brain have found dissociation between electrophysiologic, physiologic, and behavioural responses to pain in infants receiving sucrose (Slater et al., 2010a) and general anaesthesia (Hartley et al., 2014). For example, Slater and colleagues (2010a) enrolled 59 newborn infants in a double blind, randomized controlled trial and found that while 24% oral sucrose reduced behavioural pain response measured using the Premature Infant Pain Profile, it did not significantly reduce the amplitude of the pain-related ERP isolated on neonatal EEG recording when compared to those infants receiving sterile water (Sucrose: $M = 0.10$, 95% CI [0.04 – 0.16], sterile water: $M = 0.08$, 95% CI [0.04 – 0.12]; $p = 0.46$). While this study was underpowered to detect an intervention effect on the primary outcome of the pain-related ERP, the authors suggested that sucrose may have a sedative rather than analgesic effect, prompting the need for further investigation of interventions to effectively manage acute pain in infants. This research group subsequently initiated a randomized controlled trial to examine the analgesic efficacy of morphine for retinopathy of prematurity (ROP) examinations and clinical heel lancing in preterm infants by measuring pain-elicited behaviour and noxious-evoked brain activity (Hartley et al., 2018). Morphine was not found to be significantly

different than placebo in reducing noxious-evoked brain activity following heel lance (Morphine: median = 0.99, IQR [0.40 – 1.56]; placebo: median = 0.75, IQR [0.33 – 1.22], median difference = 0.25, 95% CI [-0.16 – 0.80]; $p = 0.25$). The authors reported a required sample size of 132 infants to detect a clinically meaningful reduction of co-primary outcomes of PIPP-R score and noxious-evoked brain activity. However, the study was stopped due to safety concerns surrounding morphine administration and a sample of 31 infants were recruited. Thus, this finding it likely attributable to type II error and interpretations of analgesic efficacy should be cautioned (Campbell-Yeo & Benoit, 2018).

Although numerous neurophysiologic imaging methods have been used to quantify pain responding in the literature to date, there are several benefits of using EEG ERPs in future research aimed at better understanding the relationship between behavioural pain responses, neurologic pain responses, and analgesic intervention efficacy. One of the greatest advantages of utilizing EEG is its high temporal resolution (Luck, 2013). As EEG is a direct measure of neural electrical response to a stimulus, it can capture electrical response in fractions of a millisecond (Luck, 2013; Lystad & Pollard, 2009). The ability to continuously record EEG data in real time allows researchers to accurately study the time course and relationship between neural and behavioural responses to painful stimuli. Furthermore, EEG is relatively inexpensive, is easily portable, and is non-invasive, making it possible to utilize in clinical settings to measure infant responses to medically required procedures (Lystad & Pollard, 2009). A current priority in infant pain assessment research is to utilize neurophysiologic measures as a means of advancing the appropriate utilization and interpretation of behavioural measures for use in clinical practice. Thus, the high temporal resolution and clinical

utility of EEG lends well to being collected in combination with behavioural data to advance understanding of how infants process and respond to clinically induced pain. Furthermore, the quantification of EEG ERPs as a neurophysiologic indicator of infant pain has been completed with methodological consistency across studies, complying with reporting standards for publication of ERP studies (Picton et al., 2000), and has consistently described a pain-related marker of central nervous system pain processing. While there are two published studies that attempted to quantify infant emotional response to painful stimuli using left-right EEG asymmetry, they found no evidence for reliable pain-related changes in EEG power or asymmetry indices, despite the detection of pain using behavioural measures (Maimon et al., 2013; Norman et al., 2008). Thus, in the literature to date, ERPs appear to provide the most promise as an EEG measure of pain-related activity in the infant brain.

The value of ERPs as bio-markers has been discussed in the literature (Luck et al., 2011). Luck and colleagues (2011) suggest that ERP components that have been established as sensitive and reliable responses to a particular stimulus provide a means by which to examine the influence of interventions in clinical trials. As ERPs are direct indicators of neurotransmission, they are valuable for assessing the efficacy of interventions in reducing the magnitude of pain response in the infant brain. Specifically, any effect that a treatment has on ERPs must reflect a change in neural activity, whereas the influence of a treatment on fMRI BOLD or NIRS activity may reflect changes in the hemodynamic response without modifying the underlying neural activation (Luck, 2011). Excess neuronal excitation leading to cell death in the developing infant brain is one hypothesized mechanism by which pain exposure results in adverse neurodevelopmental and behavioural outcomes (Anand & Scalzo, 2000). Thus, such a direct measure of

neuronal activation is critical for evaluating the efficacy of pain-relieving interventions in reducing transmission of nociceptive information to the brain, and their potential for preventing the adverse outcomes associated with pain exposure in infants.

Maternal acceptability. Neonatal electroencephalography is being used with increasing frequency to quantify infant nociceptive processing and the effects of pain-reducing interventions on responses to painful events. However, despite the increased use of this measure in research, no studies have specifically sought and reported on parental perceptions and acceptance of using this technology as a pain indicator. While developmentally sensitive application of neonatal EEG electrodes to the infant scalp is non-painful, it is inherently more invasive and requires more application time than the methods used to monitor other physiological indicators (such as heart rate) or infant behaviour. Previous randomized controlled trials examining the effect of pharmacologic interventions such as 24% oral sucrose (Slater et al., 2010a) and morphine (Hartley et al., 2018) on electrophysiologic brain responses and bio-behavioural pain scores have had high refusal rates of approximately 50%. This is in contrast to the relatively low refusal rates (less than 20%) in those studies examining impact of non-pharmacologic and pharmacologic interventions on behavioural and bio-behavioural pain responses (Carbajal et al., 2003; Codipietro et al., 2008). Such differences highlight the need to understand parent perceptions and acceptance of use of this technology to quantify components of their infant's pain responses in order to inform future clinical research and clinical practice integration.

Conceptual Underpinnings and Theoretical Framework: Neuromatrix Theory of Pain

Melzack's Neuromatrix Theory of Pain will provide the conceptual framework for this study (Melzack, 1999; 2005). This theory, which is an extension of Melzack and

Wall's (1965) Gate Control Theory of Pain provides an underlying framework for better understanding physiologic and conceptual factors which influence pain responding. While originally applied as a model to interpret the occurrence of post-amputation phantom limb pain (Melzack, 1999) and chronic pain conditions (Melzack, 2001; Moseley, 2003; Moseley, 2005), it has also been applied as a model to understand the mechanisms underlying the efficacy of non-pharmacologic pain-relieving interventions (Trout, 2004). This theory postulates that in addition to the neural response generated from nociceptive stimulation, pain is influenced by numerous contextual and environmental factors, such as memory for previous pain experiences and competing sensory inputs. Melzack (1999; 2005) proposes that the multiple cognitive, sensory, and emotion related factors that influence pain perception are integrated to produce a multidimensional experience of pain. This theory thus expands on the dated understanding of pain transmission as a process of central neural inhibition, excitation, and modulation of ascending and descending neural impulses that is solely occurring at the level of the dorsal horn of the spinal cord. Rather, Melzack (1999; 2001) theorizes that pain is regulated by a circuit of regulatory neural networks in the brain, the body self neuromatrix, which integrates multiple inputs to produce an output pattern resulting in pain (Melzack, 1999). This body self neuromatrix, as initially proposed by Melzack, is not localized to one brain region, but rather is made up by a widely distributed neural network that includes somatosensory, limbic, and thalamocortical components and all inform the sensory-discriminative, affective-motivational, and evaluative-cognitive aspects of each individuals' unique pain experience (Melzack, 1999). Neuroimaging studies utilizing Positron Emission Tomography (PET) and functional Magnetic Resonance Imaging (fMRI) in both experimental and clinical samples have confirmed

what Melzack initially hypothesized: The neural network involved in pain processing is diffuse, extending beyond the thalamus, anterior cingulate cortex, and primary and secondary somatosensory cortices to include the periaqueductal gray area, insula, orbitofrontal, prefrontal, motor and supplementary motor area, and the inferior parietal and anterior cingulate cortex (Derbyshire, 2000).

The Neuromatrix Theory of Pain is composed of four main assumptions: 1) That pain is multi-dimensional and produced by the synthesis of incoming stimuli via the body self neuromatrix in the brain which is a linked neural network of interconnected loops between the cortex, thalamus, and limbic systems (Melzack, 1999); 2) that continuous cyclic nerve impulses within the feedback loops of the body self neuromatrix create recognizable patterns referred to as a neurosignature – a characteristic output of a particular sensory experience; 3) that the simultaneous transmission of the neurosignature occurs to the sentient neural hub – specific regions of the brain responsible for creating ongoing awareness of pain; and 4) the communication of this neurosignature activates action centres of the neuromatrix to produce a pain response (Melzack, 1999; 2001). Thus, given the complex integration of multiple brain regions in the processing of a painful stimulus, this model moves conceptualization of pain away from the Cartesian concept of pain as a sensation that is solely influenced by tissue injury, but rather is a multi-dimensional experience that is produced and modulated by multiple contextual and genetic influences (Melzack, 1999). The body self neuromatrix and subsequent neurosignature, as proposed by Melzack (2005), is a psychologically meaningful unit in that it is influenced by both genetic components and learned experience to produce a unified behavioural and physiological pain experience dependent on the context of the painful event. Considering the guiding Neuromatrix Theory of Pain, the following

section of this literature review will discuss factors that may modulate the pain experience and response of infants in the context of acute painful procedures and the interventions most commonly used to manage said pain.

Modulation of pain response. All infants have the structural and innate biological capacity to experience stress, perceive pain, and respond to painful stimuli. Based in the tenets of the Neuromatrix Theory of Pain, the body self neuromatrix and corresponding neurosignature is one of the many regulatory systems present at birth (Melzack, 1999). Thus, while initially genetically determined, the spatial distribution and synaptic links can be shaped by sensory context and experience (Melzack, 1999). For example, in the context of adult experiences of chronic pain, mediating factors that have been identified include context, relationships, and competing multisensory inputs (Moseley, 2003). Further, multi-sensorial stimulation rather than a single sensory stimulus has been shown to be significantly more effective in managing infant pain (Bellieni et al., 2012). Thus, it is on the basis of the Neuromatrix Theory of Pain that it is believed that the multi-sensorial stimulation (closeness, tactile, auditory, olfactory) provided in the context of close maternal contact in combination with the direct active gustatory component of breastfeeding during a painful procedure may act on the body self neuromatrix to modulate and inhibit pain perception, resulting in reduced physiological, neurophysiological, and behavioural pain responses in comparison to provision of 24% oral sucrose. Empirical evidence supporting this potentially pain moderating effect will be discussed in the next section of this literature review.

Factors Underlying the Pain Moderating Effect of Breastfeeding

Closeness. Studies conducted with both animals and humans demonstrate that maternal separation induces stress, and that this stress is associated with adverse

outcomes. For example, diminished growth, accelerated neuronal apoptosis, heightened stress responses, delayed prefrontal brain growth, and disrupted orientation has been demonstrated in rat pups when separated from their mothers (Anand, 2000). Furthermore, maternal closeness and care through grooming and handling has been found to be neuroprotective (Kuhn & Schanberg, 1998; Rojas et al., 2003; Schanberg, Evoniuk, & Kuhn, 1984); promotes optimized learning, memory, and regulation (Hofer, 1994; Meaney, 2001); modulates HPA-axis responses to pain and stress (Caldji, Diorio, & Meaney, 2000; Francis, Diorio, Plotsky, & Meaney, 2002; Liu, Rovnaghi, Garg, & Anand, 2004; Meaney, 2001; Plotsky, Thrivikraman, & Meaney, 1993); and may buffer the cumulative effects of pain and stress (Walker, Kudreikis, Sherrard, & Johnston, 2003). These findings thus support the hypothesis that the close maternal contact associated with breastfeeding could potentially moderate the immediate response and long-term adverse outcomes associated with early procedural pain exposure.

Touch and skin-to-skin contact. Tactile awareness is one of the first senses of fetal development, occurring at approximately seven to eight weeks (Liaw, 2000). Thus, all newborns have the ability to perceive physical touch and demonstrate positive responses to stroking or massage (Feldman & Eidelman, 2003). For example, infants have been shown to demonstrate reduced behavioural stress and improved sleep following gentle human touch (Harrison, Olivet, Cunningham, Bodin, & Hicks,1996; Harrison, Leeper, & Yoon, 1990) and massage (Field, 2002; Field & Diego, 2008). Further, animal models of infant touch demonstrate increases in endorphins, oxytocin, and serotonin – hormones that have been associated with modulating pain response (Carden & Hofer, 1990; Nelson & Panksepp, 1998; Panksepp, Nelson, & Siviy, 1994; Panksepp, Nelson, & Bekkedal, 1997).

Full maternal infant skin-to-skin contact demonstrates significant benefit above and beyond simple touch. Positive outcomes include stabilization of physiologic parameters (e.g., temperature, heart, and respiratory rates), decreased occurrence of apneas, improved weight gain and growth, and accelerated maturation of autonomic and circadian systems (Campbell-Yeo, Disher, Benoit, & Johnston, 2015; Conde-Agudelo & Diaz-Rossello, 2014; Engler et al., 2002; Feldman & Eidelman, 2003; Johnston et al., 2003; Moore & Anderson, 2007). Furthermore, skin-to-skin contact has demonstrated benefit as a pain-relieving intervention (Johnston et al., 2017). In a recent Cochrane systematic review and meta-analysis, skin-to-skin contact significantly reduced Premature Infant Pain Profile scores at 30- (n = 267, ***MD*** = -3.21, 95% CI [-3.94 – -2.47], 60- (n = 156, ***MD*** = -1.64 [95% CI -2.86 – -0.43], and 90- (n = 156, ***MD*** = -1.28 [95% CI - 2.53 – - 0.04]) seconds following painful procedures. Primary studies exclusively reporting on full term newborns demonstrate skin-to-skin contact reduces crying, grimacing, and heart rate during heel lance procedure (Gray et al., 2000). While holding a clothed baby provides some comfort, it appears that direct skin-to-skin contact is more effective (Arditi, Feldman, & Eidelman, 2006). The underlying mechanisms of skin-to-skin contact are thought to be associated with blunting of sympathetic nervous system responses and up-regulation of parasympathetic nervous system responses through physiological neural regulators (Hofer, 1994). Specifically, skin-to-skin contact may elicit an inborn tactile receptor response that regulates vagal tone and release of endogenous opiates, oxytocin, and beta-endorphins (Michelsson, Christensson, Rothganger, & Winberg, 1996; Mooncey et al., 1997).

Olfactory recognition. Evidence now suggests that newborn infants recognize and remember their mothers' scent, demonstrate preference toward maternal scent, and

that this scent can produce pain modulating effects (Goubet, Rattaz, Pierrat, Bullinger, & Lequien, 2003; Goubet, Strasbaugh, & Chesney, 2007; Marin, Rapisardi, & Tani, 2015; Sullivan & Toubas, 1998; Varendi et al., 1998). Regardless of being breastfed (Schaal et al., 1998) or formula fed (Marlier et al., 1998), newborn infants demonstrate preference as measured by head-turning toward familiar maternal amniotic fluid smell in comparison to an unfamiliar amniotic fluid smell or formula. In a recent study, Marin and colleagues aimed to test the ability of two day old infants to recognize their own mothers' axillary odor in comparison to an unfamiliar new mother (Marin et al., 2015). Nineteen vaginally delivered, breastfeeding newborns were presented with pads of each of the odors and were videotaped to assess how long their heads were orientated toward pads carrying the scent of their mother versus the unfamiliar woman. The average time in seconds that the infants' heads were turned toward the scent of their own mother was significantly longer than the length of time they were oriented toward the scent of the unfamiliar woman (20.53 vs. 11.13, $p < 0.05$ [Marin et al., 2015]). This recognition of maternal odor is likely neurologically inborn in newborns. Regardless of whether breast or formula fed, infants exposed to the scent of maternal breast milk demonstrated a significantly larger increase in orbito-frontal oxygenated hemoglobin levels on NIRS that those infants exposed to the scent of formula (Aoyama et al., 2010).

In addition to simple recognition of maternal odor, distressed newborns demonstrate reduced crying (Sullivan & Toubas, 1998; Varendi et al., 1998) and increased sucking bursts (Sullivan & Toubas, 1998) when presented with maternal odor compared to no odor or the odor of an unfamiliar woman, suggesting that maternal odor has stress-modifying effects. Given the evidence suggesting that the memory of olfactory stimuli has behaviour modifying effects, Goubet and colleagues aimed to test if familiar

olfactory stimuli can influence responses to painful procedures in both full term (Goubet et al., 2007) and preterm newborns (Goubet et al., 2003). A sample of 51 preterm newborns were randomly assigned to undergo venipuncture or heel stick, with one-third of the infants undergoing the procedure with an odor they had previously been exposed to ($n = 17$), one-third with an unfamiliar odor ($n = 17$), and one-third with no odor ($n = 17$). Infants presented with an unfamiliar odor or no odor during the painful procedures had increased crying and grimacing, whereas infants who were presented with a familiar odor during venipuncture showed no increase in crying and grimacing during the procedure compared to baseline (Goubet et al., 2003). These findings were replicated in a sample of 44 full term newborns who were exposed to a familiarized odor (vanillin) or no odor prior to, during, and after a heel stick (Goubet et al., 2007). Infants exposed to the familiar odor demonstrated less crying and grimacing, and more oral movements during the procedure compared to the group with no odor exposure, further indicating that olfactory interventions may minimize response to pain in neonates (Goubet et al., 2007). Specifically, exposure to maternal odor during needle procedures resulted in significantly less motor agitation measured via head movements (Rattaz, Goubet, Bullinger, 2005), less change in physiologic parameters of heart rate and oxygen saturation (Neshat, Jebreili, Seyyedrasouli, Ghojazade, Hosseini, & Hamishehkar, 2016), and lower Premature Infant Pain Profile scores (Jebreili, Neshat, Seyyedrasouli, Ghojazade, Hosseini, & Hamishehkar, 2015) during the procedure compared to familiarized vanillin scent, suggesting that exposure to maternal scent may be optimal in reducing distress in response to pain. Similar findings were reported in a recent trial in preterm neonates who were randomly assigned to have maternal breast milk odor and non-nutritive sucking ($n = 16$) or no odor and non-nutritive sucking ($n = 17$) during venipuncture (de Chanville et

al., 2017). Neonates in the maternal breast milk odor group had statistically and clinically significantly different median Premature Infant Pain Profile scores (6.3 [*IQR* = 5-10]) compared to those neonates who received no treatment (12.0 [*IQR* = 7-13]).

While the mechanisms underlying the calming influence of familiar odor has not yet been fully elucidated, animal and human studies provide some evidence for opioid-mediated effects. Animal models have demonstrated that the opioid system modulates olfactory learning, odour memory, and nociceptive responses in rats (Jahangeer, Mellier, & Caston, 1997; Shide & Blass, 1991). Specifically, preference formation for certain odors in rats has been demonstrated to be dependent on the availability of endogenous opioids (Shide & Blass, 1991) and links have been drawn between the presence of opioid neurons in the olfactory bulb and limbic system and the mediation of pain through opioid mechanisms (Jahangeer, Mellier, & Caston, 1997). In humans, the gustatory systems believed to support the beneficial effects of sweet tasting solutions during painful procedures are thought to be opioid mediated and linked with the olfactory system (Stevens et al., 2016). Taken together, the studies reporting on infant odor recognition and the calming benefits of familiar odor (particularly maternal breast milk) suggest an additional potential underlying mechanism by which breastfeeding modulates the pain experience in newborns undergoing painful procedures.

Auditory stimulation. The human fetus is believed to begin to be capable of auditory perception at approximately 19 weeks' gestational age and subsequently have the capacity to form memories of auditory stimuli from the intrauterine environment (Hepper & Shahidullah, 1994). Thus, early auditory experiences have the potential to influence neurodevelopment and later regulation in infancy (Fifer & Moon, 1994). For example, very preterm infants exposed to maternal voice recordings in the neonatal

intensive care unit demonstrated significantly larger auditory cortices bilaterally on cranial ultrasound compared to those infants exposed to routine hospital environmental sounds (Webb, Heller, Benson, & Lahav, 2015), demonstrating the key role of early sensory auditory experiences in neurodevelopment. As maternal voice is reported as being the most intense acoustic sound measured in the inter-uterine environment, it is not surprising that infants demonstrate preference for maternal voice in the first days after birth (Fifer & Moon, 1994). When exposed to voices of their mothers and unrelated strangers, term newborns as young as three days old demonstrate preference for maternal voice as measured by more robust non-nutritive sucking bursts (DeCasper & Fifer, 1980) and more robust heart rate responses (Kisilevsky et al., 2003; 2009). Furthermore, a recent report comparing fetal heart rate response and newborn recognition of maternal versus paternal voice demonstrates that while heart rate responses to both voices were the same in utero, newborns demonstrated a clear preference for maternal voice as measured by more head turning toward maternal compared to paternal vocal recordings in the five days following birth (Lee & Kisilevsky, 2013).

Recognition of maternal sounds have been demonstrated in several studies to have calming effects on newborn physiological and behavioural pain responses. Exposure to maternal heartbeat recording during heel stick reduced adrenocortical response to pain in healthy full term infants as measured by infant salivary cortisol, dehydroepiandrosterone (DHEA), and dehydroepiandrosterone-sulfate (DHEA-S) in comparison to recorded drumbeat with identical rhythm and volume (Kurihara et al., 1996). Similar effects were not found in a more recent randomized crossover trial, where exposure to maternal recorded voice did not significantly reduce Premature Infant Pain Profile scores but did result in decreased oxygen saturation following a heel lace in infants 32 to 36 weeks'

gestational age (Johnston, Filion, & Nuyt, 2007). The authors of this study suggested that the high volume of the recorded sound may have been aversive for preterm newborns, thus resulting in compromised physiological status, or that familiar sound in the absence of maternal presence may not have been sufficient to significantly impact on pain responding. Given that exposure to familiar sounds have been repeatedly positively associated with improved physiological stability; such as decreased heart and respiratory rate and increased oxygen saturation (Collins & Kuck, 1991; Zimmer, Fifer, Kim, Rey, Chao, & Myers, 1993), less agitation (Standley & Moore, 1995), and more time in quiet alert sleep state (Collins & Kuck, 1991) in preterm newborns, it is reasonable to hypothesize that the auditory stimulation associated with close maternal contact through breastfeeding would positively influence such outcomes during painful procedures.

Oral stimulation. Oral stimulation through sucking on a pacifier or non-lactating nipple have demonstrated analgesic properties in newborns that are hypothesized to be the result of stimulation of oro-tactile and mechanoreceptors (Anseloni et al., 2004; Carbajal et al., 2004; Gibbins & Stevens, 2001). In rat neonates, oro-tactile stimulation with a non-lactating nipple results in decreased distress vocalization in response to thermal pain stimuli (Blass, Shide, Zaw-Mon, & Sorrentino, 1995; Gibbins & Stevens, 2001). In this study, the effect of the non-nutritive sucking was not blocked by naloxone, suggesting that the underlying mechanisms of sucking analgesia are not mediated by release of endogenous opioids (Blass et al., 1995). There is some evidence, however, that the serotonergic system promotes calming through suckling behaviours. For example, two studies have demonstrated that neonatal rat pups who receive serotonin reuptake inhibitors exhibit increased suckling time, suggesting serotonin-based modulation of

suckling behaviour (Spear, Frambes, Goodwin, Moody, 1994; Gibbins & Stevens, 2001; Williams, Rosenblatt, & Hall, 1979).

Regardless of the mechanisms of action, there is some evidence to suggest that the analgesic properties of suckling that are demonstrated in animal studies transfer to preterm and full term born human newborns. A Cochrane systematic review and meta-analysis of non-pharmacologic interventions for management of procedural pain in infants demonstrated that while non-nutritive sucking was not effective in reducing immediate pain reactivity in preterm infants, meta-analysis of five studies demonstrated a significant effect on pain regulation in preterm infants (n = 260, *SMD* 0.43, 95% CI [-0.63 – -0.23], Pillai Riddell et al., 2015). Five studies examined full term born infants in the neonatal period (birth until one month of age) and found that non-nutritive sucking was effective in reducing pain reactivity (n = 270, *SMD* -1.20, 95% CI [-2.01 – -0.38) and immediate pain regulation (n = 325, *SMD* -0.90, 95% CI [-1.54 – -0.25], Pillai Riddell et al., 2015). Only two studies examining the use of non-nutritive sucking in full term born infants older than the neonatal period have been published (Curtis, Jou, Ali, Vandermeer, & Klassen, 2007; Liaw, Zeng, Yang, Yuh, Yin, & Yang, 2011), however, when meta-analyzed they demonstrate that non-nutritive sucking is efficacious in helping older infants regulate following acute pain exposure (n = 151, *SMD* 1.34, 95% CI [-2.14 – -0.54], Pillai Riddell et al., 2015). It is important to note that the marginal effects demonstrated across age categories are based on low quality evidence suggesting a need for additional confirmatory studies (Pillai Riddell et al., 2015). However, oral stimulation in combination with adjuvant interventions is likely efficacious in facilitating human infant regulation following pain and stress.

Nutritive and non-nutritive content of breast milk. Limited research suggests that components of breast milk may contribute to its analgesic effect. As breast milk contains 7% lactose, it has been tested as a sweet tasting intervention and was not found to be as effective as more concentrated sweet solutions (Blass, 1997; Stevens et al., 2016). However, the limited literature in this area has utilized human milk substitutes to test the analgesic efficacy of the sweet tasting components of breast milk (Blass, 1997) restricting conclusions that can be drawn about the complete nutritional and immunological components of breast milk for pain reduction. For example, compared to human milk substitutes, breast milk contains high levels of tryptophan which is a precursor to melatonin (Heine, 1999). Melatonin has been shown to increase levels of endogenous opioid neuropeptides (e.g., beta endorphins) and possibly contribute to pain relief (Barrett, Kent, & Voudouris, 2000; Shah et al., 2010). However, provision of breastmilk alone without the multi-faceted maternal context of direct breastfeeding likely does not provide the same pain-reducing efficacy (Bembich et al., 2018).

Summary of Neuromatrix Theory of Pain

Full term newborns have the structural and functional neural networks, the body-self neuromatrix, necessary to perceive and respond to painful stimuli present at birth. As highlighted in the prior discussion of the Neuromatrix Theory of Pain, this body-self neuromatrix and its corresponding output in response to painful events (the neurosignature) is shaped from its original genetically determined state by the infants' sensory context and experiences. Thus, the sensory environment in which the infant experiences painful events contributes to the development of the body-self neuromatrix and neurosignature and thus influences the infants physiological and behavioural responses to current and later painful events. Based on the Neuromatrix Theory of Pain

and the previously discussed empirical literature, breastfeeding provides the optimal context for infants to experience painful events. Maternal closeness and skin-to-skin contact; and olfactory, auditory, oral, and nutritive stimulation have demonstrated analgesic effects as individual interventions. Thus, the complete multi-sensorial package of breastfeeding likely modulates the body-self neuromatrix and neurosignature such that both immediate pain reactivity, pain regulation, and responses to later pain and stress are positively altered.

A Priori Consideration of Potential Confounding Variables

In light of the research discussed throughout this literature review, as well as the Neuromatrix Theory of Pain, there are several a priori contextual factors that could influence pain responding and should be considered in studies examining infant pain. Specifically, previous exposure to pain and inflammation, infant sex, and quality of breastfeeding during the painful procedure. The following section of this literature review will discuss the literature related to these a priori factors to identify which should be controlled for in infant pain studies.

Previous exposure to pain and inflammation. Early pain exposure is associated with heightened later pain responding in full term newborns, and repeated acute procedural pain may cause altered behavioural responses (Piira, Champion, Bustus, Donnelly, & Lui, 2007; Taddio, Shah, Atenafu, & Katz, 2009; Taddio, Shah, Gilbert-MacLeod, & Katz, 2002) and dissociation between behavioural and cortical oxygenated hemoglobin responses (Ozawa, Kanda, Hirata, Kusakawa, & Suzuki, 2011). All infants underwent at least one acute tissue breaking procedure prior to participation in this study as part of routine clinical care (i.e., an intramuscular injection of Vitamin K), however, some infants were additionally exposed to repeated heel lancing for blood glucose

monitoring, bilirubin monitoring, or other blood collection. The potential influence of any group differences in prior pain exposure on subsequent pain responses and regulation were thus considered.

Infant sex. While the influence of infant sex on pain responding is not well understood, there is some evidence to suggest that male and female infants may differentially respond to acute painful procedures. In studies quantifying pain responses using behavioural measures, females have been reported to display more robust facial responses than males during intramuscular injection (Bellieni et al., 2013) and heel lance (Guinsburg et al., 2000). In contrast, males have been reported to mount more robust physiologic heart rate (Sellam et al., 2013; Valeri et al., 2014) and oxygenated hemoglobin responses than females. Therefore, while more evidence is needed to fully understand the relationship between sex and pain responding in newborns, the possible effect of sex should be considered in studies utilizing physiologic, behavioural, and brain-based indicators to quantify infant pain.

Quality of breastfeeding session. Given the proposed underlying mechanisms contributing to the effectiveness of breastfeeding in reducing newborn pain, there is potential that the quality of the breastfeeding session during pain exposure may confound its efficacy. Specifically, it is reasonable to hypothesize that variability in the length of maternal skin-to-skin contact (Gray et al., 2000; Johnston et al., 2014), active sucking (Anseloni et al., 2004; Carbajal et al., 2004; Gibbins & Stevens, 2001), and transfer of breast milk (Shah et al., 2012) may impact the analgesic efficacy of this intervention. In the literature to date, all studies examining the influence of breastfeeding on pain response in full term infants report that active breastfeeding was sustained for a minimum of two minutes prior to initiation of the painful procedure (Bembich et al., 2013; Fallah et

al., 2016; Goswani et al., 2013; Gupta et al., 2013; Modarres et al., 2013; Thomas et al., 2011) with two studies reporting initiation of breastfeeding a minimum of five minutes prior to pain exposure (Marin-Gabriel et al., 2013; Zhu et al., 2015). Two studies reported waiting to initiate the painful procedure until active sucking at the breast was observed (Modarres et al., 2013; Zhu et al., 2015). However, only one study reported a loss of participants due to ineffective breastfeeding (Marin-Gabriel et al., 2013) and no studies reported using a validated method of assessing success of the breastfeeding session under study. While breastfeeding was consistently found to be effective for full term infants across studies, every effort should be made to ensure consistency of the intervention across infants. Therefore, deviations from an optimal breastfeeding session were quantified and recorded to control for its potential confounding influence on analgesic efficacy.

Conclusion

Full term newborns are routinely exposed to painful procedures as part of clinical care. The literature to date demonstrates that untreated early pain exposure in healthy full term infants may result in adverse consequences, specifically, heighted responses to later pain in infancy. Of the available non-pharmacologic interventions for managing procedural pain in this population, breastfeeding is a multi-faceted sensorial intervention that demonstrates consistent efficacy in reducing behavioural and bio-behavioural pain scores in full term infants undergoing acute painful procedures. While such behavioural (e.g., facial actions, cry duration) and bio-behavioural (e.g., Premature Infant Pain Profile – Revised [PIPP-R]) indicators are currently the most extensively utilized pain measures in newborns (with the PIPP-R being the most extensively validated bio-behavioural measure of infant acute pain), recognized limitations associated with these indicators

(e.g., lack of specificity to pain) has prompted investigation into brain-based newborn pain assessment. However, to date, no studies have examined the influence of breastfeeding on electrophysiologic nociceptive activity, which has the greatest demonstrated potential as a reliable and valid cortical indicator of pain-related processing in the newborn brain. While oral sucrose solution has demonstrated benefit for reducing behavioural pain responses (with 24% concentration combined with non-nutritive sucking having the strongest evidence for pain-reducing benefit), research that questions its analgesic effectiveness and safety prompts an urgent need to determine interventions that function as true analgesics to prevent the adverse outcomes of pain exposure. An important step towards reaching this goal is to determine optimal ways to assess infant pain by better understanding the relationship between existing bio-behavioural and brain-based measures that act as indicators of pain processing and experience. The proposed study will address this significant knowledge gap by examining to what extent breastfeeding impacts on pain-related response in the infant brain during an acute painful procedure (i.e., a heel lance) in comparison to 24% oral sucrose. The following chapter will describe the study objectives and hypotheses as well as the methods that were utilized to address these objectives.

CHAPTER 3: METHODS

This chapter will begin with the study objectives and hypotheses, followed by a description of the methods used to address these objectives. The methods that will be outlined include the study design and participants, study variables, data collection and analysis procedures, steps that were taken to minimize bias, and ethical considerations. A preliminary knowledge translation plan is also provided. This study protocol is registered on ClinicalTrials.gov: NCT03272594.

Study Objectives and Hypotheses

Objectives. The primary objective of this study was to examine the influence of breastfeeding on pain-related activity in the healthy full term newborn brain during heel lance, compared to the administration of 24% oral sucrose and offered non-nutritive sucking while in an infant cot (considered the current standard of care). The primary outcome measure was pain-related event-related potential induced by heel lance (Slater et al., 2010a-c) and measured using a dense-array neonatal electroencephalogram (EEG) recording. Secondary outcomes compared between groups included: a) Bio-behavioural pain score measured using the Premature Infant Pain Profile – Revised (PIPP-R; Stevens et al., 2013; 2014), b) physiologic recovery, c) maternal acceptability of data collection procedures and interventions, and d) adverse events.

Primary hypothesis. Infants randomized to the breastfeeding intervention, when compared to those infants randomized to the 24% oral sucrose and offered non-nutritive sucking intervention, will demonstrate a lower amplitude pain-related event-related potential.

Secondary hypotheses. Infants randomized to the breastfeeding intervention, when compared to those infants randomized to the 24% oral sucrose and offered non-nutritive sucking intervention, will demonstrate:

a) lower bio-behavioural pain scores, measured using the PIPP-R at 30-, 60-, 90-, and 120-seconds following heel lance,
b) faster physiologic recovery measured as heart rate return to baseline,
c) greater maternal acceptability regarding the interventions and data collection procedures, and
d) fewer adverse events (e.g., fewer episodes of temperature instability, fewer rescue sucrose doses).

Design

The study utilized a single blind, randomized controlled trial design (Appendix B). Participants were randomized to have a medically indicated heel lance completed in one of two possible intervention conditions: 1) breastfeeding or 2) 24% oral sucrose and offered non-nutritive sucking while in an infant cot.

Participants and Setting

39 full term infants (20 infants in the breastfeeding group and 19 infants in the 24% oral sucrose and offered non-nutritive sucking group) were recruited from the Family Newborn Care Unit of the IWK Health Centre within the first two days of age.

Sample size. The sample size of 126 infants was calculated using a two-sided alpha error of 0.05 and a power of 80% allowing for the detection of a 30% reduction in amplitude of the pain-related event-related potential on EEG (https://www.stat.ubc.ca/~rollin/ stats/ssize/n2.html). A 30% reduction is considered clinically significant and has been utilized in prior studies (Slater et al., 2010a). Event-

related potential assumptions were based on a previous study comparing 24% oral sucrose to sterile water during heel lance in full term infants, with reported ERP mean weights of 0.10 (95% CI [0.04 – 0.16]) for the sucrose group and 0.08 (95% CI [0.04 – 0.12]) for the sterile water group (Slater et al., 2010a). Based on reported standard deviations of 0.03, a conservative standard deviation estimate of 0.06 was utilized (Slater et al., 2010a). This conservative estimate was selected as the previous work from which these effect estimates were obtained reported being underpowered to detect subtle differences in central nervous system effects of interventions (Slater et al., 2010a) and additionally because this study aimed to compare breastfeeding to 24% oral sucrose. Both are active analgesic interventions with demonstrated efficacy in reducing behavioural and bio-behavioural pain scores. A sample size of 32 infants (16 per intervention group) would be sufficient to detect a 30% difference in ERP mean amplitude if utilizing the more liberal standard deviation estimate of 0.03 reported in previous work (Slater et al., 2010a).

With a sample of 126 infants powered to detect a 0.03-point change in mean ERP amplitude, there is a power of 80% to detect a one-point difference (standard deviation of two [Obeidat & Shuriquie, 2015]) in the PIPP-R scores between groups (https://www.stat.ubc.ca/~rollin/ /ssize/n2.html). Previous literature comparing breastfeeding, maternal contact, or 24% sucrose to no treatment control groups have reported two to three point differences in PIPP scores as clinically significant (Johnston et al., 2003; Obeidat & Shuriquie, 2015). Given that this study aimed to compare breastfeeding to 24% oral sucrose, both of which have demonstrated effects in reducing behavioural and bio-behavioural pain scores, a conservative one point difference in PIPP-R score was selected, consistent with other studies using this approach (Campbell-Yeo, Johnston, Joseph, Feeley, Chambers, & Barrington, 2012). A sample size of 32 infants

would be have 30% power to detect a one-point difference (standard deviation of two) in PIPP-R score between groups.

Inclusion and exclusion criteria. Eligible participants included healthy, full term (born greater than or equal to 37 0/7 weeks' gestational age [Barrington, Sankaran, & Canadian Paediatric Society Fetus and Newborn Committee, 2016]), normally breastfeeding infants, whose mother was willing to breastfeed during the painful procedure and consented to study participation. Normally breastfeeding infants were defined as those infants who had fed directly at the breast a minimum of two times in the 24-hours prior to blood collection and whose mother and/or staff nurse reported active sucking and swallowing during those feeds. Infants who had undergone repeated heel lancing for blood glucose and/or bilirubin monitoring (e.g., small or large for gestational age infants, infants born to diabetic mothers, or with hyperbilirubinemia) were considered eligible, however, diagnosis and the number of prior painful procedures were recorded and retained to inform statistical analyses. Infants were not eligible for study participation if they were a twin birth (including all classifications of monozygotic and dizygotic twins) due to the potential for non-independence of outcomes between twin pairs, showed signs of infection, or had significant lower limb tissue damage, bruising or trauma to the scalp, previous surgery or intra-ventricular hemorrhage, were born to opioid using mothers or with significant genetic disorders, were unable to breastfeed or had contraindications to sucrose administration, or whose parents were unable to provide written informed consent.

Recruitment

The Primary Investigator (PI) or an assisting research coordinator identified eligible participants in collaboration with the clinical team leader on the Family Newborn

Care Unit at the IWK Health Centre. When the health care team identified an eligible infant, the PI or assisting research coordinator collaborated with the staff nurse caring for that infant and their family to seek permission from the family to discuss the study. Specifically, the staff nurse asked the eligible family if they were willing to speak to a researcher about the study. If they were willing, the study PI or assisting research coordinator then explained the study to the family and answered any questions. If the family wished to take part in the study, the PI or assisting research coordinator determined the timing of the infant's medically required heel lance, determined the mother's availability to breastfeed during the procedure, and obtained written informed consent. These recruitment procedures were used in previous clinical trials testing non-pharmacologic interventions for newborn pain completed at the IWK Health Centre and were compliant with Personal Health Information Act legislation governing research personnel access and use of personal health information for patients receiving health care in Nova Scotia, Canada.

Randomization

Following parental consent, infants were randomized by the PI or assisting research coordinator using a computerized off-site password protected website. Intervention allocation concealment to receive breastfeeding or 24% oral sucrose and offered non-nutritive sucking was achieved by using randomly permuted blocks of two, four, or six. Use of block randomization reduces potential bias by increasing the likelihood that intervention groups are equal in size and uniformly distributed in terms of key outcome characteristics (Efird, 2011). This is of particular importance in clinical trials with smaller sample sizes, as simple randomization may not always result in a balanced distribution of participants in the intervention groups (Efird, 2011). This

method randomized participants within blocks so that an equal number were assigned to each treatment. Specifically, a random number sequence was used to choose a particular block (e.g., two, four, or six), which set the treatment allocation order for the next two, four, or six subjects (Efird, 2011). Randomly varied block sizes of two, four, and six were selected for this trial as double blinding of the intervention is not possible, resulting in an increased potential of the assignment schedules being predicted by those recruiting and randomizing participants. By alternating both the sequence of allocation within the block and number of participants per block, the risk of allocation bias is minimized (Schulz & Grimes, 2002a;b). The PI and the assisting research coordinator were the only individuals who had permission to access the secure website to randomize consenting participants.

Outcome Measures and Variables

Sample characterization. Data characterizing the sample that were collected from maternal and infant hospital charts included: Maternal age, maternal parity, maternal experience breastfeeding previous children, maternal medications, history of maternal diabetes during pregnancy, infant gestational age at birth, infant postnatal age at time of study participation, mode of delivery, infant birth weight, infant Apgar scores, infant sex, total number of previous painful procedures, infant breastfeeding status (e.g., exclusive breastfeeding, breast milk and formula mixed feeding), characteristics of the feeding preceding the study heel lance (e.g., breastfeeding, formula feeding, time since last feeding), and any infant diagnoses (e.g., small for gestational age, large for gestational age, hyperbilirubinemia).

Pain-related brain activity. The primary outcome measure was pain-related brain activity measured using a dense array neonatal electroencephalogram (EEG)

recording that was time-locked to a medically required heel lance (Slater at al., 2010a-c). Infant EEG activity was recorded from a HydroCel Geodesic Sensor Net positioned according to the modified international 10/20 electrode placement system on a 32-channel Geodesic EEG System™ 400 series (Electrical Geodesics Incorporated [EGI], Eugene, Oregon, USA). Pain-related event-related potentials were specifically examined and isolated at the vertex of the scalp from electrode site E19 of the EGI Hydrocel Geodesic Sensor Net, as previous research has reported pain-related activity from electrodes in this region in both infants (Slater et al, 2010a-c) and adults (Truini et al., 2010).

Bio-behavioural pain response. The secondary outcome was bio-behavioural pain response, measured using the Premature Infant Pain Profile-Revised (PIPP-R). The PIPP-R, which has been revised from the original PIPP developed 14-years ago, is a 7-indicator composite pain measure consisting of three behavioural (facial actions of brow bulge, eye squeeze, and naso-labial furrow), two physiological (heart rate, oxygen saturation), and two contextual (gestational age, behavioural state) indicators of acute pain (Gibbins et al., 2014; Stevens et al, 2014). A numerical score ranging from zero to three is assigned to each indicator for a maximum score of 18 reflecting the worst possible pain in infants born at greater than 36 weeks' gestational age. A score of six or less is considered to indicate minimal or no pain, a score of six to 12 indicates mild or moderate pain, and a score of 12 or greater indicates moderate to severe pain (Schiller, Stevens, Sidani, Ballantyne, & McNair, 1999). The original PIPP has undergone extensive psychometric testing and is considered a reliable and valid bio-behavioural measure of pain in infants' 26 – 44 weeks' gestational age (Stevens, Johnston, Petryshen, & Taddio, 1996; Stevens, Johnston, Taddio, Gibbins, & Yamada, 2010). The PIPP-R was modified from the original PIPP based on feedback from researchers and clinicians to

address feasibility and validity issues. While the PIPP-R is based on the same indicators as the original PIPP, the timing of scoring of the gestational age and behavioural state items were adjusted to prevent inflating the score in the absence of an observed pain response (Stevens et al., 2014). The PIPP-R has undergone construct validity testing and inter-rater reliability testing and is considered clinically feasible (Gibbins et al., 2014; Stevens et al., 2014). Thus, as the PIPP-R is the most extensively validated bio-behavioural measure of infant acute pain, it was selected for use in this study. A copy of the PIPP-R can be found in Appendix C.

Physiologic recovery. An additional secondary outcome was infant time to physiologic recovery following the procedure. Time to recovery was considered the amount of time in seconds that elapsed until the infant's heart rate returns to baseline values. The point at which the infant's heart rate reaches baseline levels and was sustained for no less than five to seven beats following the heel lance indicated recovery.

Safety surveillance and adverse event reporting. Monitoring for adverse events for all infants enrolled in the study involved documentation of any incidence of choking, apnea (defined as unexplained cessation of breathing for 20-seconds or longer, or a shorter respiratory pause associated with bradycardia, cyanosis, pallor, and/or hypotonia [American Academy of Pediatrics Committee on Fetus and Newborn, 2004]), or bradycardia (defined as a 30 beat per minute drop from baseline, or when the heart rate is below 100 beats per minute [Canadian Paediatric Society, 2016]) following the initiation of either intervention (i.e., initiation of breastfeeding or administration of 24% oral sucrose and offered non-nutritive sucking). Need for a repeat heel lance to allow for sufficient blood collection and administration of additional doses of "rescue" sucrose was also recorded. Furthermore, as the HydroCel Geodesic Sensor Net require electrode

sponges to be soaked in warm water prior to application to the infant scalp, infant axillary temperature was monitored every 15-minutes throughout data collection to ensure normothermia (36.5 to 37.5 degrees centigrade). Infants were observed constantly during data collection in order to monitor for any risk of choking or skin irritation while wearing the HydroCel Geodesic Sensor Net. The PI and/or the assisting research coordinator checked for skin pressure points, overturned sensors, or redness under the chin strap every 15-minutes to maximize infant comfort (Electrical Geodesics, Inc., 2007). The doctoral supervisory committee served as the safety monitoring board for this study. No suspected unexpected serious adverse reactions occurred and needed to be reported to the supervisory committee or the IWK Health Centre Research Ethics Boards.

Maternal acceptability. To explore satisfaction and concerns with assigned interventions and data collection procedures, mothers were asked to complete an open-ended questionnaire (depending on assigned intervention) with seven questions following completion of the study procedures. The questionnaire was developed in consultation with the supervisory committee and was based on questionnaires previously developed and administered by this study team to examine parent and nurse perceptions of non-pharmacologic infant pain management interventions (Benoit, Campbell-Yeo, Johnston, Latimer, Caddell, & Orr, 2015; Campbell-Yeo et al., 2013). This questionnaire took approximately five-minutes for mothers to complete and focused on assessing maternal acceptability of the use of the assigned breastfeeding (Appendix D) or sweet taste (Appendix E) intervention as well as the use of neurophysiological imaging technology to measure newborn pain responding in the neonatal period.

Confounding variables.

Breastfeeding effectiveness. The LATCH breastfeeding assessment tool was used

to evaluate the effectiveness of the breastfeeding session. This tool was originally developed over 20 years ago and provides a reliable and valid method of documenting the effectiveness of a breastfeeding session (Altuntas et al., 2014; Jensen, Wallace, & Kelsay, 1994; Lau, Htun, Lim, Ho-Lim, & Klainin-Yobas, 2016). Each letter of the LATCH acronym corresponds with a different section of the assessment: L describes ability of the infant to latch to the breast, A describes audible swallowing, T describes the type of nipple, C describes maternal comfort during breastfeeding, and H quantifies the amount of help the mother required from a care provider during the breastfeeding session. A numerical score was assigned to each measure for a possible total score of 10, which represents an optimal breastfeeding session. A copy of the LATCH breastfeeding assessment tool can be found in Appendix F.

Collection of outcome measure data and study variables. The measurement of the main study outcomes relied on five data collection strategies: Continuous neonatal EEG recording, close-up video recording of infant facial actions, pulse oximeter monitoring of heart rate and oxygen saturation, observation of the effectiveness of the breastfeeding session and monitoring for adverse events, and a chart review. Collection of data related to the outcomes measures of this study took place around a medically required heel lance that all infants underwent to collect blood for routine metabolic screening. The heel lance was selected as the pain stimulus as it is a procedure that all infants are exposed to in the neonatal period at least once. Furthermore, it is not an artificial experimental stimulus and is a commonly used pain stimulus in the literature allowing for relevant clinical generalizations and comparisons across previously published studies. While full term infants are additionally exposed to an intramuscular injection of Vitamin K immediately following birth as part of clinical care at the IWK

Health Centre, and this is also a common pain stimulus reported in the full term pain literature, the timing of the Vitamin K injection immediately following birth made it impracticable to use as the pain stimulus in this study. As the heel lance completed for routine metabolic screening in this population typically occurs after 24-hours of age, it was considered more feasible to recruit participants to examine response to pain during this procedure. The following table presents details regarding the data sources and timing of collection for all study variables (Table 1).

Table 1

Key Study Variables, Measures, Data Sources, and Timing of Collection

Study Variables	Measure	Data Sources	Time of Collection
Sample characterization	Maternal and infant medical records	Chart review	Current pregnancy and hospitalization
Pain-related brain response	Pain-related event-related potential	Neonatal EEG recording	Non-painful control stimuli, heel lance
Bio-behavioural pain response	PIPP-R	Video recording (facial actions, behavioural state), Pulse oximeter (heart rate, oxygen saturation)	Baseline, 30-, 60-, 90-, 120-seconds following heel lance
Physiologic recovery	Heart rate return to baseline	Pulse oximeter	Baseline, recovery

Study Variables	Measure	Data Sources	Time of Collection
Safety surveillance	Adverse events, temperature, EEG net monitoring	Observation of data collection session, neonatal axillary temperature measurement	Baseline, heel lance, recovery
Maternal acceptability	Researcher generated questionnaire	Maternal self-report	Following completion of painful procedure/ prior to discharge
Breastfeeding effectiveness	LATCH score	Observation of breastfeeding session	30-, 60-, 90-, 120-seconds following heel lance

Data Collection Procedures and Description of Interventions

Each participating infant had one medically required heel lance completed as per the following procedures. The PI or assisting research coordinator arranged the timing of the heel lance with health centre staff and mothers, and informed participating mothers of the intervention to which their infant randomized (i.e., breastfeeding or 24% oral sucrose and offered non-nutritive sucking). Heel lancing was completed by one of the experienced lab technologists whose role in the health centre is dedicated to blood collection. As it was not possible to limit blood collection to consistent lab technologists due to the large number of lab members on staff, length of procedure and volume of blood obtained during procedure was recorded in an attempt to account for any variation in clinical practice. The infant's mother was present during the heel lance regardless of

randomization and talking to or stroking their infant throughout the procedure was not discouraged in either intervention condition. However, mothers were encouraged not to make sudden movements during the procedure to minimize the risk of movement artifact in the data. Both the PI, who is a registered nurse with experience providing breastfeeding support, and a research nurse coordinator were present constantly throughout data collection to monitor EEG, video, heart rate, and oxygen saturation recordings so as to minimize data loss, ensure infant physiologic stability and safety, as well as to ensure the assigned non-pharmacologic pain management strategies were supported and intervention fidelity was maintained. The PI or assisting research coordinator additionally monitored axillary temperature and checked for skin pressure points, overturned EEG sensors, or redness under the EEG-net chin strap every 15-minutes to maximize infant comfort (Electrical Geodesics, Inc., 2007). The maternal questionnaire was provided to the mother following completion of the heel lance procedure and was obtained in the sealed envelope by the PI or assisting research coordinator following completion.

Pain-related brain activity was recorded on EEG for the duration of the blood collection using the 32-channel HydroCel Geodesic Sensor Net positioned according to the modified international 10/20 electrode placement system on a 32-channel Geodesic EEG System™ 400 series (Electrical Geodesics Incorporated, Eugene, Oregon, USA). The HydroCel Geodesic Sensor Net offers a dense-array non-abrasion high-impedance application method, making it optimal for utilization with infants (Electrical Geodesics, Inc., 2007). Standard net application and data recording initiation procedures were followed for each infant (Appendix G). EEG activity from 0.5 to 30 Hz were recorded with a sampling rate of 1000 samples per second and a conversion of 24 bit. The heel

lance was locked to the EEG recording utilizing audio-recording equipment to time-lock based on the audible spring-blade release of the heel lance. The sound of the heel lance release was captured using a microphone, amplified through an audio-computer interface (M-Audio Fast Track, M-Audio Inc., Cumberland, Rhode Island, USA), and linked to the EEG amplifier using an EGI Audio-Visual (AV) device through an AV device DIN adaptor and hypergrip cable (Electrical Geodesics, Inc., 2019, Eugene, Oregon, USA; Appendix H). A DIN event marker was successfully and repeatedly marked on the continuous EEG recording using the audio time-locking method when the heel lance was released in proximity to the microphone. To ensure there was no latency through the audio-computer interface, the microphone input line and audio output line were connected to a digital oscilloscope and relative timing of the activity on both lines were observed during microphone activation. There was no appreciable latency between input and output, indicating a precise time-locking mechanism to isolate a pain-related event-related potential.

For calculation of the bio-behavioural PIPP-R score, infant facial responses of eye squeeze, brow bulge, and naso-labial furrow were continuously recorded throughout the baseline period, heel warming, heel lancing, blood collection, and recovery period using close-up video recording. As pain-related facial actions are exhibited bilaterally, facial actions observed from one side of the infant face are sufficient for use in the calculation of the PIPP-R score (Stevens et al., 1996; Grunau & Craig, 1987). Therefore, a single video camera was positioned to record one side of the infant's face so as not to disrupt the breastfeeding session in those infants randomized to the breastfeeding intervention. Heart rate and oxygen saturation were measured continuously throughout the procedure via a pulse oximeter placed on the infant's hand or unaffected foot and recorded on the

Geodesic EEG System™ 400 series Physio 16 II (Electrical Geodesics Incorporated, Eugene, Oregon, USA).

Breastfeeding intervention. For infants randomized to the breastfeeding intervention, data collection began with a continuous recording of a one minute baseline of all outcome measures while the infant was resting in a cot wearing only a diaper and contained in a blanket (BL1). Following this, a non-noxious (NN1) control stimulus was be applied to the infant's foot to capture a baseline response on EEG to a non-painful event prior to initiation of the assigned intervention condition. This non-noxious stimulus consisted of placing the heel lance against the foot and rotating it 90 degrees, so that when the lance was released it mimicked the sensation of the heel lance procedure without the associated tissue-breaking and pain. The infant was then placed in skin-to-skin contact with the mother for at least five-minutes prior to heel lance to allow time to settle and initiate breastfeeding. Breastfeeding position was determined based on individual maternal preference in order to optimize feeding, to facilitate ease of access to the infant's foot for blood collection, while also attempting to minimize disruption of continuous EEG, heart rate, oxygen saturation, and video recording. Active breastfeeding was facilitated to ensure it took place for a minimum of two minutes prior to heel lance and continued until the procedure was completed (Bembich et al., 2013; Fallah et al., 2016; Gupta et al., 2013; Modarres et al., 2013; Thomas et al., 2011). The PI and/or assisting research coordinator provided verbal and/or physical assistance to each mother to promote maintenance of breastfeeding throughout the procedure. All attempts were made to promote active breastfeeding (i.e., deep latch, sucking, and swallowing observed) during the heel lancing. While the infant was breastfeeding, a second one minute baseline (BL2) was recorded followed by the application of a second non-noxious (NN2) control

stimulus prior to heel lance. The pain-related event-related potential was time locked and recorded on EEG from the initiation of the heel lance and facial actions, heart rate, and oxygen saturation were recorded until at least two minutes following application of bandage, which signaled procedure end. A LATCH score was calculated by the PI or assisting research coordinator following the completion of the breastfeeding session for each infant receiving the breastfeeding intervention. If the infant did not latch to the mother's breast and active breastfeeding could not be facilitated, they received a LATCH score of zero. However, they remained in skin-to-skin contact with their mother for the duration of the procedure and were retained in the analysis as per the principles of intention-to-treat analysis (ITT [Detry & Lewis, 2014; Gupta, 2011]). The rationale for use of ITT is discussed in further detail in the statistical analysis section of this chapter. Mothers were given the acceptability questionnaire following the completion of the heel lance session and the PI or assisting research coordinator obtained the completed questionnaire in a sealed envelope prior to maternal hospital discharge. Appendix I presents the experimental timeline for those infants randomized to the breastfeeding intervention.

24% oral sucrose and offered non-nutritive sucking intervention. In the sucrose intervention, all procedures and monitoring took place while the infant was in a cot. Procedures were consistent with those outlined above with the exception of administration of 24% oral sucrose two minutes prior to the heel lance as per a study specific dosing protocol (Appendix J [Stevens et al., 2018]). Non-nutritive sucking was offered using a gloved finger or pacifier (based on parental preference) immediately following administration of the complete 24% oral sucrose dose. While the use of a pacifier is contraindicated in healthy full term newborns outside of the pain context due to

the potential for nipple confusion and interference with sustained exclusive breastfeeding (World Health Organization, 2009), it is considered acceptable when being used as a pain-relieving intervention. Prior to offering a pacifier for pain reduction to any newborn enrolled in the study, the associated risks and benefits were discussed with parents. If the infant was offered and sustained non-nutritive sucking throughout the procedure this was documented and accounted for in statistical analysis. Appendix K presents the experimental timeline for those infants randomized to the 24% oral sucrose and offered non-nutritive sucking intervention.

Rescue sucrose administration. Regardless of assigned intervention condition, the PI or assisting research coordinator was permitted to administer additional off study doses of sucrose if an infant enrolled in the study had a PIPP-R score greater than six (which indicates the presence of pain) during data collection. The PI and assisting research coordinator continuously monitored all infants' bio-behavioural pain responses following heel lance and administered these "rescue" sucrose doses to any infant with a PIPP-R score greater than six. Administration of additional sucrose doses followed study and hospital protocol and all off-study "rescue" doses of sucrose were recorded in the procedure record and in the patient's medical record.

Analysis

Analysis and inference were calculated based on the modified intention-to-treat principle (Detry & Lewis, 2014; Gupta, 2011). Application of the ITT principle to study analysis means that participants who were randomized to the breastfeeding intervention but did not successfully breastfeed during the painful procedure were retained in the final analysis if they had data for the outcome measures. Use of ITT allows for an approximation of treatment effect that reflects the variation in intervention compliance

that would typically be seen in clinical practice (Detry & Lewis, 2014). This analysis principle was used to minimize the risk of a biased estimate of treatment efficacy that is often associated with per protocol analyses in which only participants who fully adhere to the assigned intervention are retained in the analysis (Montori & Guyatt, 2001). Efforts were made to ensure that all subjects had complete follow-up and to minimize missing values for any of the subjects for outcome variables. Data editing included inspection of means and frequency distributions to identify any potential outliers or implausible values. Any outliers were manually reviewed to ensure no data entry errors occurred and any identified outliers were corrected if that information was available. Participant characteristics were summarized using descriptive statistics and differences in participant baseline characteristics were assessed with unpaired Student's *t*-tests for continuous data and chi-square tests for non-continuous (nominal and ordinal) data using the Statistical Package for the Social Sciences (SPSS, IBM Corporation, Armonk, New York, USA). For any differences noted in baseline characteristics between the two intervention groups, inferences were made on observed and linear regression adjusted differences between groups.

Primary hypothesis: Event-related potential analysis. The primary hypothesis of this study was that infants randomized to the breastfeeding intervention would demonstrate a lower amplitude pain-related event-related potential when compared to those infants randomized to the 24% oral sucrose and offered non-nutritive sucking intervention. To address this hypothesis, data from the EEG recordings were processed and statistically analyzed using both MNE-Python v0.15 (Gramfort et al., 2014) and MATLAB (MathWorks Inc., Natick, Massachusetts, USA). The continuous data from the EEG recordings were extracted into 1500-millisecond epochs corresponding to the

noxious heel lance and non-noxious control stimuli. The EEG epochs extracted included activity 500-milliseconds before the stimulus and 1000-milliseconds after the stimulus. The EEG data were filtered with a frequency pass band ranging from 1.0 – 30 Hz, referenced to electrode E17 (corresponding to electrode Fz on the international 10/20 electrode placement system, located midline on the scalp, anterior to vertex electrode Cz). and extracted segments were baseline corrected using the pre-stimulus interval. Artifact correction was completed using Independent Component Analysis (ICA). Scalp maps corresponding to each component were examined and those that represented movement artifact or excessive noise were removed. Individual infants were excluded if there was movement artifact detected (a voltage change of greater than 100 μV over 50-milliseconds) in the 1500-millisecond epochs surrounding the noxious heel lance and non-noxious control stimuli at electrode site E19 (Appendix L). Data from the EEG epochs extracted from electrode site E19 were statistically decomposed and grouped into basic waveforms using the data reduction technique of principal component analysis (PCA [Dien, 2012]) in the event-related potential (ERP) PCA Toolkit version 2.76 (Dien, 2019) in MATLAB (MathWorks Inc., Natick, Massachusetts, USA). This method of quantifying the event-related potentials following the noxious heel lance and non-noxious control stimuli was selected as it is particularly useful in analyzing noisy EEG data from developmental populations, such as infants. It was selected as a more robust analytic procedure to peak-to-peak analyses for the single trial EEG data recorded in this study. A temporal PCA with promax rotation (Dien, 2010) was completed on the 1500-millisecond epoch surrounding the noxious heel lance and the non-noxious control stimuli (Dien, 2012). Time points were considered as variables and electrode sites and subjects were considered observations. The covariance matrix was selected as the matrix of association

between variables, as covariance matrices have been found to be most appropriate in ERP PCA analyses (Dien, 2012). The resulting principal components (PCs) represent systematic variation in the amplitude of the EEG signal at different time points across the EEG tracing to help identify the constituent components of the ERP (Dien, 2012). To address the primary hypothesis of this study, six principal components were generated: Three PCs for the breastfeeding group (PCs for the two non-noxious control stimuli and PC for the one noxious heel lance) and three PCs for the sucrose group (PCs for the two non-noxious control stimuli and PC for the one noxious heel lance). Three one-way analyses of variance (ANOVAs) were run on the trimmed mean voltage amplitude of the principal components to assess the effect of stimulation type (non-noxious control stimuli, noxious heel lance) and treatment (24% oral sucrose, breastfeeding). The trimmed mean of the PC amplitude was calculated by trimming off the most extreme values contributing to the mean (removing the lowest and highest 25% of values) to produce a more robust estimate of the central tendency of the PC (Leonowicz, Karvanen, & Shishkin, 2005). With this analysis, it was possible to assess if 1) exposure to heel lance produced a significant difference in amplitude of pain-related component compared to exposure to the non-noxious control stimuli, 2) if the magnitude of the pain-related component varied between intervention groups, and 3) if the magnitude of the non-noxious component varied between intervention groups (Fabrizi et al., 2011; Slater et al., 2010a-c).

Secondary hypotheses. The secondary hypotheses of this study were that infants randomized to the breastfeeding intervention, when compared to those infants randomized to the 24% oral sucrose and offered non-nutritive sucking intervention, would demonstrate: a) Lower bio-behavioural pain scores, measured using the PIPP-R at 30-, 60-, 90-, and 120-seconds following heel lance, b) faster physiologic recovery measured

as heart rate return to baseline, c) greater maternal acceptability regarding the data collection procedures and interventions, and d) fewer adverse events (e.g., fewer episodes of temperature instability, fewer rescue sucrose doses). The analyses for these hypotheses included:

a) Bio-behavioural pain response: PIPP-R scores were calculated for each infant on the basis of the behavioural (facial) data, physiologic (heart rate, oxygen saturation), and contextual (gestational age, behavioural state) data. The continuous video recordings of the infant's facial responses were segmented into epochs corresponding to 30-seconds before the heel lance and 30-, 60-, 90-, and 120-seconds following the heel lance and 30-seconds prior to and following the non-noxious control stimulus. These PIPP-R score epochs were selected as they have been consistently utilized in clinical trials testing similar non-pharmacologic pain-relieving interventions in newborns (Campbell-Yeo et al., 2012; Johnston et al., 2003; Johnston et al., 2008). Research assistants blinded to intervention, stimuli being applied, and timing of each video epoch coded for the presence of each facial expression individually using the Pain Assessment in Newborns (PAiN) coding program (Hundert, Wozney, O'Connor, & Campbell-Yeo, 2018). The mean heart rate and oxygen saturation in the 30-seconds before the heel lance and non-noxious control stimulus and the maximum heart rate and minimum oxygen saturation in the epochs corresponding to 30-, 60-, 90-, and 120-seconds following the heel lance and 30-seconds following the non-noxious control stimulus were used to calculate the physiologic components of the PIPP-R score for each epoch. The behavioural state score was determined by observing the infants sleep

state and facial movement in the 30-seconds prior to heel lance and infant gestational age at the time of procedure were obtained from a chart review. After calculation of PIPP-R scores for each time point (30-, 60-, 90-, 120-seconds), group means were compared using unpaired Student's *t*-tests in SPSS (IBM Corporation, Armonk, New York, USA) to assess for an effect of treatment.

b) Physiologic recovery: Recovery time, defined as the period of time in seconds from the end of blood sampling until the heart rate returned to the average baseline heart rate for a minimum of five to seven beats, was calculated for each infant. Mean time to recovery between the two groups were compared using unpaired Student's *t*-tests in SPSS (IBM Corporation, Armonk, New York, USA).

c) Adverse events: Frequency of occurrence of each of the safety surveillance outcomes (i.e., choking, apnea, bradycardia, need for repeat procedure, administration of "rescue" sucrose, and incidence of hypothermia) were compared between the two groups using Student's *t*-tests for continuous data and chi-square tests for categorical data in SPSS (IBM Corporation, Armonk, New York, USA).

d) Maternal acceptability: Categorical data from the maternal questionnaire were summarized using frequencies and percentages and differences between the two groups were compared using chi-square tests in SPSS (IBM Corporation, Armonk, New York, USA).

Measures to Protect Against Sources of Bias

Due to the nature of the breastfeeding intervention, double blinding was not possible. Therefore, it was necessary to implement rigorous methods to minimize potential sources of bias. The use of a password protected website to implement randomly permuted block randomization of participants minimized risk of allocation bias. The website and randomization code were generated off site by an individual external to the study. A record of all participants who were eligible to participate, were approached, and were enrolled or refused was maintained to monitor for potential subversion bias (Campbell-Yeo, Ranger, Johnston, & Fergusson, 2009). Intervention fidelity was documented for both the breastfeeding and 24% oral sucrose and offered non-nutritive sucking interventions to ensure that the interventions remained stable over time and participants (Campbell-Yeo et al., 2009). During data collection, close-up video recording of only the infants face was completed to attempt to keep coders blind to the nature of the intervention. As previously noted, all attempts were made to limit data loss and ITT analysis was utilized to maintain statistical power and group allocation to promote balancing of confounding variables between groups (Campbell-Yeo et al., 2009). It was not possible to utilize one lab technologist for all blood collections. Therefore, length of procedure, blood volume, and need for a repeat heel lace was collected to take into account potential variation in technique as a source of bias.

Two research assistants (RA1, RA2) who were blinded to the intervention were hired to complete facial coding and calculation of infant PIPP-R scores. Each research assistant only coded data for infants in the breastfeeding intervention (RA1) or the 24% oral sucrose intervention (RA2) and were not aware of whether they were watching infant responses to noxious or non-noxious stimulation or the timing of the observation. To minimize observer bias, research assistants were not informed of the study design, were

not be permitted to attend data collection sessions, and did not share datasets or communicate with the other research assistant regarding the study. The PI and a member of Dr. Campbell-Yeo's (PI's primary supervisor) research team trained the research assistants on how to complete infant facial coding. The training and coding processes were standardized (Appendix M) and individual coding performance was assessed on standardized videos. An interclass correlation coefficient (ICC) of 0.98 (95% CI [0.86 – 0.99]) was met between the research assistants' coding for the PIPP-R behavioural components of eye squeeze, brow bulge, and nasolabial furrow. Following the initial training, the research assistants were reassessed every three months to ensure inter-rater reliability by complete facial coding of a standardized video. If the ICC fell below 0.75, retraining and recoding for the purpose of performance assessment took place. This reliability check minimized the likelihood of observer bias. To ensure intra-rater reliability, research assistants were required to re-code two randomly selected videos that they completed in the first two weeks of coding every three months. An ICC of 0.75 was considered the lowest acceptable cut off prior to retraining and reassessment of performance. Following completion of the study, the research assistants previously assigned to each intervention were required to code a random selection of videos from the alternate intervention and ICC scores were obtained. This reliability check ensured that any differences in pain response found between the intervention and control group were due to the intervention and not systematic errors in the coding techniques used by the corresponding research assistants. The ICC's between coders for the random selection of videos from the alternate intervention ranged from 0.95 to 0.97.

Ethical Considerations

Ethical approval was obtained from the Research Ethics Board of the IWK Health Centre prior to initiation of study procedures. The PI or assisting research coordinator first became aware of eligible patients through collaboration with the clinical team leader on the postpartum unit of the IWK Health Centre. The staff nurse providing care to eligible infants obtained verbal permission from each infant's mother before the PI or assisting research coordinator approached to provide information about the study. Informed consent (Appendix N) was obtained from the mother of each eligible infant prior to study entry and randomization into the intervention group. A copy of the completed consent form was provided to all participants. The consent form contained information on potential risks and benefits associated with study participation, research rights of the participant, and information on how to contact the PI, study co-supervisors, and IWK Health Centre research services department for any information on rights of study participants. Participation in the study was entirely voluntary, and mothers were informed of their right to withdraw consent to participate at any time. While this study did not provide direct benefit to the infant's or parents enrolled and compensation was not offered, participation did ensure that infants received a known effective form of analgesic intervention during a routine painful procedure. The heel lance that infants underwent as part of this study was part of routine medical care, and no painful procedures were completed solely for the purpose of this study. Study participation did not interfere with routine clinical care practices.

Provision of 24% oral sucrose and non-nutritive sucking or facilitation of breastfeeding as analgesic interventions during heel lance is not yet fully integrated as part of routine care in the unit at which this study took place. However, the completion of clinical trials that do not offer some form of pain management to infants in all treatment

arms and thus expose infants to unnecessary pain is now considered unethical (Bellieni & Johnston, 2016; Campbell-Yeo, 2016). Both 24% oral sucrose and breastfeeding have been found to be effective interventions for reducing bio-behavioural pain scores in full term newborns. Therefore, infants who were enrolled in this study received a pain-relieving intervention that has documented efficacy in the academic literature, regardless of randomization. Given that 24% oral sucrose and non-nutritive sucking is the most studied non-pharmacologic intervention (Stevens et al., 2016) and considered the current standard of care for procedural pain relief in the literature (Taddio, Yiu, Smith, Katz, McNair, & Shah, 2009), the PI or assisting research coordinator were permitted to administer additional off study doses of sucrose if an infant enrolled in the study (regardless of being in the 24% oral sucrose and offered non-nutritive sucking or breastfeeding intervention) had a PIPP-R score greater than six (which indicates the presence of pain) during data collection.

Many strategies were utilized to ensure that confidentially of all data collected for the purpose of this study was maintained. Upon recruitment into the study, infants were assigned a study identification number and all information gathered was coded with that number. All information gathered in hard copy was stored in a filing cabinet in the research lab of the study PI's primary supervisor (Campbell-Yeo) under double lock and only the PI (Benoit), study primary supervisor (Campbell-Yeo), and assisting research coordinator had access to these files. The list of participant identification codes was stored and locked separately from the participant's coded data. Only group level data will be presented, published, and shared with participants, and the information shared will not contain any identifying information. All video recordings and EEG recordings were encrypted and stored on an external hard drive under double lock at the IWK Health

Centre. Master copies of all research data will be secured under double lock for twenty-five years past the age of majority of all infants.

The Geodesic EEG System™ 400 series (Electrical Geodesics Incorporated, Eugene, Oregon, USA) is a certified medical device with Health Canada (Appendix O). A member of Electrical Geodesics Incorporated's training and technical support team installed and configured the system at the IWK Health Centre to ensure its safety for use and the system was approved by the IWK Health Centre's engineering department for use in the hospital. The HydroCel Geodesic Sensor Net offers a dense-array non-abrasion high-impedance application method, making it optimal for utilization with infants (Electrical Geodesics, Inc., 2007). Specifically, the elastomer structure of the net equally distributes the electrodes across the scalp with gentle radial pressure to promote comfort and speeds up application time (approximately five to 15-minutes). Furthermore, the net is easy to apply, requires no abrasion of the scalp, and does not use harsh chemicals, which reduces the risk of infection or skin irritation making it ideal for use in infants when compared to traditional EEG applications (Electrical Geodesics, Inc., 2016). The infant specific nets that were utilized in this study do not have face straps to simplify application and minimize stress associated with application. As previously noted, all HydroCel Geodesic Sensor Nets have a chin strap. Therefore, all infants were monitored constantly, both visually and by observing heart rate and oxygen saturation, during data collection in order to prevent any risk of choking. The PI or assisting research coordinator additionally monitored axillary temperature and checked for skin pressure points, overturned sensors, or redness under the chin strap every 15-minutes to maximize infant comfort and ensure normothermia while the infant was wearing the net (Electrical Geodesics, Inc., 2007).

CHAPTER 4: RESULTS

This chapter will begin with a flow diagram outlining participant recruitment in accordance with the Consolidated Standards of Reporting Trials (CONSORT) statement, available at http://www.consort-statement.org. Baseline characteristics of each group at randomization will be reported to highlight potential confounders that could bias study results. The results for the primary outcome of the influence of breastfeeding compared to 24% oral sucrose and offered non-nutritive sucking on pain-related event-related potential will follow. Comparisons between groups for the secondary outcomes of bio-behavioural pain score measured using the PIPP-R, physiologic recovery, maternal acceptability, and adverse events will follow.

Participant Enrolment

Participants were recruited from the Family Newborn Care Unit of the IWK Health Centre, in Halifax, Nova Scotia, Canada from November 2017 to January 2019. Of the 202 of infants who were found to be eligible during the study recruitment period, 39 had parental consent and were recruited into the study (Figure 1). Of these, 20 were randomly assigned to the breastfeeding intervention and 19 were randomly assigned to the 24% oral sucrose and offered non-nutritive sucking intervention. Two parent participants who initially provided consent to participate in the study and were randomized to the breastfeeding intervention were lost to follow-up and did not complete study procedures. Reasons for loss to follow-up were that the family was discharged home from the hospital prior to the completion of the routine heel lance ($n = 1$) and the routine bloodwork was ordered to be completed early by the medical team without notification of research personnel ($n = 1$).

Reasons for exclusion of potential eligible subjects included: Parents declining to be approached by the researcher to discuss the study (n = 31, 19%), parents declining to participate in the study after being approached (n = 59, 36%), and unavailability of the equipment and PI/assisting research coordinator (e.g., due to enrolling a participant who had their clinical heel lance scheduled for the same time as another eligible participant, coordination issues, unavailability, n = 73, 45%). The refusal rate for this study was 60%. The primary reasons for parent refusal to take part in the study after being approached by the researcher were that mothers felt too overwhelmed/tired (37%), parents were not interested in research (36%), parents preferred breastfeeding for pain management and did not want their baby to be randomized to the 24% oral sucrose and offered non-nutritive sucking intervention (13.5%), and fathers did not want their baby enrolled (13.5%).

For the primary outcome of pain-related event-related potential, failure of the equipment for time-locking the noxious heel lance and non-noxious control stimuli to the continuous EEG recording occurred in four infants (22.2%) receiving the breastfeeding intervention and four infants (21.1%) receiving the 24% oral sucrose and offered non-nutritive sucking intervention. There were additional instances where movement artifact (a voltage change of greater than 100 μV over 50-milliseconds) contaminated the EEG epochs surrounding the noxious heel lance and/or the non-noxious control stimuli. Specifically, four infants (22.2%) receiving the breastfeeding intervention and five infants (27.7%) receiving the 24% oral sucrose and offered non-nutritive sucking intervention were excluded from the analysis of the primary outcome due to movement artifact. Therefore, the final sample for the primary outcome analysis of pain-related brain activity in response to heel lance included 20 infants (10 infants who received the breastfeeding

intervention and 10 infants who received the 24% oral sucrose and offered non-nutritive sucking intervention).

For the secondary outcome of bio-behavioural pain response measured using the PIPP-R, video recording equipment failure occurred in four infants receiving the breastfeeding intervention and one infant who received the 24% oral sucrose and offered non-nutritive sucking intervention. Infants were thus excluded from the calculation of the PIPP-R score at 30- (n =3 [breastfeeding intervention]), 60- (n = 4 [breastfeeding intervention]), 90- (n = 4 [breastfeeding intervention]), and 120- (n = 5 [four breastfeeding intervention, one sucrose intervention]) seconds following the heel lance. Therefore, the final sample for the secondary outcome analysis of bio-behavioural pain score included 34 infants at 30-seconds [15 breastfeeding intervention, 19 sucrose intervention], 33 infants at 60- seconds and 90- seconds [14 breastfeeding intervention, 19 sucrose intervention], and 32 infants at 120-seconds following the heel lance [14 breastfeeding intervention, 18 sucrose intervention].

For the secondary outcomes of physiologic recovery measured as heart rate return to baseline, two infants were excluded from the analysis due to loss of heart rate data secondary to physiologic monitoring equipment failure. Therefore, 35 infants were included on the secondary outcome analysis of physiologic recovery. With the exception of those participants lost to follow-up following randomization (n = 2), complete data were obtained for the secondary outcome analyses of adverse events. Therefore 37 infants were included in the outcome analyses for adverse events. Only one mother (randomized to the breastfeeding intervention) did not return her maternal acceptability questionnaire, therefore, 36 mothers were included in the analyses of maternal acceptability of intervention and data collection procedures.

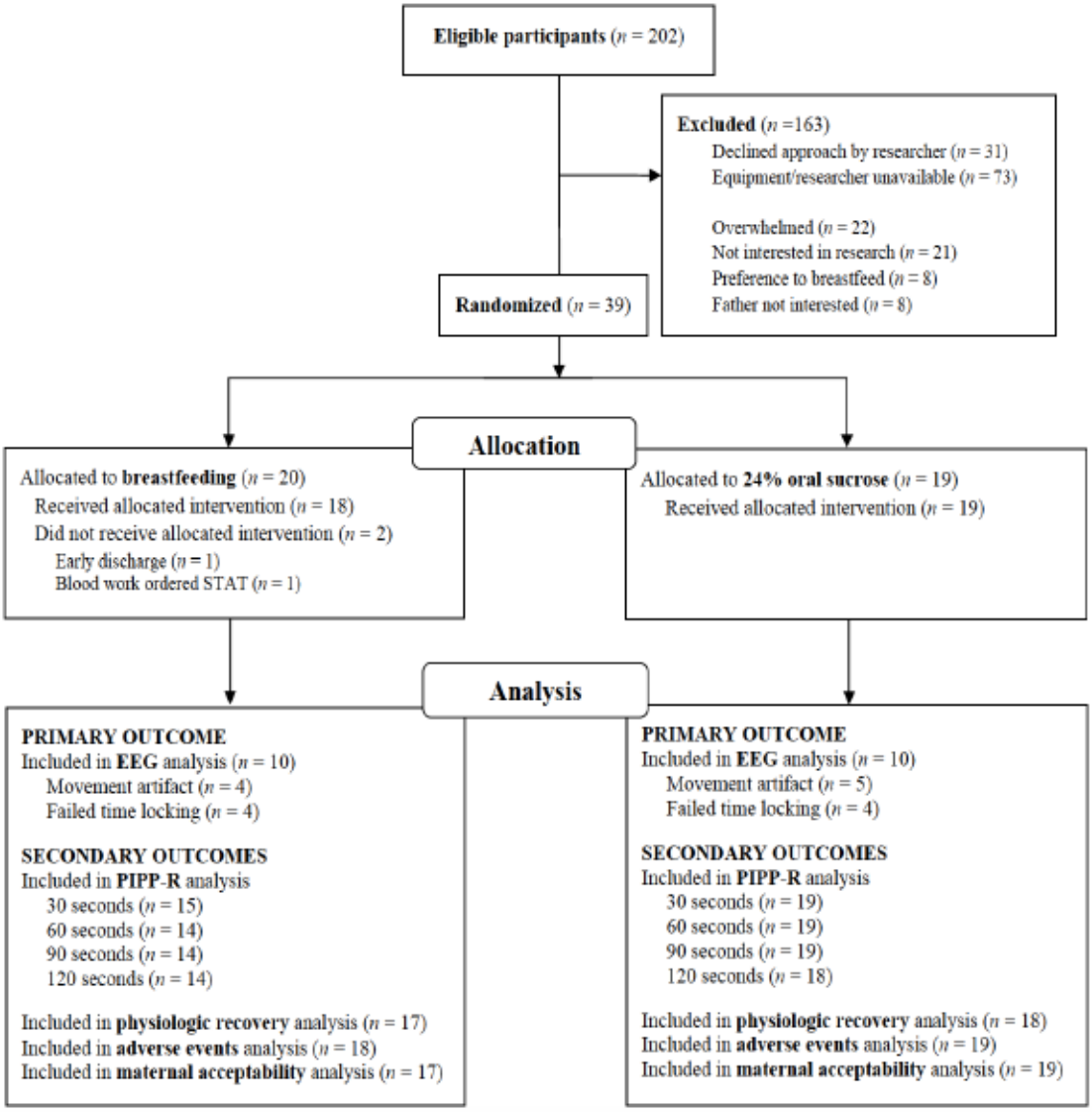

Figure 1. Participant flow diagram outlining participant enrolment, randomization, allocation, and inclusion in primary and secondary outcome analyses in accordance with the Consolidated Standards of Reporting Trials (CONSORT) statement, available at http://www.consort-statement.org.

Sample Characterization

Maternal, infant, and procedure characteristics were not significantly different between the breastfeeding and 24% oral sucrose and offered non-nutritive sucking groups at randomization (Table 2). As there were no identified differences between groups on any potential confounding variables (i.e., prior pain exposure, infant sex), no additional

statistical approaches were utilized to adjust for the potential effect of differences in covariates between groups.

Maternal characteristics. Mean maternal age was 29.8 ($SD = 4.7$) years for the breastfeeding group and 30.4 ($SD = 4.2$) years for the 24% oral sucrose and offered non-nutritive sucking group ($p = 0.644$, 95% CI [-3.59 – 2.24]). The majority of mothers enrolled in the study were Caucasian (90% in the breastfeeding group, 84.2% in the 24% oral sucrose and offered non-nutritive sucking group) with a small proportion being first time mothers (15% and 30% respectively). Approximately half of mothers had experience breastfeeding a previous baby (65% in the breastfeeding group and 52.6% in the 24% oral sucrose and offered non-nutritive sucking group). All families were two-parent families, the majority had a college or university education (70% in the breastfeeding group and 78.9% in the 24% oral sucrose and offered non-nutritive sucking group), and the majority of mothers were employed at the time that they had their baby (85% and 89.5% respectively). Very few mothers had a history of diabetes or smoking, no mothers were administered antenatal steroids, and only one mother was administered magnesium sulphate. Maternal and infant characteristics are presented in Table 2.

Infant characteristics. The infant characteristics in both groups were highly homogeneous, with no significant differences between groups with respect to gestational age (39.3 weeks in the breastfeeding group and 39.5 in the 24% oral sucrose and offered non-nutritive sucking group), birth weight (3508.7 and 3318.1 grams respectively), and Apgar score at five minutes (9 for both groups). All participating infants had the heel lance completed on postnatal day one, and a small proportion of infants were large for gestational age (15% of the breastfeeding group, 5.3% of the 24% oral sucrose group) or small for gestational age (5% and 10.5% respectively).

Table 2

Comparison of Maternal and Infant Characteristics between Breastfeeding and 24% Oral Sucrose Groups at Randomization

Maternal characteristics	Breastfeeding ($n = 20$)	24% sucrose ($n = 19$)	p	95% CI
Age in years, *M(SD)*	29.8(4.7)	30.4(4.2)	0.644	[-3.59, 2.24]
Caucasian (frequency, %)	18(90.0)	16(84.2)	0.377	
Primiparous (frequency, %)	3(15.0)	6(31.6)	0.284	
Experience breastfeeding previous child (frequency, %)	13(65.0)	10(52.6)	0.433	
Caesarean birth (frequency, %)	6(30.0)	5(26.3)	0.667	
Family arrangement, two-parent (frequency, %)	20(100.0)	19(100.0)	1.00	
Education, postsecondary graduation (frequency, %)	14(70.0)	15(78.9)	0.548	
Employment status, employed (frequency, %)	17(85.0)	17(89.5)	0.562	
Maternal diabetes (frequency, %)	0(0)	1(5.3)	0.299	
Maternal smoking (frequency, %)	2(10)	1(5.3)	0.579	
Antenatal steroids (frequency, %)	0(0)	0(0)	1.0	
Magnesium sulphate (frequency, %)	0(0)	1(5.3)	0.299	

Infant characteristics	Breastfeeding ($n = 20$)	24% sucrose ($n = 19$)	p	95% CI
Gestational age in weeks, *M(SD)*	39.3 (1.2)	39.5(1.23)	0.401	[-1.13, 0.46]
Birth weight in grams, *M(SD)*	3508.7(542.0)	3318.1(428.1)	0.232	[-127.4, 508.6]
Male sex (frequency, %)	12(60.0)	11(57.9)	0.894	
Apgar score at five minutes, *M(SD)*	9.0(0.224)	9(0)	0.336	[-127.4, 508.6]
Postnatal age at heel lance in days, *M(SD)*[a]	1.0(0)	1.0(0)	1.0	
Large for gestational age (frequency, %)	3(15.0)	1(5.3)	0.316	
Small for gestational age (frequency, %)	1(5.0)	2(10.5)	0.517	

Note. M(SD) denotes mean(standard deviation).
[a]$n = 37$ (18 in the breastfeeding group and 19 in the 24% sucrose group) as two participants lost to follow-up prior to completion of the study heel lance.
*$p < 0.05$

Procedure characteristics. The majority of infants had low procedural pain exposure in the 24-hours prior to the study heel lance. Of those babies who did have a procedure in the 24-hour period preceding the heel lance, all were tissue-breaking. The mean length of time required to complete the study heel lance was similar between groups (4.0 minutes [$SD = 0.9$] for the breastfeeding group compared to 3.4 minutes [$SD = 1.3$] for the 24% oral sucrose and offered non-nutritive sucking group, $p = 0.09$ 95% CI [-0.10 – 1.37]). The blood volume collected was also similar between groups, with a mean blood volume of 0.98 millilitres ($SD = 0.17$) being collected from the breastfeeding infants and a mean blood volume of 1.03 millilitres ($SD = 0.08$) collected from the 24% oral sucrose infants. The heel lance was completed on the left foot in 33.3% of the

breastfeeding infants and 52.6% of the 24% oral sucrose infants. The mean LATCH score for infants in the breastfeeding intervention was 4.83 (*SD* = 3.81, range = 0 – 10). Of those infants in the 24% oral sucrose and offered non-nutritive sucking intervention, 63.2% sustained non-nutritive sucking for the duration of the procedure. Procedure characteristics are reported in Table 3.

Table 3

Comparison of Procedure Characteristics between Breastfeeding and 24% Oral Sucrose Groups

	Breastfeeding ($n = 18$)[a]	24% sucrose ($n = 19$)	*p*	95% CI
Prior pain exposure				
Total number painful procedures in 24-hours prior to study heel lance, *M(SD)*	1.33(1.9)	0.89(1.4)	0.430	[-0.678, 1.55]
Distribution of painful procedure exposures in 24-hours prior to study heel lance (frequency, %)			0.791	
0	8(44.4)	9(47.4)		
1	5(27.8)	7(36.8)		
2	2(11.1)	2(10.5)		
≥ 3	3(16.7)	1(5.3)		
Prior procedure tissue breaking (frequency, %)	18(100)	19(100)	1.0	
Heel lance procedure characteristics				
Procedure length in minutes, *M(SD)*	4.0(0.9)	3.4(1.3)	0.09	[-0.10, 1.37]

	Breastfeeding ($n = 18$)[a]	24% sucrose ($n = 19$)	p	95% CI
Heel lance procedure characteristics				
Blood volume collected in milliliters, *M(SD)*	0.98(0.17)	1.03(0.08)	0.241	[-0.14, 0.04]
Heel lance completed on left foot (frequency, %)	6(33.3)	10(52.6)	0.236	
LATCH scores for breastfeeding infants, *M(SD)*	4.83(3.81)	–	–	
Non-nutritive sucking sustained for sucrose infants (frequency, %)	–	12(63.2)	–	

Note. M(SD) denotes mean(standard deviation).
[a]$n = 18$ as two participants lost to follow-up prior to completion of the study heel lance.
*$p < 0.05$

Pre-procedure feeding characteristics. The majority of infants were fed in the two hour period preceding the study heel lance, with 50% being fed less than an hour prior to the heel lance in the breastfeeding group and 57.9% being fed less than an hour prior to the heel lance in the oral sucrose group. All babies were fed directly at the breast for the preceding feeding (Table 4).

Table 4

Comparison of Pre-procedure Feeding Characteristics between Breastfeeding and 24% Oral Sucrose Groups

	Breastfeeding ($n = 18$)[a]	24% sucrose ($n = 19$)	p
Pre-procedure feeding characteristics			

	Breastfeeding ($n = 18$)[a]	24% sucrose ($n = 19$)	p
Time of feeding preceding heel lance			0.113
< 1 hour	9 (50.0)	11(57.9)	
< 2 hours	6 (33.3)	1(5.3)	
< 3 hours	3(16.7)	4(21.1)	
≥ 3 hours	0(0)	3(15.9)	
Pre-procedure feeding characteristics			
Method of preceding feeding, breast, frequency(%)	18(100)	19(100)	1.0

Note. M(SD) denotes mean(standard deviation)
[a]$n = 18$ as two participants lost to follow-up prior to completion of the study heel lance.
*$p < 0.05$

Primary Hypothesis: Event-related Potential Analysis Results

The primary outcome of pain-related electroencephalographic event-related potential following heel lance was compared between infants randomized to the breastfeeding intervention and infants randomized to the 24% oral sucrose and offered non-nutritive sucking intervention. The primary hypothesis of this study was that infants randomized to the breastfeeding intervention would demonstrate a lower amplitude pain-related potential when compared to those infants randomized to the 24% oral sucrose and offered non-nutritive sucking intervention. To address this hypothesis, data from continuous neonatal EEG recording were extracted following the application of the noxious heel lance and non-noxious control stimuli, processed, subjected to PCA, and statistically analyzed as outlined in Chapter 3.

Individual and group level average waveforms. Individual pre-processed participant waveforms during a segment of background EEG recording, in response to non-noxious control stimulus, and noxious heel lance for all participants who completed the study ($n = 37$) are provided in Appendix P. Participants with movement artifact (a voltage change of greater than 100 µV over 50-milliseconds) contaminating the epoch of EEG recording following the non-noxious control stimuli and/or noxious heel lance were excluded from subsequent analyses. Group averaging of event-related potential responses in participants with artifact-free EEG epochs ($n = 20$) were completed for response to the noxious heel lance (Figure 2), the non-noxious control stimulus prior to intervention initiation (non-noxious one [NN1], Figure 3), and the non-noxious control stimulus after intervention initiation (non-noxious two [NN2], Figure 4) for all participants and divided by treatment (breastfeeding intervention, $n = 10$, Figure 5; 24% oral sucrose and offered non-nutritive sucking intervention, $n = 10$, Figure 6) was completed. Notable differences in the morphology and latency of event-related potential waveforms evoked following non-noxious control stimuli and noxious heel lance were not clearly identifiable in the group averaged responses.

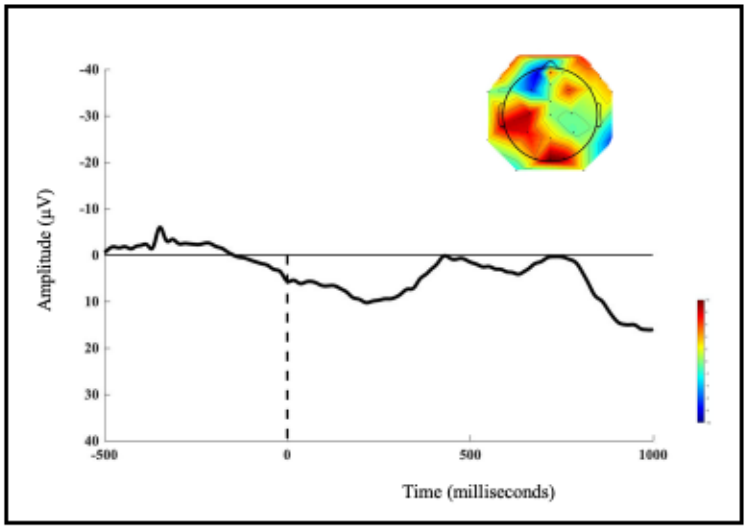

Figure 2. Average event-related potential in response to heel lance stimulus in 20 infants at vertex electrode (E19). Vertical dashed line marks the onset of the stimulus. The heel lance was applied at time 0, epoch includes 500-milliseconds prior to stimulus and 1000-milliseconds following stimulus. Negative is plotted upwards. Topographic plot at peak amplitude provided.

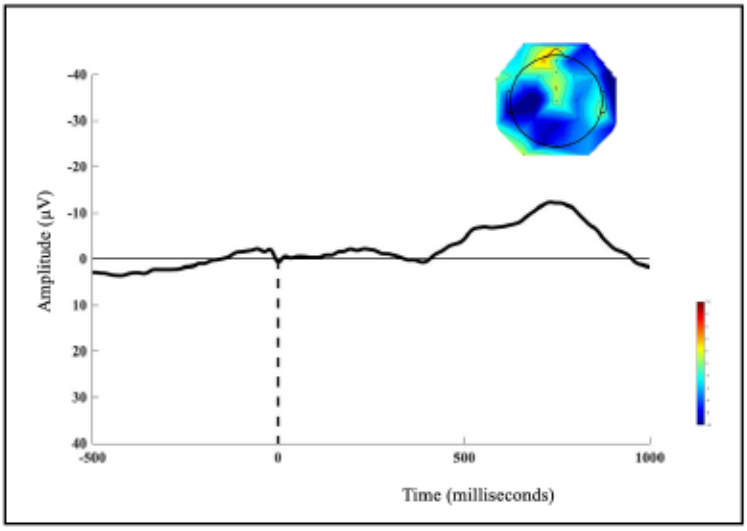

Figure 3. Average event-related potential in response to non-noxious control stimulus one in 19 infants at vertex electrode (E19). Vertical dashed line marks the onset of the stimulus. The non-noxious control stimulus was applied at time 0 (prior to intervention initiation), epoch includes 500-milliseconds prior to stimulus and 1000-milliseconds following stimulus. Negative is plotted upwards. Topographic plot at peak amplitude provided.

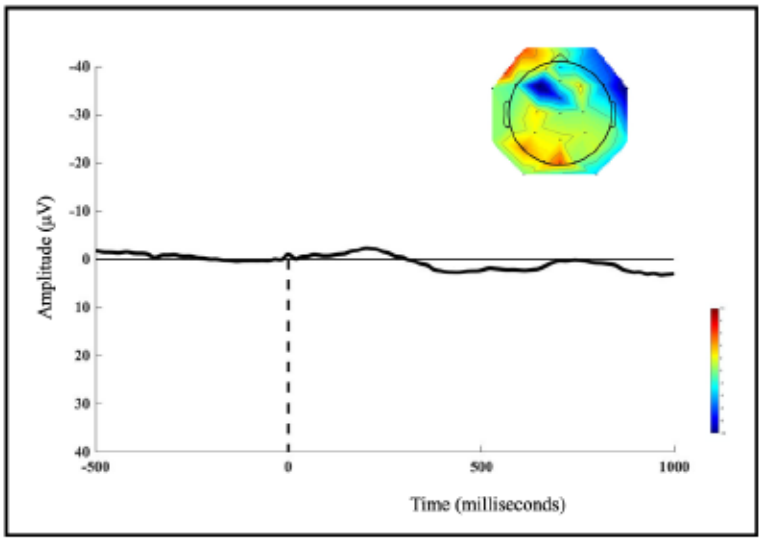

Figure 4. Average event-related potential in response to non-noxious control stimulus two in 18 infants at vertex electrode (E19). Vertical dashed line marks the onset of the stimulus. The non-noxious control stimulus was applied at time 0 (following intervention

initiation), epoch includes 500-milliseconds prior to stimulus and 1000-milliseconds following stimulus. Negative is plotted upwards. Topographic plot at peak amplitude provided.

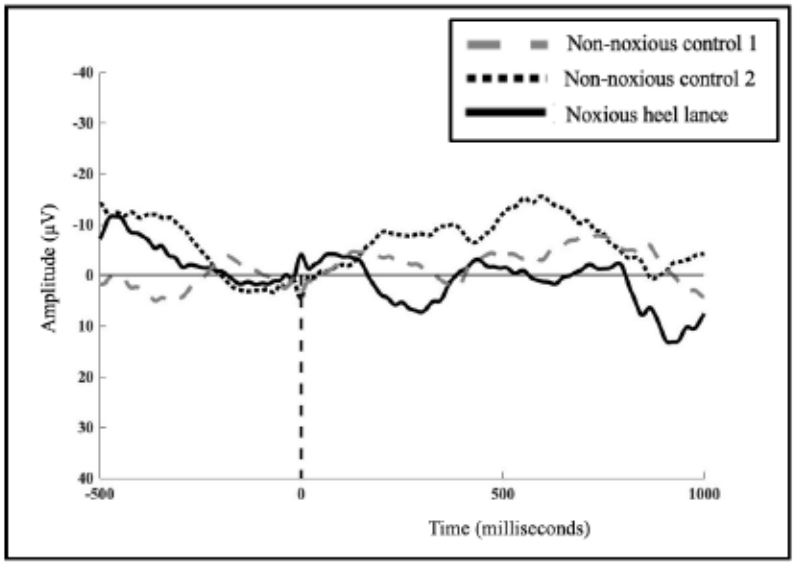

Figure 5. Average event-related potential in response to noxious heel lance, non-noxious control stimulus one, and non-noxious control stimulus two in breastfeeding infants (n = 10) at vertex electrode (E19). Vertical dashed line marks the onset of the stimulus. The heel lance and non-noxious control stimuli were applied at time 0, epoch includes 500-milliseconds prior to stimulus and 1000-milliseconds following stimulus. Negative is plotted upwards.

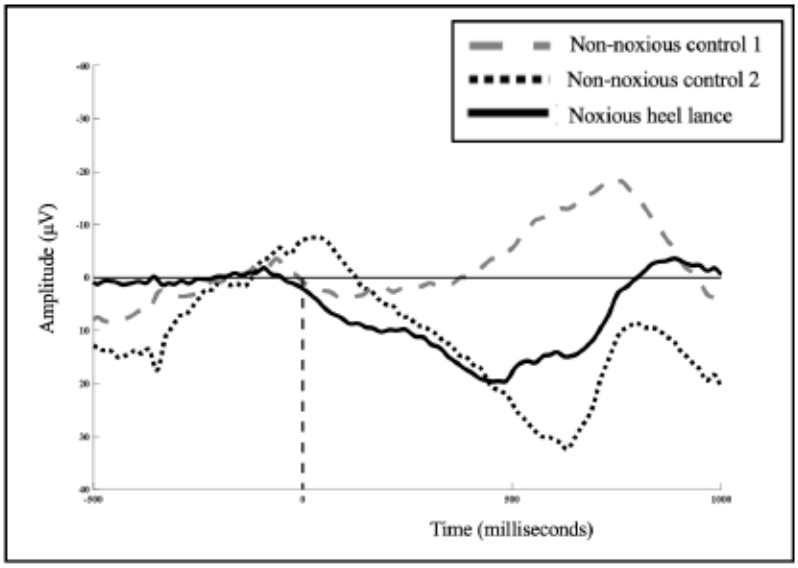

Figure 6. Average event-related potential in response to noxious heel lance, non-noxious control stimulus one, and non-noxious control stimulus two in infants who received 24% oral sucrose and offered non-nutritive sucking (n = 10) at vertex electrode (E19). Vertical

dashed line marks the onset of the stimulus. The heel lance and non-noxious control stimuli were applied at time 0, epoch includes 500-milliseconds prior to stimulus and 1000-milliseconds following stimulus. Negative is plotted upwards.

Difference waveforms between noxious heel lance and non-noxious control stimulus. The averaged data were additionally used to generate difference waveforms between the experimental stimuli. Specifically, two difference waveforms comparing the event-related potential response between the non-noxious control stimulus one and noxious heel lance were generated – one for the breastfeeding condition and one for the 24% oral sucrose and offered non-nutritive sucking condition. For those infants in the breastfeeding condition (n = 10), the difference waveform had an identifiable positive deflection of 5.7 μV 600-milliseconds following stimuli application with a peak-to-peak amplitude of 8.6 μV (Figure 7). For those infants in the sucrose condition (n = 10), the difference waveform has an identifiable positive deflection of 5.3 μV 500-milliseconds following stimuli application with a peak-to-peak amplitude of 11.46 μV (Figure 8).

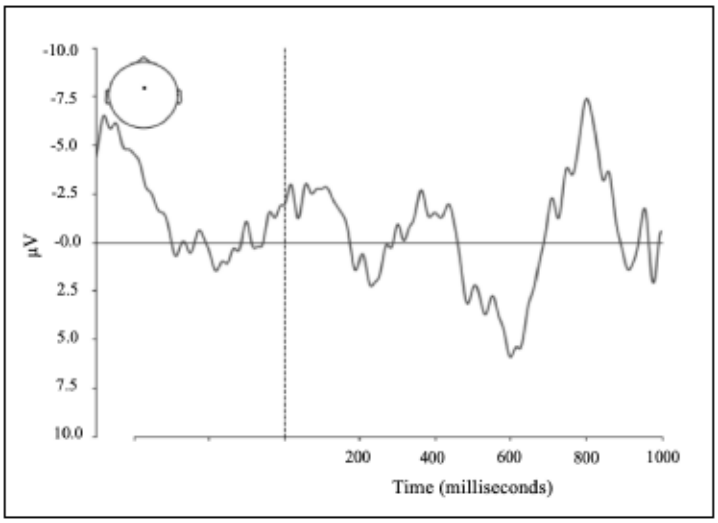

Figure 7. Difference waveform of average event-related potential response to noxious heel lance minus event-related potential response of non-noxious control stimulus in breastfeeding infants (n = 10) at vertex electrode. Vertical dashed line marks the onset of the stimulus. The stimuli were applied at time 0, epoch includes 500-milliseconds prior to stimulus and 1000-milliseconds following stimulus. Negative is plotted upwards.

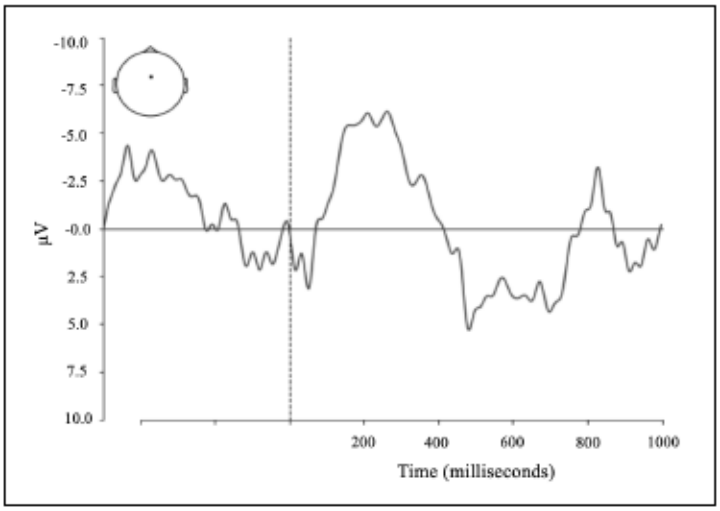

Figure 8. Difference waveform of average event-related potential response to noxious heel lance minus event-related potential response of non-noxious control stimulus in infants receiving 24% oral sucrose and offered non-nutritive sucking ($n = 10$) at vertex electrode. Vertical dashed line marks the onset of the stimulus. The stimuli were applied at time 0, epoch includes 500-milliseconds prior to stimulus and 1000-milliseconds following stimulus. Negative is plotted upwards.

Principal component analysis. Systematic variation in the amplitude and constituent components of the EEG signal in response to the noxious heel lance and non-noxious control stimuli were characterized using temporal PCA with promax rotation as outlined in Chapter 3. A twelve-factor structure was found to account for 94.7% of the variance across all variables, with the first six principal components accounting for 80.7% of variance. The determination of the twelve-factor structure was completed using a parallel test where a scree plot of the dataset was plotted against the scree plot of random data (Dien, 2012; Horn, 1965). The point of intersection between the factors in the two data sets indicate the total number of factors (components) to be retained in the factor structure of the analysis.

The individual principal components, representative variance, peak latency, and spectral topographic representation at peak latency from the twelve-factor temporal promax PCA are presented in Appendix Q. The topographic morphology, latency, and

factor structure presentation of principal component two (PC2) was consistent with previous studies characterizing pain-related potentials following heel lance and non-noxious control stimuli at vertex scalp electrodes (Slater et al., 2010c). Specifically, previous studies have reported that the PC of a positive EEG component with a latency of 560-milliseconds varied significantly across exposures to noxious heel lance and non-noxious control stimuli at vertex electrode sites of Cz and CPz of the international 10/20 electrode placement system (Slater et al., 2010c). Therefore, PC2 was selected as the focus component for further analyses at vertex electrode E19 of the EGI 32-channel electrode montage (Appendix L) and will be referred to as the PC2 waveform hereafter. Of the total variable variance represented in the twelve-factor solution of principal components, the PC2 represented 18.5% of the variable variance. Appendix R presents the individual participant principal component waveform response to the non-noxious control stimuli and noxious heel lance.

Principal component in response to noxious heel lance. Figure 9 shows the group average ($n = 20$) pain-related PC2 waveform and topographic spectral plot at vertex electrode site E19 following exposure to the noxious heel lance. The peak amplitude of this pain-related PC2 was 3.3 μV with a latency of 559-milliseconds following the application of the heel lance stimulus.

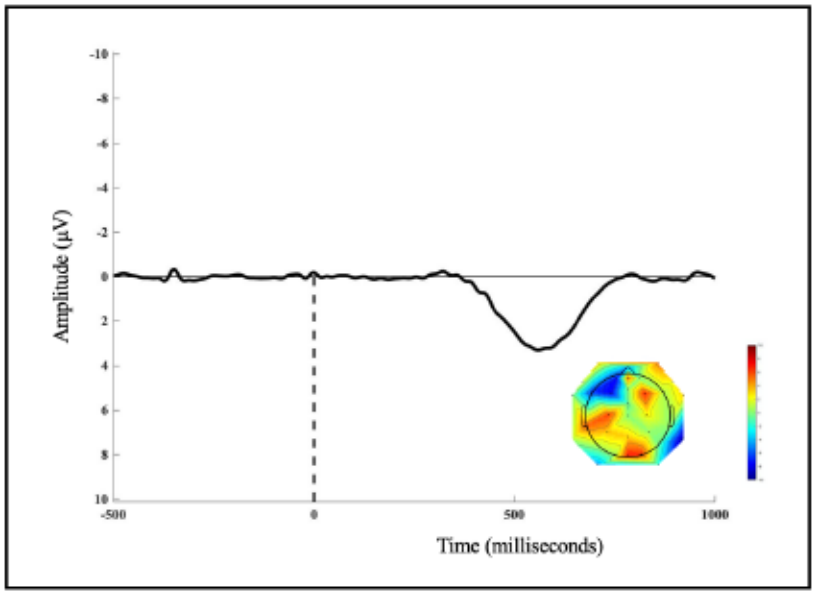

Figure 9. Average PC2 waveform and topographic spectral plot of the group data ($n = 20$) following exposure to noxious heel lance at vertex electrode E19. All infants received a pain-reducing treatment of breastfeeding or 24% oral sucrose and offered non-nutritive sucking. Vertical dashed line marks the onset of the heel lance stimulus. The heel lance was applied at time 0, epoch includes 500-milliseconds prior to stimulus and 1000-milliseconds following stimulus. Negative is plotted upwards. Topographic plot at peak amplitude provided.

Principal component in response to non-noxious control stimuli. In addition to examining the PC2 waveform in response to the noxious heel lance, the PC2 waveform in response to non-noxious control stimulation across all infants ($n = 20$) was examined. Specifically, group average PC2 waveforms in response to the non-noxious control stimulus applied prior to treatment while the infants were contained with a blanket and resting in an infant cot (NN1) and after initiation of treatment (either breastfeeding [$n = 10$] or administration of 24% oral sucrose [$n = 10$] and offered non-nutritive sucking [NN2]) were examined. Figure 10 shows the group average PC2 waveform and topographic spectral plot at vertex electrode site E19 following exposure to the initial non-noxious control stimulus with no treatment. The peak amplitude of this tactile

principal component was -6.24 μV at 559-milliseconds following application of the non-noxious control stimulus.

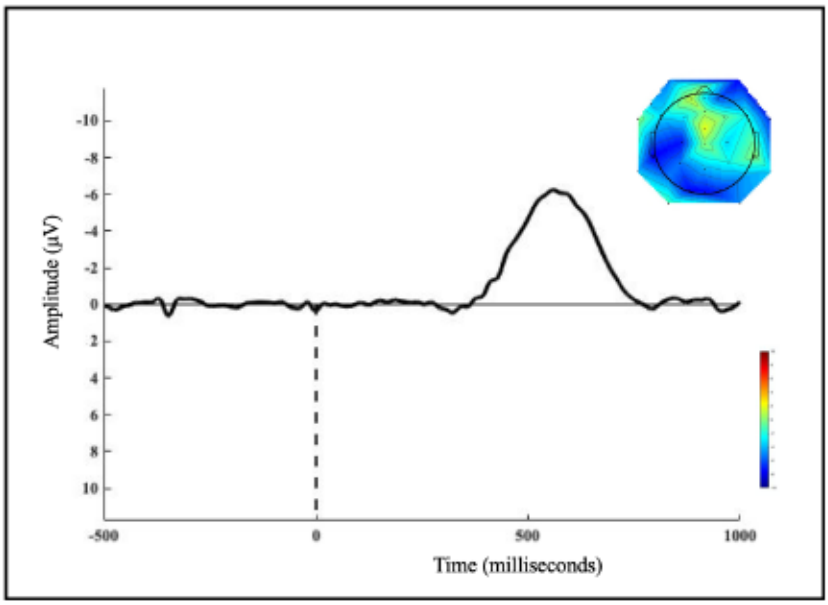

Figure 10. Average PC2 waveform and topographic spectral plot of the group data (n = 20) following exposure to non-noxious control stimulus one at vertex electrode E19. Non-noxious control stimulus one applied prior to the administration of the assigned pain-reducing intervention. Vertical dashed line marks the onset of the non-noxious control stimulus. The non-noxious control stimulus was applied at time 0, epoch includes 500-milliseconds prior to stimulus and 1000-milliseconds following stimulus. Negative is plotted upwards. Topographic plot at peak amplitude provided.

Figure 11 presents the group average (n = 20) PC2 waveform and topographic spectral plot at vertex electrode site E19 following exposure to the second non-noxious control stimulus, completed after the initiation of the assigned intervention. The peak amplitude of this tactile PC2 waveform was 2.3 μV with a latency of 559-milliseconds following the application of the non-noxious control stimulus.

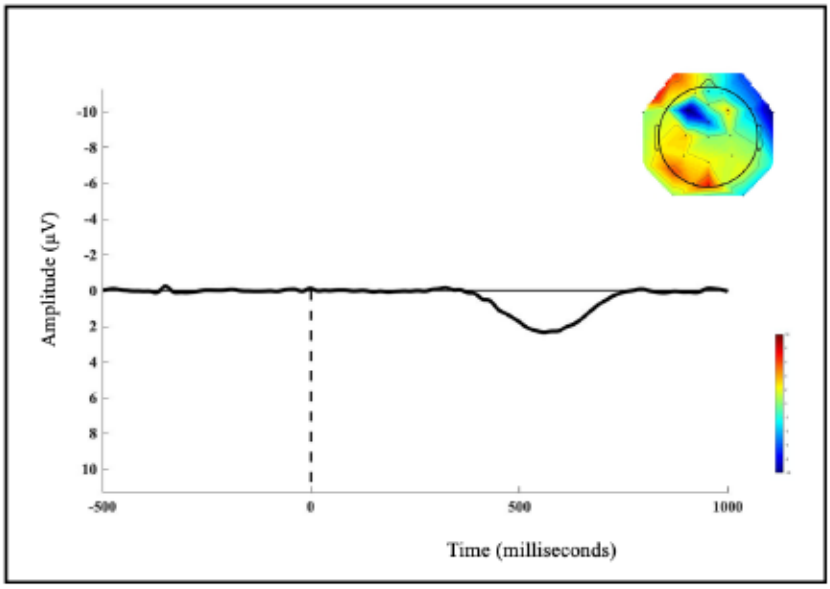

Figure 11. Average PC2 waveform and topographic spectral plot of the group data (n = 20) following exposure to non-noxious control stimulus two at vertex electrode E19. Non-noxious control stimulus applied after the administration of the assigned pain-reducing intervention. Vertical dashed line marks the onset of the non-noxious control stimulus. The non-noxious control stimulus was applied at time 0, epoch includes 500-milliseconds prior to stimulus and 1000-milliseconds following stimulus. Negative is plotted upwards.

Difference between principal component waveforms in response noxious heel lance and non-noxious control stimuli. A one-way ANOVA was run with the independent variable being type of stimulus exposure (non-noxious control stimulus with no treatment [NN1], non-noxious control stimulus with treatment [NN2], noxious heel lance [NHL]) and the dependent variable being the trimmed mean PC2 waveform amplitude. No statistically significant effect of stimulus type was found on the amplitude of the PC2 waveforms (F[2,14] = 0.58, p = 0.59, SE = 16.57).

Primary outcome comparison of principal component waveform response to noxious heel lance between intervention groups. To address the primary outcome of comparing the pain-related event-related potential response between infants who were randomized to the breastfeeding intervention (n = 10) and infants who were randomized

to the 24% oral sucrose and offered non-nutritive sucking intervention ($n = 10$), PC2 waveform in response to heel lance were compared between groups. Figure 12 presents the average PC2 waveforms and spectral topographic plots, divided by treatment. The average PC2 peak amplitude for those infants randomized to the 24% oral sucrose and offered non-nutritive sucking intervention was 8.97 μV (averaged trimmed mean 3.03 μV) at 633-milliseconds following the heel lance, whereas the peak amplitude of the average PC2 for those infants randomized to the breastfeeding intervention was 0.29 μV (averaged trimmed mean 0.40 μV). While waveforms have appreciable differences in amplitude upon visualization, one-way ANOVA comparing the effect of the independent variable of treatment (breastfeeding, 24% oral sucrose) on the dependent variable of trimmed mean PC2 waveform amplitude in response to heel lance showed no statistically significant effect of treatment ($F[1,15.9] = 0.58$, $p = 0.64$, $SE = 11.79$).

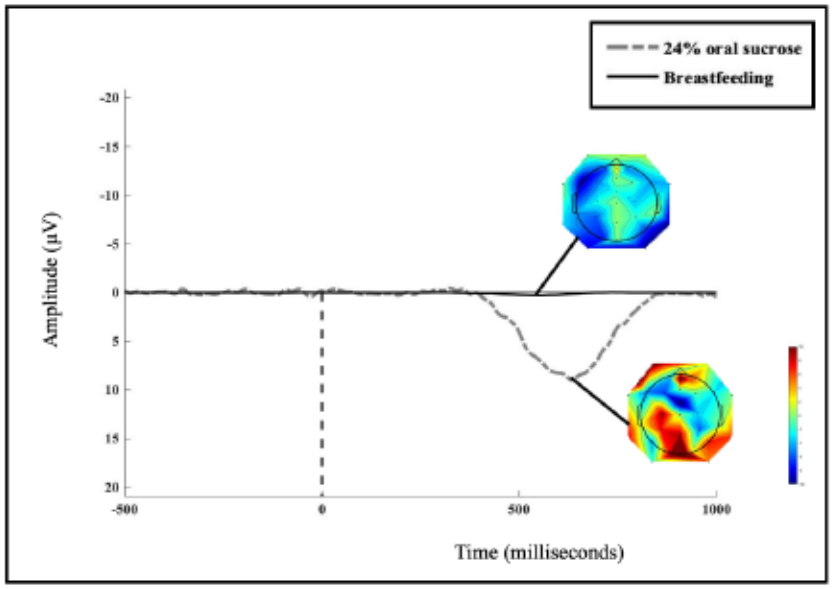

Figure 12. Average principal component waveform and topographic spectral plot following exposure to noxious heel lance at vertex electrode E19, divided by treatment with breastfeeding or 24% oral sucrose and offered non-nutritive sucking. Heel lance applied after the administration of the assigned pain-reducing intervention. Vertical dashed line marks the onset of the heel lance at time 0. Epoch includes 500-milliseconds prior to stimulus and 1000-milliseconds following stimulus. Negative is plotted upwards. Topographic plots at peak amplitude provided.

Exploratory comparison of non-noxious control stimulus between treatment groups. Exploratory analyses of the effect of treatment on the PC2 waveform response to the tactile non-noxious control stimulus two (applied after treatment initiation) was completed to further elucidate the nature of the relationship between treatment and sensory processing. One way-ANOVA of the effect of the independent variable of treatment (breastfeeding, 24% oral sucrose) on the dependent variable of trimmed mean PC2 waveform amplitude in response to non-noxious control stimulus two showed an effect of treatment ($F[1,14.9] = 7.64$, $p = 0.02$, $SE = 14.32$).

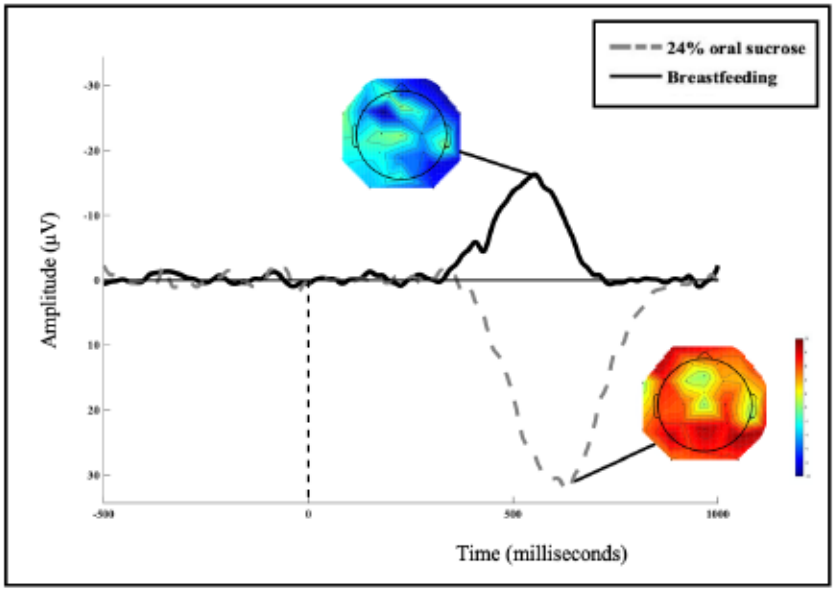

Figure 13. Average PC2 waveform and topographic spectral plot following exposure to non-noxious control stimulus two at vertex electrode E19, divided by treatment with breastfeeding or 24% oral sucrose and offered non-nutritive sucking. Heel lance applied after the administration of the assigned pain-reducing intervention. Vertical dashed line marks the onset of the heel lance at time 0. Epoch includes 500-milliseconds prior to stimulus and 1000-milliseconds following stimulus. Negative is plotted upwards. Topographic plots at peak amplitude provided.

Secondary Hypotheses

Bio-behavioural pain response. The secondary outcome of bio-behavioural pain response was measured using the Premature Infant Pain Profile-Revised (PIPP-R). The

overall PIPP-R scores were calculated by adding the scores of the behavioural, physiological, and contextual components of the measure as described in the methods chapter of this thesis. Bio-behavioural PIPP-R scores were calculated 30-seconds following the non-noxious control stimuli (NN1 completed before intervention initiation and NN2 completed after intervention initiation), and 30-, 60-, 90-, and 120-seconds following the noxious heel lance. It was hypothesized that infants in the breastfeeding group would have lower PIPP-R scores at 30-, 60-, 90-, and 120-seconds following heel lance when compared to infants who received 24% oral sucrose and offered non-nutritive sucking.

As anticipated, infants ($n = 35$) did not have PIPP-R score that would be indicative of a pain response following application of the first non-noxious control stimulus applied while all infants were contained in a blanket in an infant cot ($M = 3.23$, $SD = 2.59$). Pain scores also reflected that infants were in low or no pain following application of the second non-noxious control stimulus when the infant was in their assigned intervention condition ($n = 33$, $M = 3.39$, $SD = 2.15$). There was no statistically significant difference in PIPP-R score in response to the second non-noxious control stimulus between infants in the breastfeeding group ($n = 16$, $M = 4.13$, $SD = 1.78$) and the 24% oral sucrose and offered non-nutritive sucking group ($n = 17$, $M = 2.71$, $SD = 2.29$; $t[31] = 1.98$, $p = 0.057$, 95% CI [-0.04 – 2.88]). Table 5 presents the descriptive and inferential statistics for PIPP-R scores for infants in the breastfeeding and 24% oral sucrose groups in response to non-noxious control stimulus two applied following intervention initiation.

Table 5

Descriptive and Inferential Statistics of PIPP-R Scores for Breastfeeding and 24% Oral Sucrose Groups at 30-seconds Following Non-Noxious Control Stimulus Two

						95% *CI*	
Intervention group	*n*	*M*	*SD*	*p*	Mean difference	*LL*	*UL*
Breastfeeding[a]	16	4.13	1.78				
24% oral sucrose[b]	17	2.71	2.29				
				0.057	1.42	-0.04	2.88

Note. PIPP-R = Premature Infant Pain Profile – Revised, *sec* = seconds, *CI* = confidence interval, *M* = mean, *SD* = standard deviation, *LL* = lower limit, *UL* = upper limit.
[a]*n* = 16 as video loss during study participation prevented the calculation of PIPP-R score for two participants in the breastfeeding group at 30-seconds following non-noxious control stimulus two.
[b]*n* = 17 as video loss (*n* = 1) during study participation and withholding the non-noxious control stimuli due to baby being distressed (*n* = 1) prevented the calculation of PIPP-R score for participants in the 24% oral sucrose group at 30-seconds following non-noxious control stimulus two.

There was no statistically significant difference in PIPP-R scores between those infants in the breastfeeding group and those infants in the 24% oral sucrose group at any time point following heel lance. Specifically there was no difference in PIPP-R scores between groups at 30- ($t[32] = 0.645$, $p = 0.52$, 95% CI [-1.21 – 2.33]), 60- ($t[31] = 0.227$, $p = 0.82$, 95% CI [-1.92 – 2.41]), 90- ($t[31] = 1.36$, $p = 0.18$, 95% CI [-0.64 – 3.21]), or 120- ($t[30] = 0.116$, $p = 0.91$, 95% CI [-1.84 – 2.06]) seconds following heel lance procedure. Table 6 presents the descriptive and inferential statistics for PIPP-R scores for infants in the breastfeeding and 24% oral sucrose groups across the time phases following the heel lance procedure.

Table 6

Descriptive and Inferential Statistics of PIPP-R Scores for Breastfeeding and 24% Oral Sucrose Groups at 30-, 60-, 90-, and 120-seconds Following Heel Lance

							95% *CI*	
Phase (sec)	Intervention group	*n*	*M*	*SD*	*p*	Mean difference	*LL*	*UL*
30	Breastfeeding	15	4.67	2.16				
	24% oral sucrose	19	4.11	2.77				
					0.56	0.56	-1.21	2.33
60	Breastfeeding	14	4.71	2.43				
	24% oral sucrose	19	4.47	3.37				
					0.82	0.24	-1.92	2.41
90	Breastfeeding	14	5.07	2.81				
	24% oral sucrose	19	3.79	2.57				
					0.18	1.28	-0.64	3.21
120	Breastfeeding	14	4.50	1.87				
	24% oral sucrose	18	4.39	3.18				

							95% *CI*	
Phase (sec)	Intervention group	*n*	*M*	*SD*	*p*	Mean difference	*LL*	*UL*
					0.12	0.11	-1.84	2.06

Note. PIPP-R = Premature Infant Pain Profile – Revised, *sec* = seconds, *CI* = confidence interval, *M* = mean, *SD* = standard deviation, *LL* = lower limit, *UL* = upper limit.
[a]*n* = 15 as video loss during study participation prevented the calculation of PIPP-R score for three participants in the breastfeeding group at 30-seconds following heel lance.
[b]*n* = 14 as video loss during study participation prevented the calculation of PIPP-R score for four participants in the breastfeeding group at 60-, 90-, and 120-seconds following heel lance.
[c]*n* = 18 as video loss during study participation prevented the calculation of PIPP-R score for one participant in the 24% oral sucrose and offered non-nutritive sucking group at 120-seconds following heel lance.

Controlling for LATCH score did not influence findings, with the lack of statistically significant difference in mean PIPP-R scores between infants in the breastfeeding and oral sucrose group persisting at 30- ($F[1, 31] = 0.003$, $p = 0.598$), 60- ($F[1, 30] = 2.256$, $p = 0.144$), 90- ($F[1, 30] = 0.049$, $p = 0.827$), and 120- ($F[1, 29] = 0.256$, $p = 0.617$) seconds following lance. Furthermore, controlling for sustained non-nutritive sucking did not alter the lack of statistically significant differences in PIPP-R scores at 30- ($F[1, 31] = 0.640$, $p = 0.430$), 60- ($F[1, 30] = 0.538$, $p = 0.469$), 90- ($F[1, 30] = 0.188$, $p = 0.688$), and 120- ($F[1, 29] = 0.015$, $p = 0.904$) seconds following heel lance. Figure 13 presents a comparison to total mean PIPP-R scores in both intervention groups across time phases.

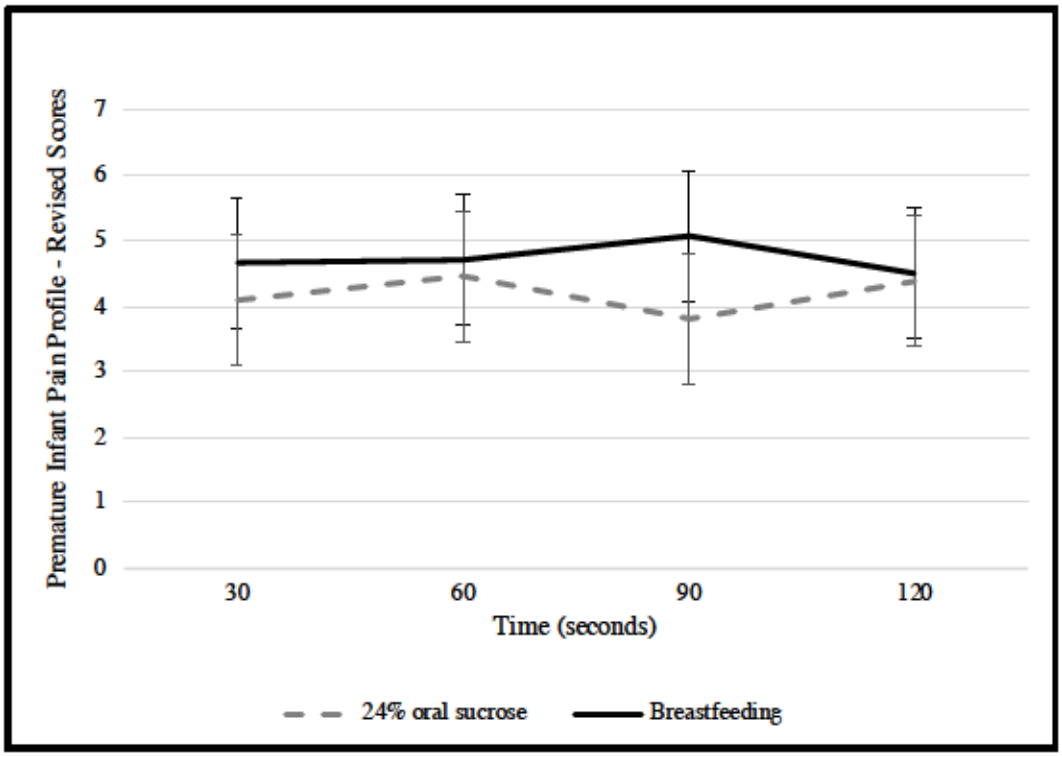

Figure 14. Comparison of mean PIPP-R scores in breastfeeding and 24% oral sucrose groups at 30-, 60-, 90-, and 120-seconds following heel lance. Mean and standard deviation for interventions groups presented at each corresponding time point.

Physiologic recovery. Recovery time (defined as the period of time in seconds from the end of blood sampling until the heart rate returned to baseline values for a minimum of five to seven beats) was calculated for each infant and mean time to recovery were compared between the breastfeeding and 24% oral sucrose and offered non-nutritive sucking groups using unpaired Student's *t*-tests. While recovery time was almost one minute shorter in the breastfeeding group ($M = 17.5$, $SD = 31.1$) compared to infants in the 24% oral sucrose group ($M = 70.8$, $SD = 144.3$), this difference was not statistically significant ($t[33] = -1.491$, $p = 0.15$ [Table 7]).

Table 7

Comparison of Mean Time to Recovery in Seconds between Breastfeeding and 24% Oral Sucrose Groups

						95% *CI*	
Intervention group	*n*	*M*	*SD*	*p*	Mean difference	*LL*	*UL*
Breastfeeding[a]	17	17.5	31.1				
24% oral sucrose[b]	18	70.8	144.3				
				0.15	-53.4	-126.4	19.7

Note. *CI* = confidence interval, *M* = mean, *SD* = standard deviation, *LL* = lower limit, *UL* = upper limit.
[a]*n* = 17 as physiologic data loss during study participation prevented the calculation of physiologic recovery for one participant in the breastfeeding group.
[b]*n* = 18 as physiologic data loss during study participation prevented the calculation of physiologic recovery for one participant in the 24% oral sucrose group.
*$p < 0.05$

Adverse events and safety surveillance. No significant adverse events or safety concerns arose in either the breastfeeding intervention or the 24% oral sucrose and offered non-nutritive sucking intervention (Table 8). No infant in this study exhibited apnea or bradycardia during or following the heel lance. Only one infant (5.3%) in the 24% oral sucrose intervention required a repeat heel lance to collect sufficient blood volume to conduct the clinically-required metabolic screening testing. Three infants (15.8%) in the 24% oral sucrose intervention required a rescue dose of 24% oral sucrose solution due to PIPP-R score greater than six as observed by the PI and/or assisting research coordinator. However, there was no statistically significant difference in frequency of administered rescue sucrose doses between groups ($\chi^2(1, 37) = 3.093$, $p =$

0.079). All infants were normothermic (36.5 to 37.5 degrees centigrade) as measured by axillary temperature from initiation of data collection procedures to study end.

Table 8

Comparison of Safety Surveillance Outcomes and Incidence of Adverse Events between Breastfeeding and 24% Oral Sucrose Groups

Safety surveillance outcomes	Breastfeeding ($n = 18$)[a]	24% sucrose ($n = 19$)	p
Incidence of apnea during heel lance (frequency, %)	0(0)	0(0)	1.0
Incidence of apnea after heel lance (frequency, %)	0(0)	0(0)	1.0
Incidence of bradycardia during heel lance (frequency, %)	0(0)	0(0)	1.0
Incidence of bradycardia after heel lance (frequency, %)	0(0)	0(0)	1.0
Need for repeat heel lance (frequency, %)	0(0)	1(5.3)	1.0
Need for rescue sucrose dose (frequency, %)	0(0)	3(15.8)	0.079
Incidence of hypothermia (frequency, %)	0(0)	0(0)	1.0

Note. [a]$n = 18$ as two participants lost to follow-up prior to completion of the study heel lance.
*$p < 0.05$

Maternal acceptability. The following section will present the findings of the maternal acceptability questionnaire administered to all mothers in the study following completion of the heel lance procedure in the assigned intervention of breastfeeding or 24% oral sucrose, prior to discharge from the hospital. A total of 36 mothers ($n = 17$

from the breastfeeding intervention and $n = 19$ from the 24% oral sucrose and offered non-nutritive sucking intervention) provided completed questionnaires. Of the 37 mothers who were randomized and completed data collection procedures, only one mother (randomized to the breastfeeding intervention) did not provide a completed questionnaire as she was discharged home from the hospital prior to returning the questionnaire.

Perceptions of breastfeeding and pain-reducing efficacy. All of the mothers ($n = 17$) who reported on their feelings surrounding breastfeeding their infant during the heel lance procedure in this study reported feeling "good" or "very good". The majority ($n = 13$, 76.5%) specifically noted that they felt "very good" about providing this intervention. Only one mother reported a concern related to breastfeeding during the painful procedure, specifically the concern that her baby would not latch to the breast prior to the heel lance. With respect to perceived effectiveness of breastfeeding as a pain-reducing intervention, all mothers similarly reported that they felt their baby did "good" or "very good" in terms of pain relief when breastfeeding ($n = 6$ [35.3%] reporting "good" and $n = 11$ [64.7%] reporting "very good").

Future utilization of breastfeeding for procedural pain management. All but one mother ($n = 16$, 94.1%) reported that they would utilize breastfeeding as a pain-reducing intervention for their infant during future procedures. When asked to describe why they would use this intervention in the future, all mothers noted the calming and pain-reducing effects of breastfeeding as the primary reason. Additionally, two mothers specifically noted that they had used with their previous children during immunizations and blood tests and found it effective. One mother indicated that it made her feel better to be able to provide comfort to her baby during pain. The mother who indicated that she would not

use breastfeeding for pain management during future procedures indicated the primary reason was that she perceived that it was not as convenient for the clinical team member to position to complete the heel lance while her baby was in her arms.

Perceptions of 24% oral sucrose and pain-reducing efficacy. The majority of mothers whose infants received 24% oral sucrose and offered non-nutritive sucking for pain management during their heel lance procedure reported positive feelings related to the use of sucrose. Specifically, 84.2% of mothers ($n = 16$) reported feeling "good" or "very good" regarding their baby receiving 24% oral sucrose. Only 15.8% of mothers ($n = 3$) reported feeling "fair", and no mothers reported feeling "not good" regarding the use of 24% oral sucrose. No mothers reported concerns related to the use of 24% oral sucrose during study participation. When asked about perceptions of the pain-reducing efficacy of 24% oral sucrose and offered non-nutritive sucking during the heel lance procedure completed for this study, all mothers reported that their baby did "good" ($n = 5$, 26.3%) or "very good" ($n = 14$, 73.7%) in terms of pain relief.

Future utilization of 24% oral sucrose for procedural pain management. When asked if they would utilize 24% oral sucrose during future procedures for pain management, the majority of mothers reported that they would ($n = 13$, 68.4%). The primary reason these mothers reported that they would use during later procedures was that it appeared to be effective in managing pain ($n = 8$, 61.5%). Three mothers (23.1%) reported that they would use 24% oral sucrose if it was recommended to them by a health professional and if the evidence supported the interventions pain-reducing efficacy. Finally, two mothers (15.4%) indicated that it provided a good alternative to breastfeeding for pain management if successfully breastfeeding during procedures was unpredictable or they were no longer breastfeeding their baby.

Of the 19 mothers whose baby was randomized to receive 24% oral sucrose during heel lance, 31.6% ($n = 6$) indicated that they likely would not use 24% oral sucrose for pain management during future procedures. Reported reasons for not using this intervention for future procedures included preferring breastfeeding and/or skin-to-skin contact ($n = 2$, 33.3%) as well as believing their baby would likely be fine without intervention ($n = 2$, 33.3%). Two mothers additionally reported that they did not have sufficient knowledge regarding access, preparation, and appropriate dosing of 24% oral sucrose to confidently use for future procedures.

Group differences in maternal perceptions of interventions. Maternal questionnaire responses regarding perceptions of intervention use, pain-reducing efficacy, and future use were compared between groups to test the hypothesis that mothers whose infants were randomized to the breastfeeding intervention would report greater maternal acceptance of the interventions. There were no significant group differences in maternal feelings related to use of either of the interventions ($\chi^2(2, 36) = 3.692, p = 0.16$) or perceived pain-reducing effectiveness ($\chi^2(1, 36) = 0.341, p = 0.56$). While there was not a statistically significant difference between groups in maternal anticipated future intervention use, there was a trend toward more mothers in the breastfeeding group reporting that they would use the intervention to manage their infant's pain in the future ($\chi^2(1, 36) = 3.782, p = 0.052$).

Perceptions of infant EEG to measure pain-related brain activity. In addition to exploring maternal perceptions of the breastfeeding and 24% oral sucrose intervention, mothers were also surveyed regarding their perceptions of having their infant wear an EEG net during study participation. When asked how they felt about their baby wearing the infant EEG net, the majority ($n = 33$, 91.7%) of mothers reported feeling "good" or

"very good". Two mothers (5.6%) reported feeling "fair" in relation to their infant wearing the EEG net and one mother reported feeling "not good". Of the 36 mothers who provided survey responses, six mothers indicated that they did have some concerns prior to taking part in the study with respect to the infant EEG net. These concerns related to ensuring their infant was comfortable wearing the net ($n = 4$, 11.1%), ensuring the net was safe ($n = 1$, 2.8%), and concerns regarding the length of time it would take to apply the net ($n = 1$, 2.8%). No mothers reported continued concerns after data collection procedures were completed. Furthermore, no mothers indicated that there were any changes that should be made to study procedures with respect to EEG net application and data collection.

Group differences in maternal perceptions of EEG. There were no significant differences in maternal feelings about their infants wearing the EEG net between the breastfeeding group and the 24% oral sucrose group ($\chi^2(3, 36) = 2.091, p = 0.554$). In the small group of mothers reported pre-participation concerns, there was a trend toward a significant difference between groups with mothers in the breastfeeding group reporting more concerns related to their infant wearing the EEG net than mothers in the 24% oral sucrose group ($\chi^2(2, 6) = 6.0, p = 0.05$).

CHAPTER 5: DISCUSSION

The following chapter of this thesis will present a discussion of the findings of this study. It will begin with a discussion of the findings of the primary outcome analysis of the effect of breastfeeding and 24% oral sucrose on pain-related brain activity in the context of the current literature, followed by a discussion of the secondary outcomes of bio-behavioural pain response measured using the Premature Infant Pain Profile – Revised, physiologic recovery, safety and adverse events, and maternal acceptance of the intervention conditions and study procedures. A discussion of the relationship between the pain-related brain response and bio-behavioural pain response will be included following the discussion of the secondary outcome of Premature Infant Pain Profile – Revised in the interest of clarity. Strengths and limitations of the work will then be examined, followed by discussion of the broader contributions of this work with respect to theory, research, and clinical practice.

To our knowledge, this is the first study to report on the comparative influence of breastfeeding and 24% oral sucrose on pain-related brain activity measured utilizing neonatal electroencephalogram event-related potentials in healthy full term newborns. This is also the first study to compare the effect of these interventions on multi-modal bio-behavioural and electroencephalographic pain measurements, as well as the first study to our knowledge to report on maternal acceptance of utilizing neurophysiologic assessment technology to measure acute procedural pain in newborn infants in the post-partum period. The sample recruited in this study was largely comparable to healthy full term infants recruited in previous work examining the effects of breastfeeding and 24% oral sucrose on multi-modal pain responses with respect to both maternal characteristics (e.g., age, previous children, education, mode of delivery) and infant characteristics (e.g.,

gestational age, birth weight, sex, Apgar scores, timing of feeding preceding heel lance). An exception to these similarities being that all infants in our study had their heel lance procedure completed on postnatal day one in contrast to postnatal day three in other studies in the healthy full term population (Carbajal et al., 2003; Codipietro et al., 2008; Slater et al., 2010a). An important consideration with respect to the timing of study participation is that infants in the first days of life are receiving colostrum as opposed to mature breast milk. Colostrum contains lower concentrations of lactose and when administered independent of maternal contact has been reported to prevent increase in heart rate but not crying and grimacing following heel lance (Blass & Miller, 2001). While the majority of studies reporting the positive influence of human milk in reducing pain responding in full term infants are completed within one to three days postpartum (Benoit et al., 2017) and prior to lactation of transitional and mature breast milk (Ballard & Morrow, 2013), consideration of the stage of breast milk when evaluating the pain-reducing effects of breastfeeding is warranted.

This study, which was underpowered to report effects of the primary and secondary pain assessment outcomes, shows that breastfeeding did not produce a statistically significant reduction in the amplitude of the pain-related event-related potential isolated utilizing principal component analysis compared to 24% oral sucrose and offered non-nutritive sucking in an infant cot. However, while not statistically significantly different, the average peak amplitude of the pain-related principal component waveform in the 24% oral sucrose and offered non-nutritive sucking group was appreciably larger (8.97 μV) compared to the average peak amplitude of the pain-related principal component waveform in the breastfeeding group (0.29 μV). This finding is consistent with the hypothesized effect that breastfeeding infants would demonstrate a

lower amplitude pain-related event-related potential than those infants who received 24% oral sucrose and offered non-nutritive sucking in an infant cot. Breastfeeding infants were not found to have significantly lower PIPP-R scores at 30-, 60-, 90-, and 120-seconds following heel lance, however, they had appreciably faster physiologic recovery and fewer identified adverse events. While mothers reported similar perceptions regarding intervention pain-reducing efficacy across groups, mothers in the breastfeeding group more frequently reported that they would use breastfeeding as a pain-management intervention for their infant during future procedures. A small proportion of mothers voiced pre-participation concerns related to their infant wearing the EEG net as part of the study procedure, however, these concerns did not persist after study completion. Thus, we have shown that breastfeeding may be associated with modulated pain-related neurophysiologic processing when compared to 24% oral sucrose with no adverse effects, and positive maternal acceptance.

Primary Outcome: Pain-related Brain Activity

The primary hypothesis of this study was that infants who were randomized to the breastfeeding intervention would have lower amplitude pain-related brain activity following heel lance procedure than those infants who received 24% oral sucrose and offered non-nutritive sucking while in an infant cot. Pain-related brain activity was measured using neonatal EEG recording and statistically summarized using principal component analysis, consistent with the methodological approach of previous work published in this area (Fabrizi et al., 2011a; 2011b; Green et al., 2019; Hartley et al., 2017; Hartley et al., 2018; Slater et al., 2010a; 2010b; 2010c). This study revealed that there was no statistically significant difference between intervention groups. Despite this lack of statistical differentiation between groups, there was an appreciable difference in

the amplitude of the average principal component waveforms, with those infants in the breastfeeding group having a lower amplitude average principal component response to heel lance compared to infants who received 24% oral sucrose and offered non-nutritive sucking. An additional interesting exploratory finding of this study was that there was a statistically significant difference between groups in the mean principal component waveform response to the non-noxious tactile stimulus, suggesting a potential effect of breastfeeding in modulating response to tactile stimuli.

This study makes a valuable contribution to the current literature in that it provides a replication of methods being utilized to characterize pain-related electrophysiologic responses in the infant brain. It additionally is the first study to provide maternal feedback on the acceptance of conducting this type of electrophysiologic data collection with their newborns. A group of researchers collaborating across two labs have characterized electrophysiologic brain activity following exposure to the non-noxious tactile and noxious stimuli specifically used in this study (Fabrizi et al., 2011a; Hartley et al., 2015; 2016; Jones et al., 2017; Slater et al., 2010a; 2010b; 2010c; Verriotis et al., 2016; 2018), and are also attempting to elucidate the effects of analgesic interventions (Hartley et al., 2017; 2018; Slater et al., 2010a) on such evoked brain responses. To our knowledge, we are the first to replicate these methods in infants undergoing heel lance outside of these two collaborative research labs and are further the first to examine if breastfeeding and 24% oral sucrose differentially modulate electrophysiologic brain responses to these tactile and painful stimuli. Our finding of a pain-related waveform isolated using principal component analysis from a vertex electrode site with a peak positive latency 559-milliseconds following heel lance is highly consistent with the pain-related waveform reported in previous studies (Fabrizi et

al., 2011b; Hartley et al., 2015; 2016; Slater et al., 2010a; 2010b; 2010c). Specifically, Slater and colleagues (2010c) were the first to utilize principal component analysis as a means of characterizing electrophysiologic brain responses to heel lance in infants. They reported a pain-related component waveform with a peak positive latency 560-milliseconds following application of a single-trial heel lance at vertex electrodes, that was significantly greater following heel lance compared to background EEG activity and the non-noxious tactile control. These authors have argued that this component is indicative of a sensory-discriminant response to noxious stimulation generated from the primary and secondary somatosensory cortices, anterior cingulate cortex, and insula (Garcia-Larrea, Frot, & Valeriani, 2003; Fabrizi et al., 2011; 2016; Slater et al., 2010c), while others have argued that such conclusions are premature and overstated based on the current evidence-base (Mouraux & Iannetti, 2018). Our study was not designed to support localization of the specific neural generator of this electrophysiologic activity. However, the presence of a stimulus-related principal component waveform with a latency and amplitude consistent with previous studies nonetheless lends support to the presence of a characteristic underlying electrophysiologic response to noxious and tactile stimulation in infants.

While a potential with comparable amplitude and latency to past reports following heel lance was identified in our study, our findings deviate from previous work in that there was no effect of stimulation modality on the principal component waveform. Specifically, no statistically significant difference in the principal component waveform response to non-noxious tactile stimuli and the noxious heel lance across all infants. Previous event-related potential studies aiming to characterize the effect of noxious and non-noxious stimulation have found statistically significant differences in the amplitude

of the waveform response to the two types of stimuli (Fabrizi et al., 2011a; 2016; Hartley et al., 2017; Slater et al., 2010a; 2010b; 2010c), which was interpreted as nociceptive specificity. No studies in this area have completed power calculations for statistical analyses of the amplitude differences of this pain-related potential response between noxious and non-noxious stimuli exposures. Despite this, relatively large and statistically significant differences in principal component amplitude response to noxious and non-noxious stimuli have been observed in a previous report (e.g., 9 μV in response to heel lance compared to 3 μV in response to non-noxious control, $n = 44$ infants [Slater et al., 2010a]). This is in contrast to a relatively small amplitude difference observed in response to the comparable stimuli in our study (e.g., 3.3 μV in response to the heel lance compared to 2.3 μV in response to non-noxious control, $n = 20$ infants). As the noxious heel lance response in our study was appreciably (albeit only slightly) larger than the response to the preceding control stimulus, it is possible that this small amplitude difference is related to a large amount of individual variability in a small sample of infants, and a more robust effect would be observed in a larger sample.

From a more critical perspective, our finding that there was no statistical difference in waveform amplitude across stimulation types in this small sample of infants also raises questions regarding the reliability of interpreting such a component as a discriminant response to noxious stimulation. The concern that these "pain-specific" responses are in fact not specific to pain but rather are elicited by a broad range of salient somatosensory stimuli has been raised by others (Iannetti, Hughes, Lee, & Mouraux, 2008; Mouraux, Diukova, Lee, Wise, & Domenico, 2011; Mouraux & Iannetti, 2009; Mouraux & Iannetti, 2018). Non-nociceptive somatosensory, auditory, and visual stimuli have all been reported to result in a vertex potential that has a similar latency and scalp

topography to those elicited by painful stimuli (Garcia-Larrea, Frot, & Valeriani, 2003; Mouraux & Iannetti, 2009), with some suggesting that the magnitude of these responses is likely related to stimuli salience and temporal expectancy rather than pain-specificity. For example, in a sample of healthy adults ($n = 7$) Iannetti and colleagues (2008) delivered experimental noxious laser pulse stimuli to the dorsum of the hand and found that higher participant subjective perception of laser stimuli intensity was positively correlated with the amplitude of the laser-evoked vertex waveforms. However, repeating the laser stimulus at regular intervals both significantly reduced the magnitude of the laser-evoked waveforms and disrupted the relationship between pain-perception and waveform magnitude (Iannetti et al., 2008). These authors went on to further examine the influence of salient stimuli (nociceptive laser, non-nociceptive pulses, visual, auditory) and participant subjective experience of saliency on this laser-evoked potential (Mouraux & Iannetti, 2009). Using a blind-source separation algorithm with probabilistic independent component analysis, they were able to demonstrate that the potentials generated from the battery of sensory stimuli were explained by multi-modal, as opposed to nociceptive-specific, neural activities (Mouraux & Iannetti, 2009). Similar to their previous work, participant subjective rating of the stimulus saliency was positively correlated with the magnitude of the neural response regardless of the type of sensory stimulation (Mouraux & Iannetti, 2009). Taken together, these findings suggest that conclusions regarding the nociceptive specificity of these potentials may be premature and that subjective experience and attentiveness to stimuli be more influential in predicting response magnitude and potential treatment effects. Such a conceptualization of the contributions to the pain experience being influenced by multiple physiological and psychological processes, not simply limited to the pain intensity of a stimulus, is directly

aligned with the Neuromatrix Theory of Pain and prompts the need for future work attempting to elucidate the relationship between sensory processing, saliency, and pain responding to advance the field.

In our study, the non-noxious control stimulus and noxious heel lance were expected to produce similar somatosensory experiences including the tactile contact of the lancet, holding and pressure applied to the infant foot, and vibration of the spring blade release of the lancet. The nociceptive input of the blade penetrating the foot only occurred when the noxious heel lance was completed for clinical blood collection. It is possible that this component of the stimulus was not uniquely salient over and above the somatosensory experience of the control stimulus to produce a significant difference in the evoked electrophysiologic response. Further, the need to physically restrain and hold the foot to complete both the non-noxious and noxious stimuli may represent a salient somatosensory experience for some or all infants, confounding our capacity to isolate a pain-specific response from a somatosensory response related to distress. Individual variability in infant behavioural and physiological responses to non-noxious and noxious heel lance stimuli has also been reported and may lend explanation to our findings. In a recent study, only 67% of infants exhibited "noxious-specific" brain activity following heel lance (Green et al., 2018), and of those who were classified as having more mature responses (specifically, an identifiable event-related potential following lance) only 75% exhibited pain-related facial actions (eye squeeze, brow bulge, nasolabial furrow). This finding that there are "non-responders" on measures of nociceptive-brain activity as well as infants who do not exhibit pain-related behaviour following nociceptive stimuli despite the presence of evoked brain activity has been reported in several other studies (Fabrizi et al., 2011b; Hartley et al., 2018; Jones et al., 2018; Slater et al., 2008). These authors have

concluded that future work is needed to understand why pain-related brain activity and behavioural responses are not present or dissociated in some infants (Hartley et al., 2018). It is possible, and aligned with the guiding Neuromatrix Theory of Pain, that infant individual contextual (e.g., pain-relieving intervention received), experiential (e.g., time since last painful procedure), and genetic differences (e.g., different stress responses) contribute to variability in nociceptive sensitivity and salience attributed to painful and non-painful sensory inputs, obscuring differences in stimuli responses as observed in our study. Indeed, individual infant factors such as higher stress (measured as salivary cortisol levels) have been related to larger amplitude electrophysiologic potentials in one study (Jones et al., 2018). However, it is also possible that the pain-related waveform that was examined in our study and others is not sensitive and specific to nociceptive processing and thus is not always present following nociceptive stimuli.

Our study also showed a lack of statistically significant effect of treatment on the principal component waveform following the painful heel lance. As this study is underpowered to detect a difference in the primary outcome, this was anticipated. Despite this, the finding that breastfeeding did not result in a statistically significant difference in pain-related brain activity when compared to oral sucrose is aligned with reports of previous studies showing lack of effect of 24% oral sucrose (Slater et al., 2010a) and morphine (Hartley et al., 2018) on nociceptive-related electrophysiologic potentials when compared to control intervention during heel lance. While both of these studies were also underpowered, with the latter study (Hartley et al., 2018) being dramatically so due to early cessation, these findings provide weak evidence for the possibility that both non-pharmacologic and pharmacologic pain-relieving interventions may not modulate nociceptive electrophysiologic brain responses in some infants. In

contrast, Hartley (2018) reported that topical anesthetic (4% tetracaine gel) resulted in a significant reduction in evoked brain activity in response to experimental noxious stimulation compared to no treatment. However, this finding was reported from a small observational sample of 12 infants exposed to a low-intensity experimental quantitative sensory stimulus that has been cited as not inducing a bio-behavioural pain response in infants despite evoking an event-related potential (Hartley et al., 2015). As the pain stimulus examined in our study was a heel lance, which is arguably a much more salient and distressing painful clinical event, it is possible that the treatments used in our study do not have the same effectiveness in diminishing nociceptive responses to a stimulus with this higher intensity and saliency.

Although there was no statistically significant difference between interventions, there was an appreciable difference in the peak amplitude of the principal component waveform following heel lance, with infants who received 24% oral sucrose having a visibly more positive peak amplitude compared to the breastfeeding infants. There was additionally a significant effect of treatment on the response to the non-noxious tactile control stimulus, with infants who received 24% oral sucrose also having a more positive peak waveform amplitude in response to this stimulus. Such findings are aligned with the literature proposing that maternal contact reduces pain intensity and stress reactivity in newborn infants through release of endogenous opiates, oxytocin, and beta-endorphins (Michelsson, Christensson, Rothganger, & Winberg, 1996; Mooncey et al., 1997). In particular, there is compelling evidence of the relationship between maternal touch and smell, oxytocin release, and processing of painful and tactile stimuli (Walker, Trotter, Swaney, Marshall, & Mcglone, 2017). In a recent review, Walker and colleagues highlight the role of physical touch, particularly gentle stroking, in stimulating C-tactile

afferent neurons and promoting oxytocin release (Walker et al., 2017). These authors went on to demonstrate that stroking at a rate that optimally stimulated C-tactile afferent fibers resulted in a reduced amplitude vertex potential in response to painful and non-painful tactile stimuli, as well as reduced facial grimacing following heel lance in healthy term infants compared to infants who received no touch (Gursul et al., 2018). Such findings suggest that C-tactile afferent fibers may represent a neurophysiological mechanism by which interventions such as infant massage and skin-to-skin contact promote pain reduction (Gursul et al., 2018; Walker et al., 2017). While the work of Gursul and colleagues (2018) was a small observational sample of infants, findings align with previous studies suggesting a role of touch, oxytocin, and mediation of pain responding (Mazzuca et al., 2011; Matthiesen, Ransjö-Arvidson, Nissen, Uvnäs-Moberg, 2001; Morhenn, Beavin, & Zak, 2012; Rash, Aguirre-Camacho, & Campbell, 2014; Walker et al., 2017). While mothers were not specifically instructed to stroke their infants in our study and these patterns were not documented or controlled for, all infants were in skin-to-skin contact and stroking and other affiliative behaviour (e.g., talking to their infant during the procedure) was not discouraged. Parents instinctively stroke their infants at a rate that provides optimal stimulation of C-tactile afferent fibers (Croy, Luong, Triscoli, Hofmann, Olausson, & Sailer, 2016). The Neuromatrix Theory of Pain proposes that pain perception and experience is produced by complex interactions between sensory inputs and genetic and contextual factors that influence stress regulation following painful events (Melzack, 1999). Thus, C-tactile afferent stimulation and oxytocin release elicited by maternal contact and stroking may be one mechanism which could have mediated response to the salient tactile stimuli in our sample of infants. Taken

together, such evidence provides justification for further examination of the mechanisms underlying modulation of pain responding through maternal contact and breastfeeding.

An unfortunate similarity between our work and that of others is a high incidence of data loss from the participant electrophysiologic recordings. In our study, 48% of participants were not included in the analysis for the primary outcome due to data loss secondary to electrophysiologic noise contamination or failed time-locking of the clinical heel lance stimulus to the EEG recording. This issue appears to be pervasive in studies examining infant pain-related brain responses to a single heel lance event, with EEG data loss ranging from 15% (Verriotis et al., 2017) to 46% (Verriotis et al., 2016) of infants included in previously published reports. While the high number of participant EEG recordings lost in our work reflects the complexity of implementing a single-trial event-related potential trial protocol in infants, it highlights an important concern with respect to reliability and generalizability of study findings that has not been discussed in other studies. Electrophysiologic data loss was balanced across the intervention groups in our study, however, it is possible that the intervention effect estimate between the groups is biased due to systematic variability in the infants who did not have primary outcome data. For example, infants with higher behavioural pain reactivity and subsequent movement (which could be related to individual variability or the effectiveness of the assigned intervention) could be hypothesized to have higher levels of EEG data loss due to movement artifact. To test this, PIPP-R scores for those infants who were not included in the primary outcome EEG analysis were examined. Mean scores across all infants were indicative of low to no pain ($M = 4.35$, $SD = 2.50$) and there was no statistically significant difference in the 30-second PIPP-R scores of infants with incomplete EEG data between the breastfeeding intervention ($M = 4.33$, $SD = 2.53$) and the 24% oral

sucrose intervention ($M = 4.33$, $SD = 3.08$), $t(13) = 0.00$, $p = 1.00$ [95% CI -2.99 – 2.99]. Further, there was no statistically significant difference in 30-second PIPP-R scores between infants who had complete EEG data ($M = 4.37$, $SD = 2.54$) versus those who did not ($M = 4.33$, $SD = 2.52$), $t(32) = 0.04$, $p = 0.97$ [95% CI -1.75 – 1.82]. However, despite the reassurance of these exploratory analyses with respect to the EEG data likely being missing at random, such large loss of data is cause for concern and any interpretation of treatment effects, sensitivity, and specificity of the pain-related potential in our study and others should be done cautiously.

Secondary Outcomes

Bio-behavioural pain response. It was hypothesized that breastfeeding would have superior pain-reducing effects on bio-behavioural PIPP-R scores when compared to those infants who received 24% oral sucrose. Scores were compared in 30-second epochs following the heel lance stimulus and no statistically significant effect of treatment was found. While likely due to the small sample size and lack of statistical power to determine differences in effect estimates, this finding may also reflect a comparative efficacy between the two interventions in reducing infant behavioural and physiological reactivity to heel lance. Meta-analysis of the effects of breastfeeding and sweet taste interventions on bio-behavioural (e.g., PIPP) and solely-behavioural (e.g., DAN, NFCS, NIPS) pain scores has shown that breastfeeding is at a minimum comparable and more often superior in terms of pain-reducing efficacy (Shah et al., 2012). In a larger sample of full term infants ($n = 89$) with similar characteristics to the infants in our sample (i.e., gestational age, birth weight, mode of delivery, Apgar scores, timing of preceding feed), Carbajal (2003) reported that no statistical difference in mean PIPP scores between breastfeeding infants ($n = 44$, $M = 5.18$, $SD = 3.86$) and those who received glucose

combined with non-nutritive sucking ($n = 45$, $M = 4.38$, $SD = 3.82$). Codipietro (2008) has reported contrasting findings in a comparable sample of 101 full term infants, with breastfeeding infants having significantly lower PIPP scores ($n = 51$, $M = 3.9$, $SD = 2.3$) compared to those who received sucrose alone without the additive non-nutritive sucking ($n = 50$, $M = 8.5$, $SD = 2.7$). However, a notable difference between these two study protocols is the use of non-nutritive sucking as an adjuvant intervention combined with sweet taste, which was completed in the work of Carbajal (2003) but not by Codipietro and colleagues (2008). Similar to the study protocol of Carbajal and colleagues (2003), infants in the sweet-taste intervention arm of our study were contained in a blanket and offered non-nutritive sucking on a pacifier or gloved finger, with the majority sustaining sucking for the duration of the procedure (63.2%). Furthermore, infants in our study and those in the sample recruited by Carbajal and colleagues (2008) were only one postnatal day old at the time of the painful procedure and thus received colostrum when breastfeeding. The concept that the combination of multiple pain-reducing modalities is more effective than use of single treatments alone is not novel, with a synthesis of eight studies showing that "sensorial saturation" using the combination of containment, touch, non-nutritive sucking, and sweet taste is more effective than sweet taste alone in reducing scores on validated behavioural and bio-behavioural pain measures (Bellieni et al., 2012). Our findings lend support to the conclusion that multi-modal pain management approaches likely offer the greatest benefit to infants, and that the intervention combination of sweet taste, non-nutritive sucking, and containment presents a comparative reduction of bio-behavioural pain scores to the multi-modal intervention offered by breastfeeding in the first day postnatally.

An important point to note in our findings is that regardless of intervention condition or time following heel lance, PIPP-R scores were in the lowest possible range indicating low to no pain. Specifically, the mean PIPP-R scores at 30-seconds following the heel lance were 4.67 ($SD = 2.16$) in the breastfeeding group and 4.11 ($SD = 2.77$) in the 24% oral sucrose group. These mean PIPP-R score values are consistent with other studies comparing breastfeeding and sweet taste intervention effects on PIPP scores (Carbajal et al., 2003; Codipietro et al., 2008) and suggest that both breastfeeding and 24% oral sucrose combined with non-nutritive sucking and containment offer pain-reducing benefit during heel lance. At 90-seconds following heel lance infants who received the 24% sucrose intervention had a PIPP-R score that was over one-point lower than those who were breastfeeding, however, as both groups of infants had PIPP-R scores that were indicative of low to no pain, this difference is not clinically meaningful and suggest that both interventions had similar pain-reducing efficacy across the time phases of the procedure.

While average pain scores were generally low, at 30-seconds following heel lance, 13.3% ($n = 2$) of infants in the breastfeeding group and 26.3% ($n = 5$) of infants in the 24% sucrose group had PIPP-R scores greater than six (ranging from 6 – 10), indicating moderate pain. While this is an overall small proportion of infants in our study, it suggests that these interventions did not ameliorate pain in some infants. The idea that there are individual differences in infant acute pain responding has been previously raised (Pillai Riddell et al., 2013). In a large cohort of older infants undergoing immunization at 2-, 4-, 6-, and 12-months, Pillai Riddell and colleagues (2013) demonstrated that there are stable patterns in the way groups of infants react immediately following a painful procedure and regulate following procedure end. In contrast, Cignacco and colleagues

(2009) reported that there was a high level of individual variability in response to heel lance, with the greatest amount of variability present during the procedure, in a small pilot sample of preterm infants undergoing five repeated heel lances. While these samples of infants are different from those in our study, these findings bring into question the representativeness of comparing mean group level pain scores from a single procedure when aiming to determine the immediate and sustained effects of non-pharmacologic infant pain treatments. Future research with more robust and comparable samples is needed to better understand and discriminate any individual and environmental factors contributing to variability in pain and treatment responsiveness.

An important consideration is that although infants in the breastfeeding and 24% oral sucrose arms of this study had comparable PIPP-R scores in response to the non-noxious control stimulus and the noxious heel lance, pain-related principal component waveforms had an appreciably more positive amplitude in the sucrose arm in response to both stimuli. This finding is very much in line with other work in this area that has demonstrated that while behavioural and cortical responses to painful stimuli are generally well correlated, behavioural responses may be absent despite the presence of an evoked brain response (Green et al., 2018; Slater, Cantarella, Franck, Meek, Fitzgerald, 2008). This lack of behavioural manifestation of pain responses brings question to the validity of using behavioural responses to measure infant pain, as they may underrepresent pain and over-represent treatment efficacy (Green et al., 2018; Meesters, Dilles, Simons, & van Dijk, 2019; Pillai Riddel et al., 2016). However, similarly, most studies measuring nociceptive-related brain activity demonstrate a lack of measurable cortical response to painful stimuli in sub-sets of infants (Fabrizi et al., 2011b; Green et al., 2018; Hartley et al., 2018; Jones et al., 2018; Slater et al., 2008) and suggest that

individual infant factors, such as stress, may dissociate the relationship between behavioural and brain reactivity (Jones et al., 2018). While this evidence suggests that pain-related brain potentials and behavioural responses are not dependent on one another in the manifestation of a pain response, debate regarding the "optimal" indicators to measure pain in pre-verbal infants is ongoing. This is driven by a crucial clinical need for sensitive and specific tools and measures that are responsive to the effects of pain-reducing treatments. The Neuromatrix Theory of Pain proposes that the outputs in response to a pain stimulus are diverse, including voluntary and involuntary action patterns (e.g., behavioural responses) and stress-regulation programs (e.g., physiologic changes in cortisol, norepinepherin, and endorphins [Melzack et al., 2005]). In line with this guiding theoretical framework, our findings support the conclusion that multi-modal measurement of diverse physiological and behavioural parameters that respond to painful and stressful stimuli is needed to provide the most comprehensive possible understanding regarding pain responding, analgesic needs, and treatment efficacy.

Physiologic recovery. An important and interesting finding in this study was that the infants in the breastfeeding group had a clinically relevant (albeit not statistically significant) difference in recovery times – as measured by heart rate return to baseline values. Specifically, infants in the breastfeeding group recovered almost one minute faster than those infants who received 24% oral sucrose and offered non-nutritive sucking while contained in a blanket in an infant cot. While not statistically powered to detect this effect, this result is consistent with Shah and colleagues' (2012) meta-analysis of studies reporting on breastfeeding for pain management in the neonatal period, which predominantly reported that breastfeeding infants had lower heart rate increases following painful procedures when compared to control interventions such as sweet taste, non-

nutritive sucking, and being held. Another finding in this sample that is consistent with previous literature is the high amount of individual variability in infant time to recovery – particularly in those infants in the 24% oral sucrose group. The majority of infants in the breastfeeding group recovered to baseline heart rates values within 17-seconds following procedure completion, however, several infants in the 24% oral sucrose group took over 150-seconds to recover or never returned to baseline heart rate values. While this variance may reflect a lack of precision given the small sample size, it is also suggestive of an effect of close-maternal contact and breastfeeding in regulating infant physiologic homeostasis following a painful and stressful event.

Promotion of autonomic nervous system development through the thermal, tactile, and nutritive components of close maternal contact and breastfeeding has been reported (Hofer, 1987). Feldman and colleagues examined the influence of maternal-infant skin-to-skin contact in early life on autonomic functioning and cognitive development at term equivalence (Feldman & Eidelman, 2003) and at 10-years of age (Feldman, Rosenthal, & Eidelman, 2014) in preterm infants who received a minimum of one hour of ventral maternal-infant skin-to-skin contact for at least 14-days in the neonatal period. These authors reported that infants who received this dose of maternal contact had a significant increase in vagal tone (measured as the amplitude of respiratory sinus arrhythmia) when compared to control infants (Feldman & Eidelman, 2003), and that this positive effect on autonomic functioning was sustained to 10-years of age (Feldman, Rosenthal, & Eidelman, 2014). It has been hypothesized that there are mechanisms specific to maternal-infant contact, such as regulation of corticotrophin releasing hormone in the central nervous system (Meaney, 2001), that promote this autonomic development and subsequent ability to regulate during stressful events. While the exact mechanisms of

maternal care in improving infant physiologic regulation in the context of pain has not been specifically examined, spectral analysis of heart rate variability in breastfeeding infants has showed improved autonomic cardiac reactivity. In a large prospective study of 180 healthy full term newborns, Weissman and colleagues (2009) reported that breastfeeding infants ($n = 31$) and infants being held by their mother and receiving formula ($n = 30$) had better autonomic regulation and lower heart rate increases when compared to those infants who received no intervention ($n = 29$), non-nutritive sucking ($n = 30$), oral glucose ($n = 31$), or simple holding ($n = 29$). Such findings are consistent with those of our study and lend support to the cumulative influence of maternal proximity, touch, and scent; along with the nutritive and gustatory stimulation that the breastfeeding context provides, to positively influence infant parasympathetic control and cardiac regulation following painful events.

Adverse events and safety surveillance. There were no statistically significant differences found between groups with respect to the safety surveillance items of incidence of apnea, bradycardia, repeat heel lancing, required repeat "rescue" 24% oral sucrose doses, or temperature instability. These findings are consistent with evidence syntheses reporting on the immediate safety of using non-pharmacologic pain management interventions, such as breastfeeding (Benoit et al., 2017; Shah et al., 2012), maternal-infant skin-to-skin contact (Johnston et al., 2017), and oral sweet solutions (Bueno et al., 2013; Stevens et al., 2016) to manage needle-related procedural pain in neonates. Short term minor adverse events of administering oral sweet solutions have been reported in some studies, and include self-resolving episodes of oxygen desaturation, choking, and spitting up following administration of glucose (Bueno et al., 2013) and sucrose (Stevens et al., 2016). However, no infants in our study experienced

physiologic instability following administration of the study dose of 24% oral sucrose. This may be related to the small sucrose volume administered as per the study protocol (0.24 milliliters), which was based on data published by Stevens and colleagues (2018) indicating that 0.1 millilitres of 24% oral sucrose administered two minutes prior to heel lance has the same efficacy in reducing bio-behavioural pain intensity as larger doses of 0.5 millilitres or 1.0 millilitres in a largely preterm infant sample (gestational age in weeks, $M = 32.6$, $SD = 4.2$). As previous studies reporting minor adverse effects in full term infants have administrated larger volumes of sweet solutions prior to painful procedures (e.g., 0.5 millilitres [Tutag Lehr et al., 2015], 2.0 millilitres [Thakkar et al., 2016]), provision of smaller minimally effective volumes of sweet solutions may ameliorate this safety concern. Such findings may suggest that provision of a minimally effective dose may be an important consideration in both full term infants and preterm infants to minimize risk of minor adverse events.

It was hypothesized that infants who were randomized to the breastfeeding group would experience fewer adverse events (e.g., fewer episodes of temperature instability, less required rescue sucrose doses). This was based on the hypothesized superior pain-reducing efficacy of breastfeeding (Benoit et al., 2017; Shah et al., 2012; Melzack, 2005), as well as studies reporting the positive effects of maternal-infant skin-to-skin contact and closeness on physiologic stability and homeostatic regulation in both preterm (Campbell-Yeo, Disher, Benoit, & Johnston, 2015) and full term born infants (Moore, Bergman, Anderson, & Medley, 2016). Although this study was not powered to detect differences in the measured adverse events and safety surveillance items, there was an appreciable difference in the number of infants in the 24% oral sucrose group who required off-protocol "rescue" dosing of sucrose following the heel lance as they exhibited a PIPP-R

score greater than six, indicating pain (15.8% in the sucrose intervention compared to no infants in the breastfeeding intervention). This finding may represent further evidence that breastfeeding provides superior pain-reducing effects when compared to 24% oral sucrose. However, given the small sample included in this study such a conclusion is largely based on the broader evidence base reporting equivalent or superior pain-reducing efficacy of breastfeeding when compared to sweet tasting solutions (Benoit et al., 2017; Shah et al., 2012).

Finally, monitoring of infant axillary temperature prior to application of the HydroCel Geodesic Sensor Net and throughout data collection demonstrated that all infants maintained normothermia for the duration of study participation. To our knowledge, no previous safety evaluation of infant temperature stability has been completed in infants wearing these EEG nets (Electrical Geodesic Inc., 2019) which are soaked in warm water prior to application on the infant's head. These findings suggest that, regardless of being in skin-to-skin contact with a mother or contained in a blanket in an infant cot while wearing the net, use of this net for the monitoring of infant pain-related brain activity is safe with respect to infant temperature regulation and stability. Furthermore, this finding would suggest that both interventions have equivalent safety in maintaining normothermia in healthy newborn infants in the immediate post-partum period during a routine painful procedure.

Maternal acceptability. There is a dearth of available evidence regarding parental perceptions of the use and effectiveness of interventions for procedural pain in healthy full term infants in the immediate post-partum period. The majority of studies to date have focused on neonatal nurse and parent perceptions of the use of interventions in the context of neonatal critical care (Benoit et al., 2015; Pölkki, Korhonen, & Laukkala,

2018a; Pölkki, Korhonen, & Laukkala, 2018b; Pölkki, Korhonen, & Laukkala, 2016) or primary care (Ouach et al., 2018; Taddio, Manley, Potash, Ipp, Sgro, Shah, 2007). In our sample of mothers, there were positive perceptions of both breastfeeding and 24% oral sucrose and offered non-nutritive sucking with respect to pain-reducing efficacy. These findings are consistent with those of Ouach and colleagues (2018) who completed surveys with parents of infants who were undergoing immunization in a primary care clinic to evaluate perceptions of infant distress and the efficacy of utilized pain-relieving interventions. In their study, 100% of parents whose infant received breastfeeding, skin-to-skin contact, or sucrose to manage needle-pain reported that they perceived the interventions as somewhat or very effective (Ouach et al., 2018). Such reports lend support to our findings that mothers generally perceive non-pharmacologic and sweet taste interventions as positive and effective strategies to manage pain associated with acute procedures, such as heel lance and intramuscular injections.

The majority of mothers indicated that they would utilize the intervention to which they were assigned (either breastfeeding or 24% oral sucrose) to manage pain during subsequent painful procedures. Not surprisingly, more mothers in the breastfeeding group indicated they would use the assigned intervention during later procedures (94.1% of mothers in the breastfeeding group compared to 68.4% in the sucrose group), emphasizing their perception of its pain-reducing efficacy and previous experience using breastfeeding to manage immunization pain as the primary reasons. Mothers in the sucrose intervention more commonly voiced concerns related to access, appropriate dosing, and lack of knowledge regarding safe and effective sucrose administration. Furthermore, several mothers in the 24% oral sucrose group noted that they would utilize it for future procedures if it was recommended by a health care

professional – particularly a physician – and supported by evidence. Primary and tertiary care physicians and nurses have previously been reported as the most highly trusted sources of infant pain management information (Orr, Campbell-Yeo, Benoit, Hewitt, Stinson, & McGrath, 2017; Parvez et al., 2010). However, similar to the findings of our study, parents commonly report needing more information to inform their involvement in and use of appropriate pain-reducing strategies for their infant during acute procedures (Franck, Oulten, & Bruce, 2012; Orr et al., 2017). Taken together, such reports emphasize the important role that trusted clinical care providers play in providing parents with sufficient information and support regarding the application of evidence-informed pain management strategies for infants undergoing procedures in both tertiary and primary care contexts. To effectively integrate and engage parents in the research development and provision of effective infant pain care, clear and consistent communication and a collaborative family-integrated culture is needed (Marfurt-Russenberger, Axelin, Kesselring, Franck, & Cignacco, 2016; Franck, Oulten, & Bruce, 2012).

It is important to note that 13.5% of families approached refused participation in this study as they preferred to breastfeed their baby during the heel lance procedure and did not want to be randomized to the 24% oral sucrose group. Thus, the sample that was recruited in this study may not be representative of broader parent perceptions and acceptance of non-pharmacologic interventions for infant pain management. However, as parents who participated in this study consented to random assignment to breastfeeding or 24% oral sucrose, it is likely that they were open to diverse approaches to pain management and are reflecting on their experiences with the interventions in this study.

In addition to the overall positive perceptions of breastfeeding and 24% oral sucrose, mothers had positive perceptions of their infant wearing the EEG net as part of the study procedure. Of the small proportion of mothers who reported concerns prior to study participation, none reported continued concerns regarding the safety and comfort of their infant. To our knowledge, this is the first study utilizing neurophysiological techniques to measure pain in infants that has reported on parent experiences and perceptions with the use of this method of pain measurement. Numerous reports have spoken to the use of electroencephalogram as a clinically feasible and objective measure to advance understanding of cortical nociceptive processing in preterm and full term infants undergoing clinical painful procedures (Hartley & Slater, 2014; Fitzgerald, 2015). However, even when completed in a developmentally sensitive way, application of neonatal electroencephalogram requires more handling and is more intrusive than alternative pain measurement methods, such as observing neonatal behaviour and other physiological indices (e.g., heart rate, oxygen saturation). Parent acceptance of the use of this technology will critically influence its utilization in clinical research and any future integration in clinical practice for monitoring of acute or persistent infant pain states. The findings of our study highlight that parents are generally accepting of the use of this technology in clinical research, with the expected caveat that infant comfort and safety while wearing the electroencephalogram net is of high priority.

It is also important to note that there was a high refusal rate for this study, with 60% of families who were approached refusing to take part. This finding is consistent with previous randomized controlled trials that have evaluated the effect of 24% oral sucrose in healthy full term infants (Slater et al., 2010a) and morphine in preterm neonates (Harley et al., 2018) on multi-modal bio-behavioural and

electroencephalographic pain outcomes, where approximately half of families approached to take part in the studies refused participation. Thus, there is a strong likelihood that the maternal perceptions of use of neonatal electroencephalogram to monitor pain-related brain activity in this study are not representative of the post-partum population generally.

Strengths and Limitations

To our knowledge, this is the first study to examine the influence of breastfeeding on electrophysiologic brain responses to painful and tactile stimuli in full term infants undergoing clinical heel lance in the immediate post-partum period. This study utilized rigorous methods to minimize the risk of bias associated with clinical intervention trials (Campbell-Yeo et al., 2009). Strict and clearly defined inclusion and exclusion criteria were implemented and infants across intervention groups were balanced in terms of key confounding variables (e.g., prior pain exposure) that could influence outcomes. A password protected website that was generated by an individual not involved in the study was utilized to randomize consenting participants to ensure random sequence generation and promote concealment of group assignment. Intervention conditions were clearly defined to promote consistency in delivery and deviations from the assigned intervention were documented and controlled for where possible and appropriate. While the PI, assisting research coordinator, and participating families could not be blinded to the intervention infants received, the research assistants who coded the behavioural components of the PIPP-R were blinded to the study objectives and intervention conditions to minimize the risk of bias associated with single-blind intervention trials. These research assistants were rigorously trained and evaluated to ensure inter- and intra-rater reliability (with interclass coefficients ranging from 0.95 to 0.97). Participant loss to follow-up and missing outcome data was transparently recorded and reported, and a

modified intention-to-treat approach was utilized for analyses to minimize the risk of bias associated with having incomplete participant outcome data or deviations in intervention fidelity.

This study presents multi-modal measurement of infant responses to tactile and painful stimuli in the context of several established pain-reducing interventions. There are several strengths of this study with respect to the measurement of pain responding. To our knowledge, we are the first to replicate this research protocol approach of time-locking a clinically required heel lance to continuous EEG recording for measurement of tactile and nociceptive event-related potentials in this population. In doing so we have developed a precise auditory time-locking method to link the heel lance and EEG recording that can be used in future research in this area. Replication of these methods allows for indirect comparison to other work in this area to contribute to the current literature regarding feasibility, reliability, and validity of using these methods. Furthermore, using a well-established bio-behavioural pain measure as a secondary outcome further enhances reliability, validity, and relevance of findings, as well as comparisons across studies measuring multi-modal responses to pain and pain treatments. As single-trial event-related potential recordings in infants present a challenge with respect to low signal-to-noise ratio, principal component analysis was utilized to enhance the interpretability of the underlying component response to stimuli. Furthermore, average trimmed means of the principal component peak amplitude were used in inferential analyses to minimize the effect of outliers in skewing effect estimates. Physiological data and behavioural responses to the painful procedure were recorded with continuous pulse oximeter and video recording equipment to minimize risk of data manipulation, increase precision, and reduce measurement bias. Two study personnel

were present for every data collection session in order to optimize data capture and support intervention delivery.

While these are the numerous strengths of this study, there are several limitations that influence interpretation of study findings that should be noted. One of the most considerable limitations is that due to a smaller than anticipated recruited sample size and loss of outcome data, we are underpowered for our primary and secondary outcomes. Having been involved in similar protocol on the post-partum unit of the IWK Health Centre with healthy full term newborns (Campbell-Yeo et al., 2013), it was anticipated that it would be feasible to both recruit participants and facilitate breastfeeding during the medically required heel lance procedure for the purpose of this study. However, this smaller sample size is a result of a higher than anticipated participant refusal rate, a limited study budget, and closing of recruitment to meet graduate program deadlines. Thus, our ability to make strong conclusions regarding presence and lack of treatment effects from this sample is limited. While all attempts were made to minimize data loss and optimize data quality, approximately half of those infants who completed study procedures did not have usable data for the primary outcome measure. This data loss is consistent with previous clinical trials measuring both bio-behavioural data and pain-related electroencephalographic brain potentials in neonates (Hartley et al., 2018; Slater et al., 2010a) and highlights a substantial limitation of completing single-trial event-related potential studies in infants given the electrophysiologic noise and variability inherent to such an outcome measure. While there were no differences in baseline characteristics and mean bio-behavioural responses between infants with complete and incomplete EEG data, or in loss between intervention conditions, such data loss introduces concern regarding representativeness of the included infants. Additionally, video-recording

equipment failure occurred in a disproportionate number of infants in the breastfeeding intervention. While a modified intention-to-treat analysis approach was used, where all available data was analyzed in the intervention group to which the infant was assigned, such data loss introduces concern regarding risk of bias associated with loss of outcome data. Finally, while it was possible to control for the influence of breastfeeding effectiveness (measured using the LATCH score) and non-nutritive sucking status on PIPP-R scores, software limitations precluded controlling for the influence of confounders on the pain-related brain activity. While there were no significant differences between intervention groups on a priori established confounding variables (i.e., previous pain exposure, infant sex), it is possible that the lack of statistically significant difference in intervention effects on pain-related brain activity were related to the inability to control for the influence of breastfeeding effectiveness and non-nutritive sucking status during the procedure.

Clinical intervention trials testing the effects of breastfeeding and maternal-infant skin-to-skin contact for pain management cannot be double-blinded. Thus, there is concern that investigator, research personnel, and participant knowledge of the intervention condition to which participants were allocated represent a source of bias. Although research assistants who were coding the behavioural components of the PIPP-R were blinded to the nature of the two intervention groups, the study personnel and clinical staff caring for the infant were not blinded. The lack of significant difference between groups on the primary and secondary pain assessment outcomes reduces concern related to biased estimates of treatment effects. However, it is possible that research personnel biased perceptions regarding the superiority of breastfeeding for pain management

influenced care of infants during study participation (i.e., administering a higher frequency of rescue sucrose doses to infants in the 24% oral sucrose arm of the study).

Our study did not have a no-treatment control arm. This could be considered a limitation of the study as it enhances the complexity of interpreting the relative effects and mechanisms of the interventions. Without the use of a no-treatment control arm, or a maternal-infant skin-to-skin contact arm, it is not possible to determine from our study whether the effects of breastfeeding are driven by maternal contact alone or in combination with the gustatory aspect of breastfeeding. The two-treatment arm design of this study was selected to enhance study feasibility and clinical utility – as breastfeeding and 24% oral sucrose have the strongest evidence for pain-reducing efficacy in full term infants in the empirical literature. The use of breastfeeding as a pain-relieving intervention was specifically chosen as it is a multi-modal pain-relieving intervention that incorporates maternal-infant contact, and olfactory and gustatory stimulation. Past literature has identified this intervention as the most effective for reducing behavioural pain responses in full term neonates (Benoit et al., 2017; Shah et al., 2012). While maternal-infant contact alone has been demonstrated to be an effective pain-relieving intervention in neonates (Johnston et al., 2017), the purpose of this study was not to isolate the mechanism of effectiveness of breastfeeding from skin-to-skin contact. Alternatively, this study aimed to determine optimal clinical practice in comparing breastfeeding to what is currently considered the standard of care for managing newborn acute pain in the hospital setting (24% oral sucrose provided in combination with non-nutritive sucking while infant is contained in a blanket in an infant cot). Furthermore, it is our belief that infant pain intervention trials with no treatment control arms are unethical given knowledge of the adverse physiological and behavioural outcomes of untreated

pain and the ready availability of evidence-informed interventions that reduce bio-behavioural pain reactivity. Our findings, in this small cohort of infants, suggest that there may be some aspect of breastfeeding that modulates infants cortical processing of tactile and painful stimuli. Therefore, future studies that attempt to isolate and test the underlying components of this intervention that contribute to its effectiveness (e.g., by comparing direct breastfeeding with skin-to-skin contact with no breastfeeding) are warranted.

Clinical Implications

The findings of this dissertation provide several important clinical implications for consideration in the nursing management of acute procedural pain in full term newborns in the post-partum period. This study provides further evidence that breastfeeding and 24% oral sucrose (combined with non-nutritive sucking and containment) provide comparable and effective reduction of pain measured using bio-behavioural pain scores. Our results additionally implicate maternal-infant skin-to-skin contact and breastfeeding with a potential modulation of electrophysiologic processing of non-painful tactile stimuli and painful heel lance. Furthermore, this study associates maternal-infant skin-to-skin contact and breastfeeding with potential improved physiologic regulation following heel lance. Infants who were breastfed during the heel lance had fewer adverse events and mothers reported higher likelihood of using breastfeeding as a pain reducing strategy during future procedures due to the belief that it was effective and accessible. Taken with the larger body of evidence that breastfeeding and 24% oral sucrose (combined with adjuvant interventions such as non-nutritive sucking and containment) effectively reduce bio-behavioural pain scores and improve regulation in full term infants during acute procedures, they should be integrated as part of routine practice of nurses and clinicians

providing care to this population. Full term neonates in the post-partum period have been a largely neglected population with respect to pain prevention initiatives and clinical practice change policies and programs. However, they are vulnerable to the immediate and long-term physiological and behavioural effects of untreated painful procedures. The findings of this study provide further evidence to support the use of these interventions in clinical nursing care to optimize infant health outcomes.

While definitive conclusions cannot be drawn from this study, our findings implicate breastfeeding in the modulation of tactile and pain-related brain potentials following heel lance and improved infant physiologic recovery. This finding is aligned with our hypotheses that breastfeeding provides the optimal multi-sensorial context for reducing pain reactivity and improving regulation, as it enables maternal closeness and skin-to-skin contact; and olfactory, auditory, oral, and nutritive stimulation during the painful event. These findings, along with the fewer documented adverse events in breastfeeding infants in our study and others (Benoit et al., 2017; Marin Gabriel et al., 2013; Shah et al., 2012), suggests that nurses should support breastfeeding as the first line treatment for painful procedures in the post-partum period. In the event that breastfeeding is unavailable, 24% oral sucrose combined with adjuvant interventions to promote sensorial saturation (e.g., containment, non-nutritive sucking, maternal skin-to-skin contact) should be utilized as part of the nursing plan of care for infants undergoing routine procedures. It is not possible to make conclusions regarding the comparative pain-reducing effects of maternal infant-skin-to-skin contact alone or combined with 24% oral sucrose from the sample of infants in our study. However, the combination of sweet taste and non-nutritive sucking within the context of maternal skin-to-skin contact (i.e., maternal closeness, containment, and olfactory and auditory stimulation) likely provides

equal or superior pain-reducing efficacy compared to sweet taste and non-nutritive sucking provided in an infant cot.

Supporting the active integration and involvement of mothers and families in infant care, notably pain care, is a current clinical priority and holds benefits for infants, mothers, and families. Those mothers who breastfed their infant during the heel lance procedure more frequently reported that they would use breastfeeding for future painful procedures, voicing their positive perceptions of its pain-reducing efficacy and intervention accessibility as the primary reasons. Such findings align with previous studies highlighting parent preference to be actively engaged in their infant's care during painful procedures (Franck, Oulten, & Bruce, 2012; Orr et al., 2017). Nurses are optimally positioned and should make every effort to support the meaningful engagement of families in their infant's pain care in both tertiary and primary care settings to optimize health outcomes. Parents have previously reported requiring more information and more opportunities to be optimally involved in their infant's pain care, particularly recognizing the important role of nurses in providing sensitive and consistent information and support to effectively implement parent-led pain management techniques (Franck, Oulten, & Bruce, 2012). As such, clinical educator, administrator, and policy maker prioritization of nursing-specific and interdisciplinary clinical education, family education, and contextual and environmental support and recognition of the importance of nurse-family collaboration in infant pain care delivery is warranted and necessary to support sustained practice integration (Franck, Oulten, & Bruce, 2012; Marfurt-Russenberger et al., 2016).

Theoretical Considerations and Directions for Future Research

This doctoral research is of theoretical importance as it contributes to the literature in an area of important need – advancing our understanding of and capacity to

comprehensively assess and manage pain in vulnerable pre-verbal infants. The findings of this work provide valuable preliminary information to guide our understanding of potential neurologic, physiologic, and behavioural mechanisms of the pain-reducing effects associated with 24% oral sucrose and breastfeeding. The Neuromatrix Theory of Pain, which provided the conceptual underpinnings of this study, describes the concept of pain as a multi-dimensional experience that is produced and modulated by the complex integration of multiple factors acting on an interconnected neural network (Melzack, 1999; 2001). The subsequent pain experience – the neurosignature – is thus influenced by innate and learned sensory, affective, and cognitive processes that blend together to produce a unique pain experience that is dependent on context (Melzack, 2005). The breastfeeding context in particular, which integrates maternal closeness and skin-to-skin contact; and olfactory, auditory, oral, and nutritive stimulation provide a multi-sensorial intervention package to modulate the neurosignature and subsequent pain reactivity and recovery through numerous potential bio-psycho-social regulators. The Neuromatrix Theory of Pain further proposes that response outputs to pain are diverse, and include neural, hormonal, and behavioural activity patterns aimed at mitigating physical and psychological stress and maintaining homeostasis (Melzack, 2005; 1999). Taken together with the findings of the broader literature, the differential influence of breastfeeding on neurophysiologic reactivity and physiologic regulation in the small group of infants in our study implicate some component of the breastfeeding intervention in modulating autonomic and hormonal nervous system responses to painful and stressful stimuli. Our findings are the first to implicate the multi-sensorial experience of direct breastfeeding with modulated neurophysiologic response to heel lance and stimulate exciting directions for future research related to the effects of maternal-infant contact and sensorial

stimulation pain responding. Our findings further highlight the diverse patterns of infant pain reactivity and recovery in response to tactile and painful stimuli and underscore the importance of multi-modal measurement of physiological, neurophysiological, and behavioural responses to fully interpret and appreciate the infant pain experience.

From our work and others (Bellieni, 2012), it appears that multi-sensorial stimulation, particularly in the maternal context, provides superior pain reducing and regulating effects. However, it is not possible to elucidate from our study if maternal skin-to-skin contact alone would provide comparable reduction of neurophysiologic responses, behavioural responses, and time to physiologic recovery in full term infants. Future work examining the effects of maternal-infant skin-to-skin contact alone or combined with sweet taste interventions on these multi-modal pain responses would be a valuable area of investigation. Carefully designed and adequately powered studies that aim to tease apart the underlying mechanisms contributing to the effectiveness of multi-sensory pain-reducing interventions like breastfeeding are needed to understand how to best optimize treatment efficacy. There is also a need to determine the sustained efficacy of breastfeeding in modulating multi-modal pain responses in order to best tailor pain assessment and management across repeated procedures.

Given the gaps in our current understanding of the pain-specificity of the neurophysiologic potential examined in this work, future studies attempting to characterize the selectivity and sensitivity of this response are needed. Examining the neural responses to varying stimulus modalities and intensities in infants would be valuable to elucidate if the presence and intensity of evoked responses are related stimuli salience, intensity, or nociceptive pain. Additionally, use of neurophysiologic measurements to examine evoked responses that may represent the potential mechanisms

underlying modulation of pain response (such as components associated with cognitive attendance to a salient stimulus) may provide an interesting area of investigation. Such work could provide exciting insights into the nuanced relationship between subjective and somatosensory pain experiences, as well as factors that contribute to individual and group-level variability in neurophysiologic and behavioural correlates of painful and stressful sensory events and comforting interventions (Mouraux & Iannetti, 2018). Furthermore, given the current limitations of neurophysiologic imaging technology with respect to clinical utility, such an avenue of investigation may support the refinement and development of measures to consider individual and contextual factors that selectively or differentially influence pain reactivity and regulation. Ongoing examination of parent and clinician perspectives of the acceptance and feasibility of applying such pain measurements and technologies in the care of diverse populations of infants should be integrated into such work to enhance relevance and promote later ease of integration into clinical care.

Loss of participant data and poor data quality in studies using electroencephalography to quantify pain responding in infants is a significant concern that has not been given its corresponding credence in the literature to date. There is a need for continued methodological development and application to promote retention of high-quality data for participants enrolled in such studies in order to promote representativeness and generalizability of findings. Such data retention and improved data quality should be considered an essential pre-requisite for all future clinical research attempting to draw conclusions regarding the specificity and sensitivity of this pain-related potential, as well as the neuro-modulatory effects of interventions, given the substantial risk of bias associated with such data loss. A collaborative effort from diverse

interdisciplinary groups of scientists and clinicians to implement rigorous studies with transparent reporting of methods is needed to truly advance methodological quality and our understanding and application of these measures and interventions to optimize care of infants during pain and stress.

Knowledge Translation

Knowledge translation initiatives were ongoing throughout the research process, with initial emphasis on raising healthcare professional and public awareness and interest in infant pain management. Regular updates on the progress of the study were provided to health professionals and administrators on the participating unit via email and face-to-face meetings. As results from the study are now available, focus will be placed on targeting researchers, healthcare providers, administrators, and decision makers with new knowledge to inform infant pain assessment and management practices, as well as subsequent research in the field. Three reports (one, three, and 10 pages) will be generated for key clinical and policy stakeholders (e.g., members of the Baby Friendly Hospital Initiative Committee; unit managers, physicians, and leaders within the IWK Health Centre Women and Newborn Health Program and the Nova Scotia Health Authority) to disseminate the findings of the study. These reports will be disseminated nationally and internationally though affiliations with the Canadian Neonatal Network and Children's Healthcare Canada (previously the Canadian Association of Pediatric Health Centres), the Canadian Child Health Clinician Scientist Program (CCHCSP), and the Pain In Child Health (PICH) program. Traditional end-of-grant knowledge translation initiatives targeting researchers and knowledge users will include publication in academic peer-reviewed journals (e.g., submission to *Lancet, JAMA Pediatrics, Pediatrics, PAIN, Birth*), presentation at national and international conferences (e.g., International

Symposium on Pediatric Pain, Canadian Pain Society Annual Scientific Meeting, Pediatric Academic Society, Council of International Neonatal Nurses), presentation to healthcare providers in the clinical setting (e.g., lunch-and-learn presentations to clinicians on the Family Newborn Care Unit and Neonatal Intensive Care Unit), presentation to key clinical decision makers through affiliation with the IWK Health Centre Baby Friendly Hospital Initiative Committee, and presentations to primary care providers through affiliation with the Dalhousie University Building Research for Integrated Primary Healthcare – Nova Scotia (BRIC-NS) group. Webinars directly targeting pediatric pain researchers will be delivered through affiliation with the Pain In Child Health (PICH) program to disseminate knowledge related to research process and findings. Information will be targeted at parents through collaborations with the IWK Health Centre Family Centered Care Council, through the "It Doesn't Have to Hurt" initiative (an established partnership with YummyMummyClub.ca), and to local parenting and family centers through talks, guest blogs, magazine articles, and twitter posts. Additional knowledge translation initiatives that will be implemented include use of social media (e.g., Twitter) and press releases to raise widespread public, healthcare provider, and parent awareness related to infant pain assessment and management.

CHAPTER 6: CONCLUSION

This study has shown that while breastfeeding and 24% oral sucrose combined with non-nutritive sucking and containment have similar pain-reducing effects as measured using bio-behavioural pain scores, breastfeeding may differentially modulate evoked neurophysiologic responses following tactile and painful stimuli. Furthermore, breastfeeding may provide greater benefit in supporting infant physiologic recovery following painful procedures, may have fewer associated adverse events, and is associated with more positive maternal perceptions regarding its future utilization as a procedural pain-management strategy. These findings, while from a small sample, support the preferential use of breastfeeding for reduced multi-modal pain reactivity and improved regulation. These findings additionally support the continued use of 24% oral sucrose combined with non-nutritive sucking and containment for the reduction of bio-behavioural pain scores when breastfeeding is unavailable.

This work has also highlighted the substantial complexity of measuring and interpreting diverse physiologic and behavioural indicators of procedural pain in infants. It further highlights the critical need for continued evaluation of the optimal combination of assessment techniques to appreciate infant pain experiences and the efficacy of pain-reducing treatments in both research and clinical practice contexts, with patient and clinician engagement and feedback representing a key area of focus in this evaluation. Replication and advancement of this work with a particular focus on optimized data quality is needed to understand the underlying mechanisms of maternal care and sweet taste interventions, individual variability in response to pain and pain treatments, and the pain-selectivity of neurophysiologic indicators. Such knowledge would greatly support our capacity to provide optimal pain care to infants during painful procedures.

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APPENDICES

Appendix A

Characteristics and Risk of Bias of Studies Examining Breastfeeding for Pain Management

Author, year, country	Study objective	Study design	Study setting	Painful procedure	Participants	Intervention group	Comparison group	Risk of bias
Bembich (2013) Italy	To evaluate cortical and behavioural responses to a painful procedure during either breastfeeding or glucose	RCT	Hospital	Heel lance	30 full term neonates less than 3-days PNA	Breastfeeding initiated 2-minutes prior to procedure	2 mL bolus of 20% oral glucose 2-minutes prior to procedure	HIGH
Boroumandfar (2013) Iran	To compare vaccine pain between infants who receive vapocoolant spray or breastfeeding	RCT	Hospital	Intra-muscular injection	144 full term born infants at 2-, 4-, and 6-months of life	Breastfeeding during procedure	a) Vapocoolant sprayed 10-seconds pre-procedure b) No-treatment control	HIGH
Fallah (2016) Iran	To compare the analgesic effect of KMC, breastfeeding, and swaddling	RCT	Hospital	Intra-muscular injection	120 full term neonates less than 24-hours PNA	Breastfeeding initiated 2-minutes prior to procedure	a) KMC 10 minutes before, during procedure b) Swaddled 10-minutes before, during procedure	MOD

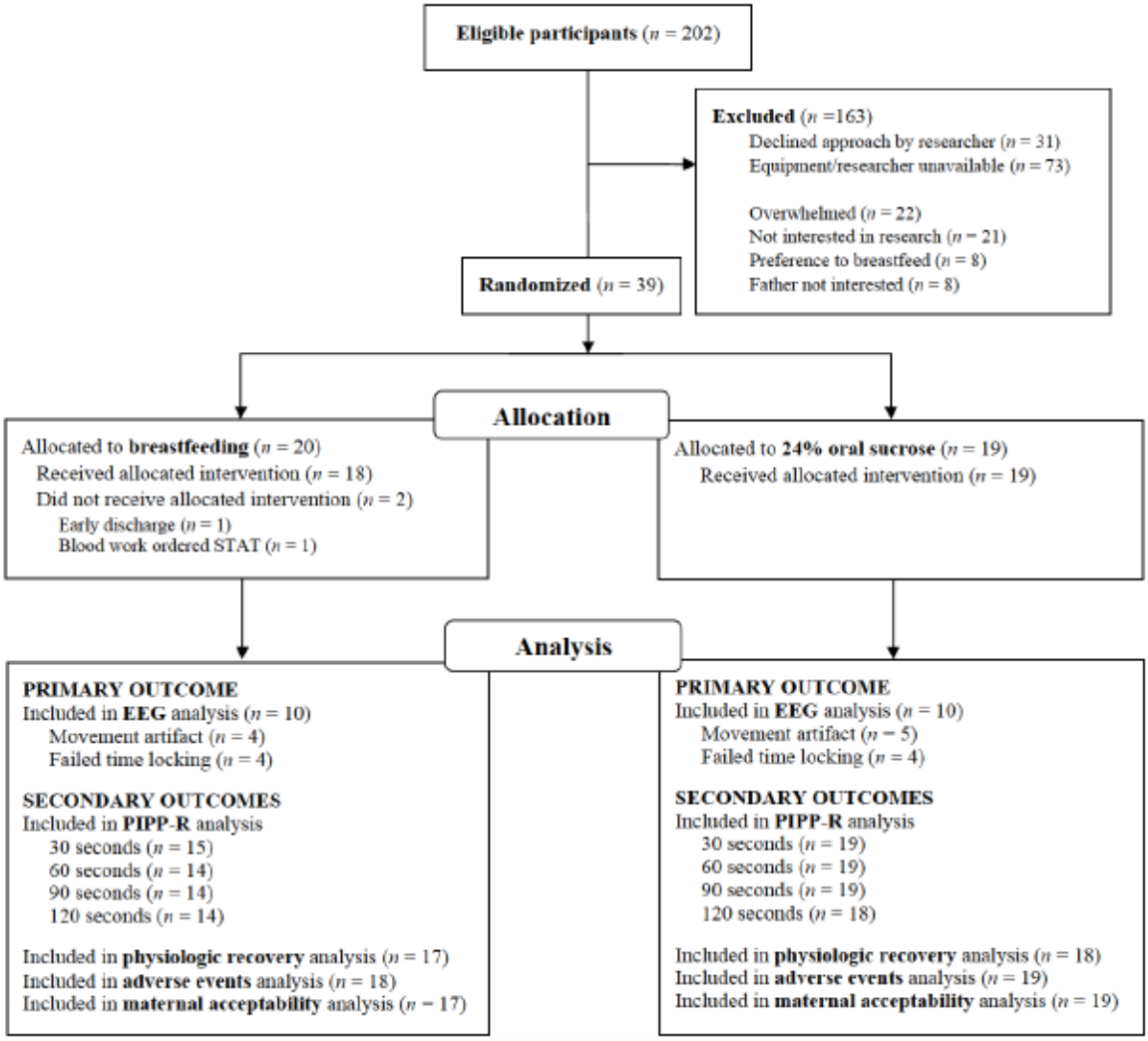
Goswani (2013) India	To compare the analgesic effect of breastfeeding, 25% dextrose, and placebo	RCT	Clinic	Intra-muscular injection	120 full term infants less than 3-months PNA	Breastfeeding initiated 2-minutes prior to procedure	a) 2 mL bolus of 25% dextrose 2-minutes prior to procedure b) 2 mL bolus distilled water 2-minutes prior to procedure	LOW
Gupta (2013) India	To evaluate the analgesic effect of EMLA and breastfeeding	RCT	Clinic	Intra-muscular injection	90 full term infants less than 3-months PNA	1-gram EMLA 60-minutes prior to procedure + breastfeeding 2-minutes prior to procedure	a) EMLA + 2 mL bolus distilled water 2-minutes prior to procedure b) Vaseline + 2 mL bolus distilled water 2-minutes prior to procedure	LOW
Marin Gabriel (2013) Spain	To evaluate the analgesic effect of breastfeeding combined with SSC vs. other non-pharmacologic analgesics	RCT	Hospital	Heel lance	136 full term neonates in first days of life	Breastfeeding initiated 5-minutes prior to procedure + SSC	a) 2 mL bolus 24% sucrose 2-minutes prior to procedure + SSC 5-minutes prior to procedure b) SSC contact 5-minutes prior to procedure c) 2 mL bolus 24% sucrose 2-minutes prior to procedure	HIGH
Modarres (2013) Iran	To examine the effect of	RCT	Hospital	Intra-muscular injection	130 full term neonates less	Breastfeeding initiated 2-	Held by mother with no breastfeeding	MOD

	breastfeeding on pain				than 24 hours PNA	minutes prior to procedure		
Obeidat (2015) Jordan	To determine the efficacy of breastfeeding with maternal holding to holding alone	RCT	Hospital	Heel lance	128 full term neonates less than 6-days PNA	Breastfeeding while held in mothers lap	Held in mothers lap with no breastfeeding	MOD
Thomas (2011) India	To compare pain response between infants who are breastfeeding and not breastfeeding	Quasi-experi-mental	Not stated	Intra-muscular injection	40 full term infants less than 15-weeks PNA	Breastfeeding initiated 2-minutes prior to painful procedure	No breastfeeding	HIGH
Zhu (2015) China	To test the effect of breastfeeding, music therapy, and combined breastfeeding and music therapy on pain	RCT	Hospital	Heel lance	288 full term neonates less than 4-days PNA	a) Breastfeeding initiated 5-minutes prior to procedure + classical music 5-minutes prior to procedure b) Breastfeeding initiated 5-minutes prior to procedure alone	Classical music playing 5-minutes prior to procedure	MOD

Note. RCT = Randomized controlled trial; GA = Gestational age; PNA = Postnatal age; KMC = Kangaroo mother care; EMLA = Eutectic mixture of local anesthetics (EMLA); SSC = Skin-to-skin contact

Appendix B

Study Flow Diagram for Randomized Controlled Trial Examining the Influence of Breastfeeding on Pain-related Potentials and Bio-behavioural Indicators of Procedural Pain in Newborns



Appendix C

Premature Infant Pain Profile – Revised

Infant Indicator	Indicator Score				Infant Indicator Score
	0	1	2	3	
Change in heart rate (bpm) Baseline: _____	0 – 4	5 – 14	15 – 24	> 24	
Change in O_2 saturation (%) Baseline: _____	0 – 2	3 – 5	6 – 8	> 8 or increase in O_2	
Brow bulge (sec)	None (< 3)	Minimal (3 – 10)	Moderate (10 – 20)	Maximal (> 20)	
Eye squeeze (sec)	None (< 3)	Minimal (3 – 10)	Moderate (10 – 20)	Maximal (> 20)	
Naso-labial furrow (sec)	None (< 3)	Minimal (3 – 10)	Moderate (10 – 20)	Maximal (> 20)	
*Sub-total score:					
Gestational age (weeks + days)	> 36	32 – 35 $^6/_7$	28 – 31 $^6/_7$	< 28	
Baseline behavioural state	Active awake	Quiet awake	Active asleep	Quiet asleep	
**Total score:					

Note. Adapted from Gibbins, S., Stevens, B. J., Yamada, J., Dionne, K., Campbell-Yeo, M., Lee, G., … Taddio, A. (2014). Validation of the Premature Infant Pain Profile-Revised (PIPP-R). *Early Human Development*, *90*(4), 189–193. doi:10.1016/j.earlhumdev.2014.01.005

Appendix D

Maternal Acceptability Questionnaire – Breastfeeding

You and your baby participated in a study entitled "The influence of breastfeeding on cortical and bio-behavioural indicators of procedural pain in newborns".

During this study, you were breastfeeding your baby during their routine blood work and during this, your baby was wearing a newborn electroencephalogram (EEG) cap to measure pain activity in the brain.

The questions below ask about how you think things went during the procedure. Please answer the questions below to the best of your ability and place the completed form in the sealed envelope provided. A member of the research team will pick up this completed questionnaire before you and your baby are discharged from the IWK Health Centre.

1. **How did you feel about breastfeeding your baby during this procedure?**
    a. **Not good**
    b. **Fair**
    c. **Good**
    d. **Very good**

2. **How do you think your baby did in terms of pain relief?**
    a. **Not good**
    b. **Fair**
    c. **Good**
    d. **Very good**

3. **How did you feel about your baby wearing the infant EEG cap?**
    a. **Not good**
    b. **Fair**
    c. **Good**
    d. **Very good**

4. **Did you have any concerns about breastfeeding your baby during the painful procedure?**

    If so, do you still find it concerning?

5. **Did you have any concerns about your baby wearing the infant EEG cap during the procedure?**

If so, do you still find it concerning?

6. **Do you think you will use breastfeeding for pain relief in the future (for example, during your baby's immunizations or any other procedures)?**

    a. **If yes, why?**

    b. **If no, why?**

7. **Is there anything that could have been changed about this study to improve the experience?**

    a. **Changes to the breastfeeding part of the study:**

    b. **Changes to the EEG part of the study:**

Appendix E

Maternal Acceptability Questionnaire – Sucrose

You and your baby participated in a study entitled "The influence of breastfeeding on cortical and bio-behavioural indicators of procedural pain in newborns".

During this study, your baby received a sweet tasting solution (24% sucrose) during their routine blood work. Your baby was also wearing a newborn electroencephalogram (EEG) cap to measure pain activity in the brain.

The questions below ask about how you think things went during the procedure. Please answer the questions below to the best of your ability and place the completed form in the sealed envelope provided. A member of the research team will pick up this completed questionnaire before you and your baby are discharged from the IWK Health Centre.

1. **How did you feel about your baby receiving sucrose during this procedure?**
    a. **Not good**
    b. **Fair**
    c. **Good**
    d. **Very good**

2. **How do you think your baby did in terms of pain relief?**
    a. **Not good**
    b. **Fair**
    c. **Good**
    d. **Very good**

3. **How did you feel about your baby wearing the infant EEG cap?**
    a. **Not good**
    b. **Fair**
    c. **Good**
    d. **Very good**

4. **Did you have any concerns about your baby receiving sucrose during the painful procedure?**

    If so, do you still find it concerning?

5. **Did you have any concerns about your baby wearing the infant EEG cap during the procedure?**

If so, do you still find it concerning?

6. **Do you think you will use sucrose for pain relief in the future (for example, during your baby's immunizations or any other procedures)?**

    a. **If yes, why?**

    b. **If no, why?**

7. **Is there anything that could have been changed about this study to improve the experience?**

    a. **Changes to the sucrose part of the study:**

    b. **Changes to the EEG part of the study:**

Appendix F

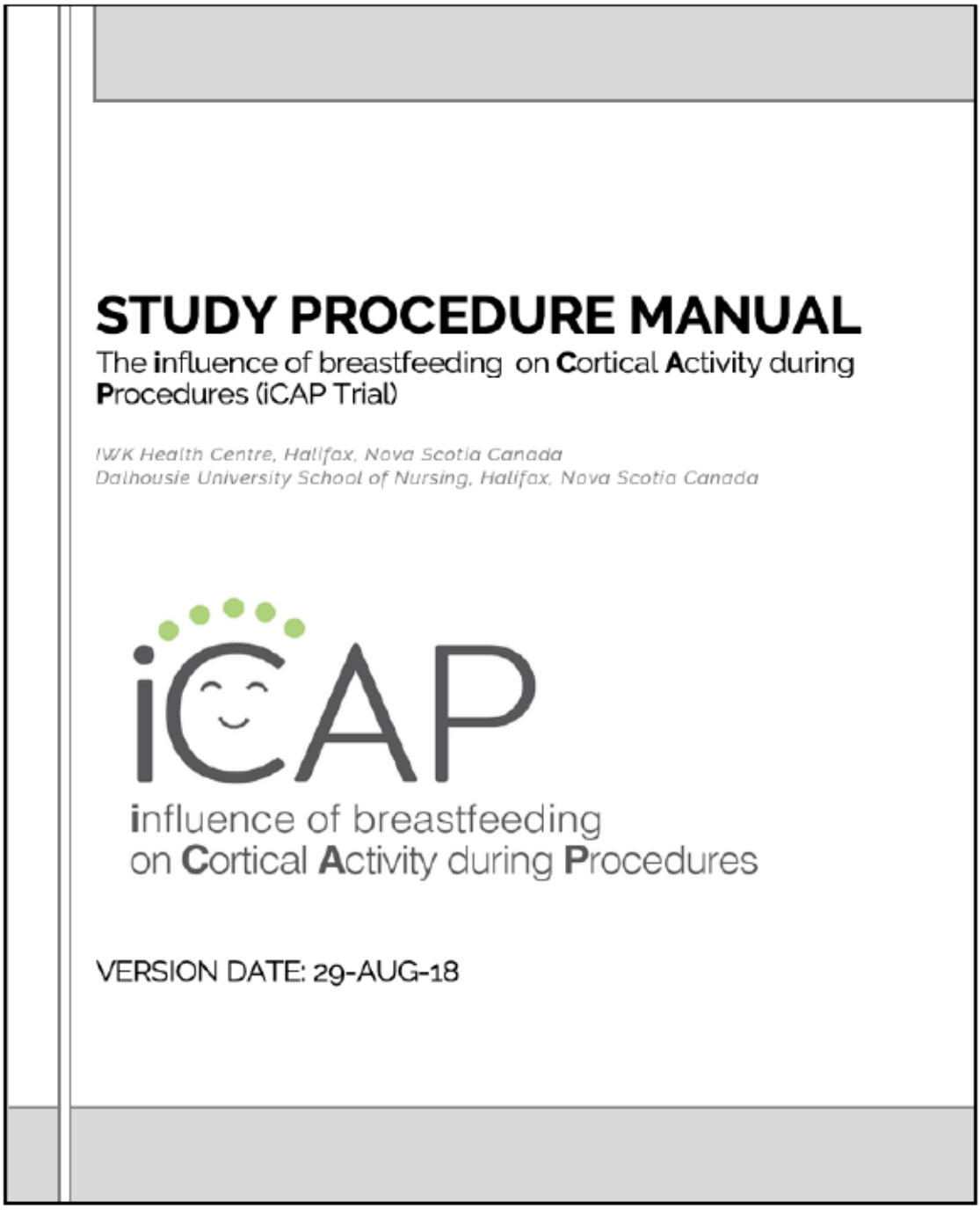
LATCH Breastfeeding Assessment Tool

LATCH SCORE			
	0	1	2
L Latch	Too sleepy or reluctant No latch achieved	Repeated attempts Hold nipple in mouth Stimulate to suck	Grasps breast Tongue down Lips flanged Rhythmic sucking
A Audible swallowing	None	A few with stimulation	< 24 hrs old: Spontaneous and intermittent > 24 hrs old: Spontaneous and frequent
T Type of nipple	Inverted	Flat	Everted (after stimulation)
C Comfort (breast/nipple)	Engorged Cracked, bleeding, large blistered or bruises Severe discomfort	Filling Reddened/small blisters or bruises Mild/moderate discomfort	Soft Non-tender
H Hold (positioning)	Full assist (staff holds infant at breast)	Minimal assist (staff provides some teaching/support)	No assist from staff Mother able to position/hold infant

Note. Adapted from Jensen, D., Wallace, S., & Kelsay, P. (1994). LATCH: A breastfeeding charting system and documentation tool. *Journal of Obstetric, Gynecologic, and Neonatal Nursing*, *23*, 27–32. doi:10.1111/j.1552-6909.1994.tb01847.x

Appendix G

iCAP Study Data Collection and Geodesic EEG System™ 400 Series HydroCel Geodesic Sensor Net Application Manual



STUDY PROCEDURE MANUAL

The influence of breastfeeding on Cortical Activity during Procedures (iCAP Trial)

IWK Health Centre, Halifax, Nova Scotia Canada
Dalhousie University School of Nursing, Halifax, Nova Scotia Canada

iCAP

influence of breastfeeding
on Cortical Activity during Procedures

VERSION DATE: 29-AUG-18

1. ABOUT THIS PROCEDURE MANUAL

This procedure manual was developed to guide execution of all aspects of the study, as a means of standardizing research practices across participants according to the research protocol. It is a meant to be used as a training and reference tool.

2. STUDY SITE, TEAM MEMBERS, & FUNDING AGENCIES

2.1 STUDY SITE

IWK Health Centre, Halifax Nova Scotia Canada

2.2 TEAM MEMBERS

Britney Benoit*, MScN RN PhD(c), Principle Investigator
Marsha Campbell-Yeo, PhD NNP-BC RN, Supervisor
Ruth Martin-Misnener, PhD NP RN, Co-Supervisor
Aaron Newman, PhD, Committee Member
Margot Latimer, PhD RN, Committee Member

*This work constitutes Benoit's dissertation research in the PhD in Nursing program at the Dalhousie University School of Nursing.

2.3 FUNDING

This research is funded by the IWK Health Centre Category A Operating Grant (Grant #: 20285) and the Dalhousie University School of Nursing, Nursing Research Fund Operating Grant (Grant #: 36683).

3. STUDY BACKGROUND

Even the healthiest of infants undergo routine painful procedures as part of universal medical care. In addition to causing unnecessary suffering to the smallest and most vulnerable of our population, unmanaged early pain exposure is associated with adverse neurological consequences, such as heightened pain response during subsequent procedures in infancy (Grunau, Holsti, & Peters, 2006; Taddio, Shah, Gilbert-MacLeod, & Katz, 2002). Therefore, utilizing effective pain reducing interventions is imperative.

Summative data from 75 randomized controlled trials demonstrates that 24% oral sucrose solution reduces behavioral pain scores during acute procedures (Stevens, Yamada, Lee, & Ohlsson, 2016). Despite this compelling evidence, recent data questions the analgesic efficacy of this intervention. Slater and colleagues (2010) enrolled 59 newborn infants in a double blind, randomized controlled trial and found that while 24% oral sucrose reduced behavioral pain response, it did not reduce pain-specific activity in the infant brain measured on electroencephalogram. The authors of this study concluded that the reduction of behavioral pain scores should not be interpreted as pain relief and that further investigation is necessary (Slater et al., 2010). As such, research examining the analgesic properties of alternate interventions, such

as breastfeeding, is needed to inform optimal care of newborns undergoing painful procedures.

Evidence supports the effectiveness of breastfeeding as a pain relieving intervention. A recent Cochrane systematic review of 20 studies demonstrates that behavioral pain scores are significantly lower in infants who are breastfed during heel lance or venipuncture when compared to those who are positioned in their mothers' arms, or receive oral sucrose, placebo, or no intervention (Shah, Herbozo, Aliwalis, & Shah, 2012). While breastfeeding shows great promise as an intervention to reduce behavioral indicators of pain, no studies to date have examined the effect of breastfeeding on pain-specific brain response in the newborn.

Through innovative and rigorous application of experimental methodologies, the proposed study will address this significant knowledge gap by examining to what extent breastfeeding impacts on pain-specific response in the infant brain during an acute painful procedure (i.e., a heel lance procedure).

3.1 iCAP STUDY DESCRIPTION

3.11 STUDY OBJECTIVES & HYPOTHESES

Objectives. The primary objective of this study is to examine the influence of breastfeeding on pain-specific activity in the healthy full term newborn brain during heel lance, compared to the administration of 24 percent oral sucrose and offered non-nutritive sucking while in an infant cot (considered the current standard of care). The primary outcome measure will be a pain-specific event-related potential induced by heel lance and measured using a dense- array neonatal electroencephalogram (EEG) recording (Slater et al., 2010a-c). Secondary outcomes that will be compared between groups will include: a) Bio-behavioural pain score measured using the Premature Infant Pain Profile – Revised (PIPP-R; Stevens et al., 2013; 2014), b) physiologic recovery, c) maternal acceptability of data collection procedures and intervention conditions, and d) adverse events.

Primary hypothesis. Infants randomized to the breastfeeding condition, when compared to those infants randomized to the 24 percent oral sucrose and offered non-nutritive sucking condition, will demonstrate a lower amplitude pain-specific event related potential.

Secondary hypotheses. Infants randomized to the breastfeeding condition, when compared to those infants randomized to the 24 percent oral sucrose and offered non-nutritive sucking condition, will demonstrate:

a) lower bio-behavioural pain scores, measured using the PIPP-R at 30, 60, 90, and 120 seconds following heel lance,

b) faster recovery measured as heart rate return to baseline,

c) greater maternal acceptability regarding the data collection procedures and interventions, and

d) fewer adverse events (e.g., fewer episodes of temperature instability, fewer rescue sucrose doses).

3.12 DESIGN

The study will utilize a single blind, randomized controlled trial design (Appendix B). Participants will be randomized to have a medically indicated heel lance completed in one of two possible intervention conditions: 1) breastfeeding or 2) 24 percent oral sucrose and offered non- nutritive sucking while in an infant cot.

3.13 PARTICIPANTS & SETTING

We plan to recruit one hundred and twenty-six healthy full term infants (63 infants per intervention group) will be recruited from the Family Newborn Care Unit of the IWK Health Centre within the first three days of age.

3.14 INCLUSION & EXCLUSION CRITERIA

Inclusion criteria Healthy, full term (born greater than 37 0/7 weeks' gestational age; Barrington, Sankaran, & Canadian Paediatric Society Fetus and Newborn Committee, 2007) normally breastfeeding infants, whose mother is willing to breastfeed during the painful procedure and consents to study participation. Normally breastfeeding infants will be defined as those infants who had fed directly at the breast a minimum of two times in the 24-hours prior to blood collection and whose mother and/or staff nurse reported active sucking and swallowing during those feeds. Infants who have undergone repeated heel lancing for blood glucose and/or bilirubin monitoring (e.g., small or large for gestational age infants, infants born to diabetic mothers, or with hyperbilirubinemia) will be considered eligible, however, diagnosis and the number of prior painful procedures will be recorded and retained for statistical analysis.

Exclusion criteria Infants will not be eligible for study participation if they are a twin birth (including all classifications of monozygotic and dizygotic twins) due to the potential for non-independence of outcomes between twin pairs, show signs of infection, significant lower limb tissue damage, have had previous surgery or intra-ventricular hemorrhage, are born to opioid using mothers or with significant genetic disorders, are unable to breastfeed or have contraindications to sucrose administration, or whose parents are unable to provide written informed consent.

4. RECRUITMENT

This section of the procedure manual addresses how potential research participants will be identified/screened and enrolled. Only authorized team members (as per the IWK Health Centre Research Ethics Board Study Task Delegation Log) should screen potential participants and obtain consent.

4.1 SCREENING

The Primary Investigator (PI) or an authorized team member (as per the study task delegation log) will identify eligible participants by routinely (i.e., daily whenever possible) consulting with the clinical team leader on the Family Newborn Care Unit at the IWK Health Centre. The clinical team leader or designate will identify eligible infants from the FNCU admission log/equivalent source that records all unit admissions using the eligibility criteria stated in section 3.14 of this study procedure manual.

The PI will not require personal health information to facilitate the screening and recruitment process. Rather, the clinical team leader will be provided the eligibility criteria for the study and will screen the list of current admissions on the unit utilizing the criteria. The clinical leader will then provide a list of potential participants' room numbers to the study PI. The study PI will then identify the staff nurse caring for the patients in each of these rooms utilizing the assignment board in the nursing station (which does not provide patient names, but rather the list of staff nurses on shift and the room numbers for the patients they are caring for). The PI will then request the staff nurse caring for a potential participant ask that potential participant if they would allow the PI to approach to discuss a study (e.g., the PI will ask the staff nurse caring for the patient in room 512 bed A). If that potential participant agrees to discuss the study with the PI, then they will be approached.

4.2 ENROLLMENT

When the health care team identifies an eligible infant, the PI will collaborate with the staff nurse caring for that infant and their family to seek permission from the family to discuss the study, will explain the study to the family, and answer any questions.

If the family wishes to take part in the study, the PI will determine the timing of the infant's medically required heel lance, determine the mother's availability to breastfeed during the procedure, and obtain written informed consent as per the criteria outlined below.

Components of Properly Obtained and Documented Consent:

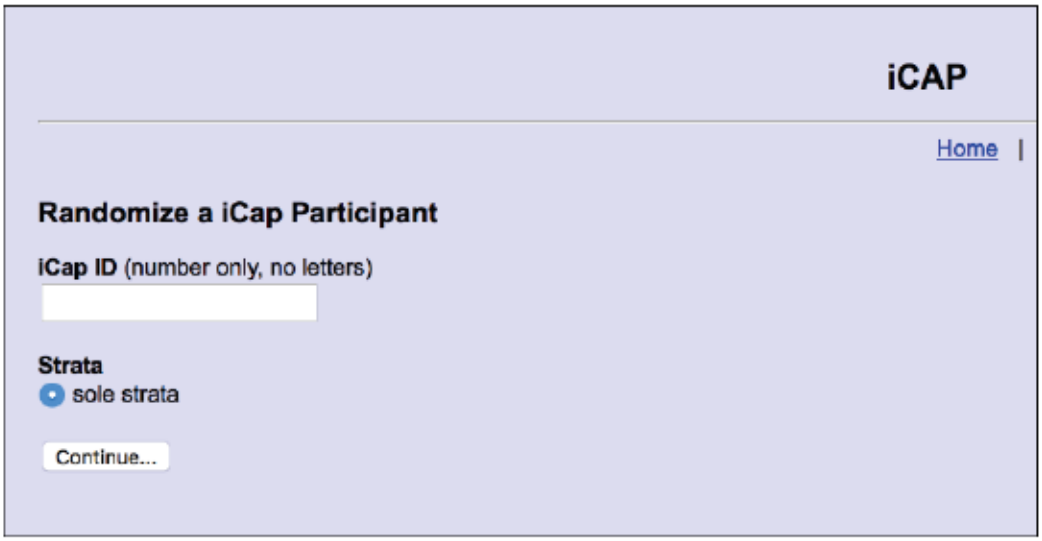
- Full and frank disclosure of all information relevant to free and informed consent (i.e., as laid out in the Research Ethics Board approved ***Consent Form***.
- Being aware when a potential participant is in a dependent relationship to you (i.e., when you are a primary caregiver) and therefore may consent under duress; in such cases consent should be obtained by another authorized team member who is completely independent of this relationship.
- Ensuring potential participants are given adequate opportunities to discuss and contemplate their participation, including opportunity and encouragement to ask questions about the research study.
- Explaining orally what is contained in the consent form as well as providing the written consent form as a description of the research study.
- Determining whether the potential participant is fluent in the language of the consent form or requires an interpreter.

- Being attentive to cultural differences which may lead to unexpected interpretations or unintentional coercion (e.g., in cultures where people unquestionably obey health care professionals).
- Retaining the original signed consent form for the research study files, filling a copy in the patient health record, and providing a copy to the authorized third party who signed it.

4.4 RANDOMIZATION

Once written informed consent is obtained, randomized the baby to one of the two treatment groups by logging in to: https://www.bringinghealthhome.com/iCAP/ with username and password.

Click randomize a participant, enter the iCAP study number (e.g., 001) and click continue.



Documentation of randomization should be entered in the confidential master list in the iCAP study information and consent form binder. A copy of the randomizations to date (downloaded from the menu page of the iCAP randomization website, see above) should be included in the iCAP information and consent form binder (stored in room K8011, IWK Health Centre).

In the event of any issues with the iCAP randomization website, questions can be directed to Shawn Wilson at shawn.wilson@iwk.nshealth.ca or + 1 902 470 7400. Password access to this randomization website can be accessed through the PI (Benoit) or via contacting Shawn Wilson.

4.5 ADDITIONAL ENROLLMENT REQUIREMENTS

- Make a copy of the signed information and consent form for family.
- Place infant hospital sticker, complete relevant information, sign, and scan sucrose order to pharmacy upon enrollment and consent (Refer to section 7 of this manual for information on the sucrose pre-printed order and administration of 24% oral sucrose for study purposes).
- Notify lab regarding blood collection for iCAP study (with mothers initials, room number, blood work time, and baby K#). Call resource lab tech at +1 902 229 4583 if patient enrolled before 14:45 the day prior to blood collection. If patient enrolled in evening, email Heather MacNeil at heather.macneil@iwk.nshealth.ca with information above.
- Notify attending physician or midwife providing newborn care of the infants enrollment and time of collection.

7

4.6 WITHDRAWAL CRITERIA

A parent/authorized third party may choose to withdraw their infant from the study at any time. Likewise, the Principal Investigator, Britney Benoit, and/or study Primary Supervisor, Marsha Campbell-Yeo, may choose to withdraw an infant from the study if there is concern about exposure to an unacceptable level of risk from participating in study procedures. In such cases, the Principal Investigator/study Primary Supervisor: (a) will keep for analysis any information that has already been collected about the infant for the purpose of the study at the consent of the parent/authorized third party.

5. DATA COLLECTION & MANAGEMENT

5.1 REDCap

Study data will be collected and managed using Research Electronic Data Capture (REDCap) software hosted at the IWK Health Centre. REDCap is a secure, web-based application designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources. The Principal Investigator and/or a research assistant at the IWK Health Centre will be responsible for cleaning the electronic data.

REDCap for the iCAP study can be accessed through the following link: https://research-survey.nshealth.ca/redcap_v6.0.18/ProjectSetup/index.php?pid=69&msg=projectmodified

Only authorized members of the study team will have access to this site through the use of a username and password set up through the Principal Investigator. Refer to Appendix A for instructions on how to access REDCap for the iCAP study.

iCAP study information that should be entered into REDCap by the authorized members of the study team pertain to the below listed data collection forms:

1. Study Day Information
2. Feeding Information
3. Adverse Events
4. Breastfeeding Intervention Information
5. Sucrose Intervention Information
6. Procedure Information
7. Infant Chart Review
8. Maternal Chart Review
9. Maternal Acceptability Questionnaire - 24% Sucrose Intervention
10. Maternal Acceptability Questionnaire - Breastfeeding Intervention

5.2 DATA STORAGE AND RETENTION

All information collected for the purpose of this study will be marked with a participant number. The only document that links the participant number to the personal health information and study data will be the original copy of the information and consent form. This information and consent form will be stored under double lock (in a locked filing cabinet in room K8011) at the IWK Health Centre. The information and consent form is stored in a separate filing cabinet from any other participant data.
The personal health information will be kept under double lock (in a locking filing cabinet in a locked office) at the IWK Health Centre. Specifically, it will be kept in the research lab of the study primary supervisor where the study principle investigator is based at the IWK (Room K8011).

Following study closure, Iron Mountain Data storage will be used to store information collected for study purposes. Records will be stored for 10 years past the age of majority of all infants participating. All paper and video materials will be destroyed according to secure Iron Mountain protocols.

6. DATA COLLECTION PROCEDURES

All data collection for this study is being completed with the 128 Channel Geodesic EEG System™ 400 series (Electrical Geodesics Incorporated, Eugene, Oregon, USA). Prior to commencing data collection, it is critical that the use manual for this system is review to familiarize with the equipment and safety requirements for using this system. The user manual can be found using the following link:
https://drive.google.com/file/d/0B388xdH0Vxl2LVJjUmU5WXhzMWM/view.

6.1 MEASURE NEWBORN HEAD CIRCUMFRENCE & DETERMINE NET SIZE

- After obtaining informed consent and enrolling a participant, carefully measure the circumference of the infant's head around the glabella and occipital protuberance (widest part of the head on top of ears and eyebrows). If you have trouble finding the occipital protuberance, you can start at the base of the spine and feel upward for the bump/ledge on the back of the skull.
- Measure from the nasion to the occipital protuberance, the center point of this measurement will be Cz.
  *For babies, you do not need to mark the actual vertex. If you do, tell parents that marker is washable and use a marker that is safe for the infant's skin. Many infant EEG labs will just measure to get an approximation for cap placement, but you can also use circle labels until you get the cap on and then remove prior to initiating data collection.
- Measure from one temporomandibular joint to the other, across the top of the head. Place one end of the measuring tape in front of ear, and use your pinky finger to hold end of tape and thumb to hold tape around top of head so that is does not slide forward (the further forward you are, the less accurate your measurement will be).
  *If the measurement is between 2 sizes, go up a size so not too tight for infants. Cap measurements can be found on the connector box at the end of the cord on each cap (all measurements are in centimeters).

6.2 GATHER NECESSARY MATERIALS

- Hydrocel Geodesic Sensor Net of appropriate size
- Tape measure
- Plastic bag or towel to cover the end of connector
- 1L of warm water
- Control III Test Strips
- Control III disinfectant (concentrated or premixed)
- 2 buckets (electrolyte/rinse, disinfectant)
- Timer
- Clean, dry towels
- Clean sink area
- IWK Health Centre approved baby shampoo
- Sucrose
- Sucrose Medication Administration Record stickers
- Participant study day data collection form
- Heel warmer
- Extra lancets
- Pacifier
- Chart review form
- Maternal acceptability questionnaire

Ensure all GES equipment is operating correctly and prepared for the recording session. Confirm that there is enough disk space for acquisition data. Ensure the infant's head is free of open wounds, clean and dry.

6.3 PREPARE SOLUTION TO SOAK NET IN

- Ensure the electrolyte bucket has been disinfected since it's last use.
- For infants, mix 1L of warm water and 1 tsp baby shampoo with no potassium chloride (scoops are labeled for convenience).
- Ensure electrolyte is prepared and computer is running before you begin next steps.

6.4 PREPARE SOLUTION TO SOAK NET IN

⚠ **CAUTION**: Ensure that all infant is not exposed to any electrode barbs due to missing or loose sponges.

- Make sure to keep cord and connector dry, keep around shoulder, or wrap them in a towel or plastic bag around it with an elastic. The cord can wick moisture up cord and into connector, so be sure to only submerge cap and wires into the electrolyte solution.

SOAK THE NET:

1. Immerse the sensor end of the Net in the electrolyte.

2. Soak net for 5 minutes..
3. Put lid on bucket while soaking Net to prevent Net from slipping in or out of bucket.

⚠ **CAUTION:** to prevent damage to the Net's cable and connector, at all times: ensure that no liquid drips down the cable to the connector and hold the connector high above the Net while handling.

- After 5 minutes, remove the Net from the electrolyte bucket
- Shake net and gently tap net against sides of bucket to remove excess electrolyte.
- Blot the excess electrolyte from the outside of the Net with a towel to prevent liquid from 1) dripping in the infant's eyes, 2) dripping into the infant's clothing or bedding (especially behind the neck, shoulders and back), which can cause temperature instability and 3) increasing the chance of bridging between sensors.

  *Leads will start to dry out after about 1 hour, they can be re-wet with a pipette.

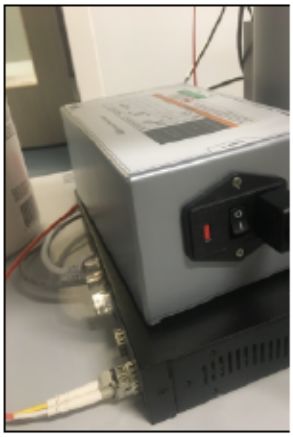
6.5 POWER ON EGI SYSTEM

1. Plug main grey power cord into main power source, ensuring that main power cord is not posing a tripping hazard to the patient or clinical/research staff.
2. Turn on a) main power supply at equipment base (green power light will turn on) and then turn on b) GES external power supply box (NOTE: straight line - power on. At this time, the green lights on the left side of the c) amplifier at the back of the tower should be blinking.
3. Power on iMac (power button on back of screen, left side).

1. **Main power cord**

a) Main power supply

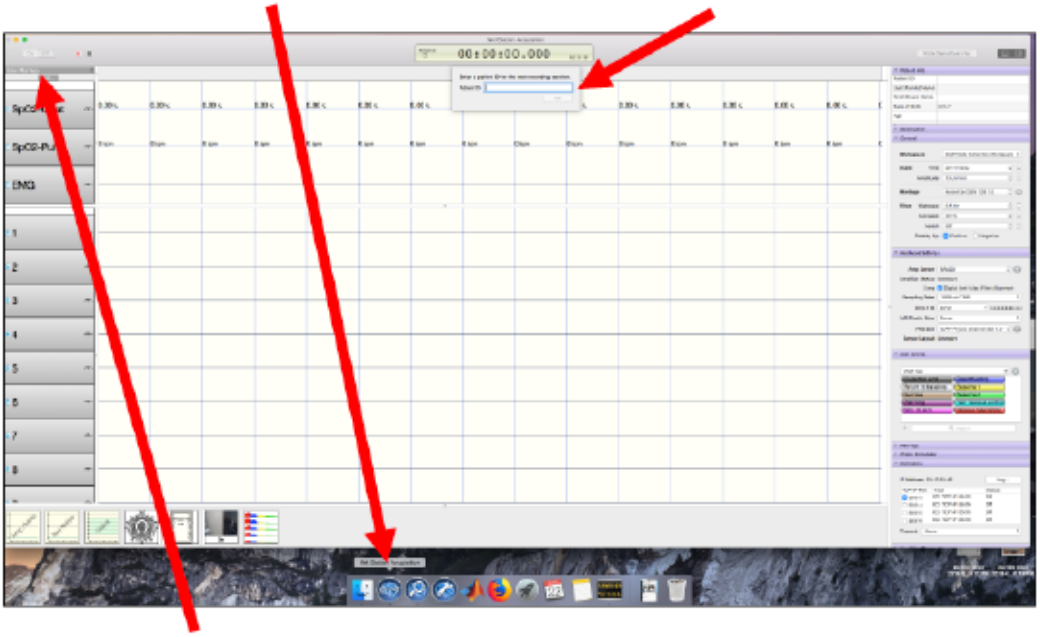
c) Amplifier "on"



b) External power supply

6.6 LOGIN TO EGI SYSTEM & START NETSTATION ACQUISITION

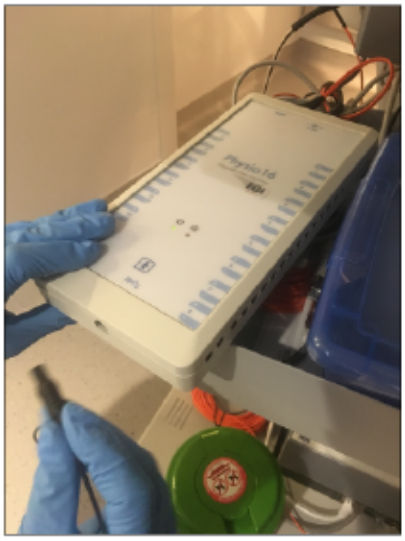
1. Log in to "EGI" on computer main screen using password: ***Geodesic***
2. After logging in, move mouse to hover at bottom of main screen and click on NetStation Acquisition, acquisition will open.
3. Enter patient ID to progress to recording (e.g., iCAP_001)



4. Press "**On**" on top left corner of NetStation Acquisition screen to start initialization (**NOTE**: this does not start recording, but starts the initialization of the amplifier. You will need to press record after setting up video, EEG, and SpO2/heart rate monitoring equipment).

6.7 SET UP SPO2/HEART RATE MONITORING & VIDEO EQUIPMENT

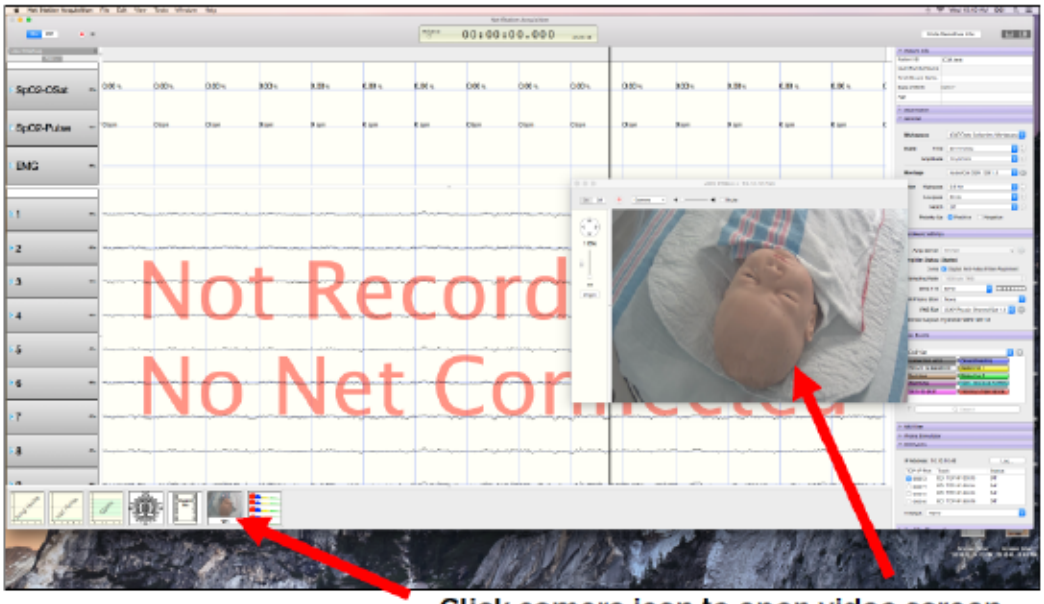
1. With baby positioned comfortably and bundled in the cot/incubator, plug the NONIN 8001JFW Neonate sat probe into the Physio 16 and secure on baby's wrist (with the sat probe light and sensor on opposite side well-aligned on either side of the wrist) using the NONIN Flexi-wrap stickers (pictured) or the appropriate wraps from the unit supply carts. You will need to verify that the heart rate and oxygen saturation are picking up on NetStation acquisition and adjust the sat probe as needed to ensure a good signal.



Sat probe port on Physio 16

NONIN Neonatal Sensory (8001JFW) Flexi-wrap for securing neonatal sensor

2. Click on camera icon on bottom left of NetStation Acquisition and adjust camera to ensure that you are able to visualize the baby's face. It is critical that you are able to see the baby's brow, eyes, and cheeks



Click camera icon to open video screen

6.8 PLUG IN THE NET

- To ensure that the Net does not present a risk to the infant or risk electrolyte dripping or flowing back to the connector, it is plugged into the Net Amps amplifier or articulated arm before applying it to the patient. **You may wish to ask a second person to plug in the connector while the other holds the Net.

⚠ **WARNING:** Ensure the Net's connector and cable never come into contact with infant: never position the Net's connector or cable over infants to avoid physical injury if dropped or entanglement and confirm that the Net's connector is securely seated and locked in place to avoid physical injury if pulled loose.

- Plug the Net's connector into the articulated arm: The connector is easy insertion, if it feels like its resisting, pull back and check to see if something is out of place. Then turn lock pin 180 degrees clockwise to lock connection (to detach lever, turn lock pin counter clockwise).

6.9 APPLY THE NET

'Net application requires two qualified staff - One to support the infant's neck and one to apply the EEG net

- Identify nasion sensor at the center of front of net.
- Count 2 sensors away from the nasion sensor on either side and hook your thumbs under the double lines of elastomer.
- Hold net at 180 degrees while positioning thumbs, this allows you to extend your pinkies to the back of the Net more easily later.
- Take chin strap off of wrists and place over the back of net so it does not hit the infant's face during application.
- Position your pinkie fingers in the bottom back row of the Net, opposite to your thumbs at the front of the Net.
- Ensure electrodes are all in correct orientation: If you can see the sponges, the electrode is inside out.
- Expand the Net enough for it to easily fit over the infant's head without dragging across the scalp.
- Face the infant to apply the Net: The goal is to put the Net on symmetrically, standing to the side may result in crooked application.
- Support the infant's head and neck.
- Gently lower hands until the Net surrounds the infant's head.
- Place electrode CZ on the vertex you identified during measurement.
- Gently fasten chinstrap to secure Net while maintaining tension.
  - Hold all chin straps close to the end of the strap to maintain tension across the Net and adjust large central white bead to the appropriate tightness below the infant's chin.
- If needed, place a pad of gauze between chin and the chinstrap for comfort.

DO AN INITIAL POSITION CHECK: CZ should be aligned, ears in ear holes, front electrodes in line with brow bone, and back electrodes in a straight line across nape of neck and tight enough to the head. RM and LM should be on the right and left mastoid bone, respectively. Adjusting the chin straps can help facilitate this positioning. Electrode 17 should be as close to the nasion as possible. Electrodes can be within 0.5 cm off of correct point and still get a proper read.

CHIN STRAPS ADJUSTMENTS

- **Blue bead**: holds tension across the top of the head (either side goes through center cord lock).
- **White bead**: holds tension around the back of the neck (either side goes through side cord lock).

To adjust chin straps, hold the non-adjusting beads with one hand, and then use other hand to pull the strap you wish to adjust. Pull first through side cord lock, then through the bottom part with the bead.

6.10 ADJUST THE NET

- Always move the sensors by lifting them away from the scalp and pulling them to protect the infant's fragile skin. To adjust, grasp groups of sensors, lift and move together, do not move the Net by individual sensors.
- Ensure the Net is positioned symmetrically on the head, the Net's midline is straight, and the ear holes are correctly surrounding the ears.
- Use a pipette to move hairs out from under the leads, which can interfere with impedance. Lift each electrode individually, and gently use end of pipette to brush hair away. Be careful to not scratch the scalp. Can work from inside outward or go number by number to be thorough.
- Adjusting the Net properly can take 15-30 minutes.

6.11 CHECK DATA QUALITY AND SENSORY IMPEDENCES

Within NetStation Acquisition:

- Check data by clicking "Stream On" and scrolling through the EEG waveforms
  - Look for noise or anything that looks out of place, could be a dry lead or muscle tension.
  - A perfect signal looks very calm, almost like straight lines.
  - Stepping on the power cord, leads making improper contact, or leads that are touching can produce heavy black EEG readings.
- Check the impedance values by clicking the Impedance view button.
- Set the Impedance value to 100 kΩ, unless advised otherwise:

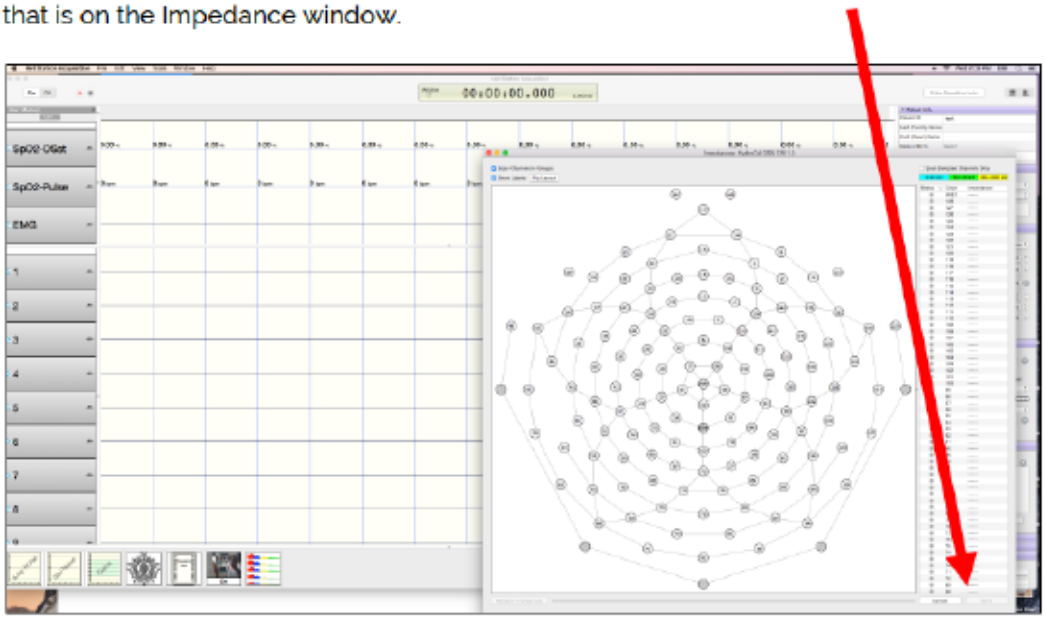
  Anything **blue** is **great**
  Green is okay but **can be lower**
  Yellow or red **needs to be fixed**

  **When working with infant's look at level of impedance for yellow electrodes. It is more important to fix electrodes that are further from 100 than those closer to it as infants may grow impatient.

- If the impedance is too high for an electrode, use a pipette full of electrolyte to re-wet the electrode.
  - The useful life of electrodes is 3-5 years.
  - If impedance remains too high, the sponge or wire may need to be replaced.

- Make sure most impedances are below the threshold and that they do not vary greatly between sensors.
  **Note: Hair thickness, cleanliness, and skin texture can affect impedance.

Save the impedance values in the Net station session by clicking the **"Save"** button that is on the Impedance window.



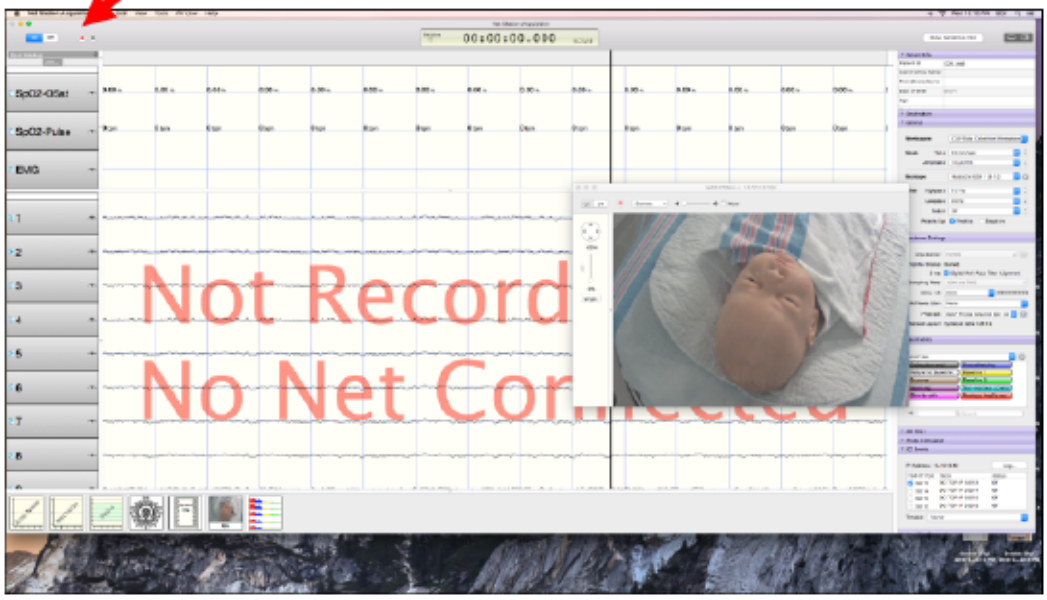
⚠ **CAUTION:** It is ***essential*** that you ***close the impedance window after saving the impedance values*** so that the impedance measuring signal does not override the EEG data during recording

For any sensors that cannot be fixed, mark those channels as bad within Net Station and note them in your logbook.

6.12 START RECORDING & COMPLETE STUDY PROCEDURES

Start recording data by clicking Record on the dashboard and monitor the infant closely during the recording. Use event buttons to enter events where and when necessary. Stop recording by clicking "Stop" on the dashboard and click "Stream Off" to stop streaming data.

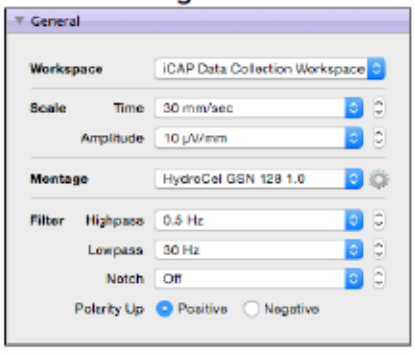
Click **Red** button to start recording, then entire acquisition screen will turn red.
Do not pause recording at any point during data collection
(only at collection end).



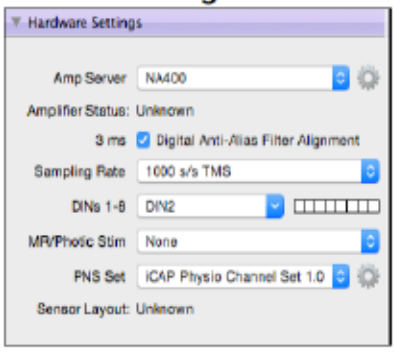
NOTE: It is important to continuously monitor the acquisition screen during data collection. Ensure the baby's entire face is visible in the video screen, and that the physiologic data (heart rate, SpO2, EEG) are picking up well. The entire acquisition screen should appear **RED** the entire time that data is being recorded.

****THE FOLLOWING SETTINGS MUST BE MAINTAINED** on NetStation Acquisition:

General settings:

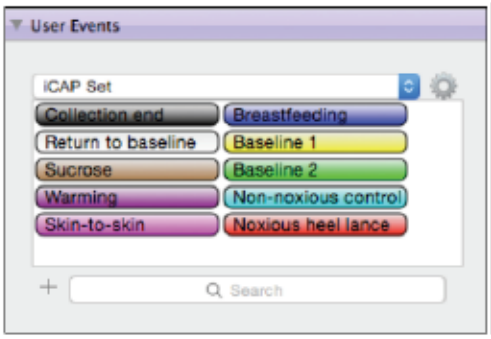


Hardware settings:

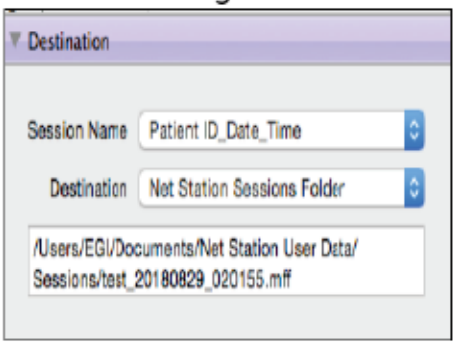


19

User event settings:



Destination settings:



6.13 COMPLETE STUDY RECORDING & INTERVENTION PROCEDURES

The measurement of the main study outcomes will rely on five data collection strategies:

1. Continuous neonatal EEG recording
2. Close-up video recording of infant facial actions
3. Pulse oximeter monitoring of heart rate and oxygen saturation
4. Observation of the effectiveness of the breastfeeding session
5. Monitoring for adverse events
6. Chart review

Collection of data related to the outcomes measures of this study will take place around a medically required heel lance that all infants undergo to collect blood for routine metabolic screening. The heel lance was selected as the pain stimulus as it is a procedure that all infants will be exposed to in the neonatal period at least once.

⚠ **CAUTION:** Before commencing data collection procedures, **measure infants axillary temperature to ensure normothermia**. If the infant temperature is < 36.5 degrees Celsius, place infant in skin-to-skin contact with mother/care giver and monitor temperature every 15 minutes ensure temperature is normal. In the event that infant does not sustain a temperature > / - 36.5 degrees Celsius, infant should have not have geodesic sensor net applied.

BREASTFEEDING CONDITION

After starting recording on NetStation Acquisition, data collection takes place as follows:

1. **Baseline 1 (BL1)**: Complete continuous recording of a two-minute baseline of all outcome measures (EEG, HR, O2 saturation, facial actions) while the infant is resting in a cot wearing only a diaper and contained in a blanket. Manually mark the start of BL1 with the user even 'Baseline 1' and document the start time of the BL1, infant heart rate, and oxygen saturation on the Study Day Phase Marker Form (below).
2. **Non-noxious control stimulus 1 (NN1)**: Apply the non-noxious (NN1) control

stimulus to the infant's foot to capture a baseline response on EEG to a non-painful event. This non-noxious (sham) stimulus consists of placing the heel lance against the foot and rotating it 90 degrees, so that when the lance is released it mimics the sensation of the heel lance procedure without the associated tissue-breaking and pain. Two research team members are needed to apply the NN1 stimulus:

   i. ***Team member 1:*** Applies the stimulus to the foot and releases the lancet.
   ii. ***Team member 2:*** Turn on the microphone used to time lock the NN1 stimulus to the EEG recording, hold the microphone close to the newborns foot where the lancet will be released, and manually mark the timing of the NN1 release using the 'Non-noxious control' user event. The second research team member should additionally document the approximate timing of the NN1 stimulus on the Study Day Phase Marker Form. For a more detailed description of the microphone set-up used to time lock the lancet to the continuous EEG recording see Appendix G.

3. **Skin-to-skin contact initiation (SSC):** Following completion of NN1, transfer the infant to ventral skin-to-skin contact with the mother at least five minutes prior to heel lance to allow time to settle and initiate breastfeeding. Infant should have full ventral SSC with mother, however, should be positioned to enable infant access to the breast in order to initiate breastfeeding. It is important to additionally ensure that the infant feet are available to the lab staff to complete the heel lance procedure.
4. **Baseline 2 (BL2):** Complete continuous recording of a second two-minute baseline of all outcome measures (EEG, HR, O2 saturation, facial actions) while the infant is resting in SSC. Manually mark the start of BL2 with the user even 'Baseline 2' and document the start time of the BL2, infant heart rate, and oxygen saturation on the Study Day Phase Marker Form (below).
5. **Breastfeeding:** Support mother to initiate breastfeeding a minimum of two minutes prior to the heel lance procedure. Ensure mother is positioned comfortably and support infant to be latched to the breast. Observe for active sucking and swallowing and document LATCH score for entire breastfeeding session following completion of the heel lance. Ensure the babies feet are accessible to the lab staff and bed is raised to enable ease of blood collection.
6. **Non-noxious control stimulus 2 (NN2):** Repeat NN control stimulus in same procedure as detailed above while infant in SSC.
7. **Heel lance:** Support mother to breastfeeding throughout the heel lance. Time-lock the heel lance procedure (completed by lab staff) to the EEG recording using the micro-phone audio time-locking method detailed for the NN1 And NN2 stimuli. Manually mark in recording using the 'Noxious heel lance' user event and document heart rate and oxygen saturation on phase marker sheet.
8. **Recovery:** Following completion of heel lance, continue recording for a full 5-minutes or until heart rate returns to baseline values.

BREASTFEEDING PARTICIPANT ID: _____

TEMP 1:_____ TEMP 2:_____ TEMP 3:_____

BASELINE 1 (2 minutes): _____ HR:_____ O_2: _____

NN CONTROL: _____

SSC INITIATED (5 minutes): _____

BASELINE 2 (2 minutes):_____ HR:_____ O_2: _____

WARM (5 minutes): _____

BREASTFEEDING INITIATED (2 minutes): _____

NN CONTROL: _____

HEEL LANCE: _____ HR:_____ O_2: _____

RECORD TO RECOVERY (5 minutes minimum)

START RECOVERY: _____ HR:_____ O_2: _____

END RECOVERY:_____ HR:_____ O_2: _____

NOTES:

24% ORAL SUCROSE CONDITION

All procedures in the sucrose condition are completed in the same manner as the breastfeeding condition (above) with the exception that the infant remains in their cot for the duration of the procedure. Ensure infant in contained in a blanket. Administer 0.24mL of 24% oral sucrose 2-minutes prior to heel lance procedure (as per study specific pre-printed order, see section 7.3 of this procedure manual). Offer a pacifier or gloved finger for non-nutritive sucking immediately following sucrose administration and documented if it is sustained.

SUCROSE PARTICIPANT ID: _____

TEMP 1:_____ TEMP 2:_____ TEMP 3:_____

BASELINE 1 (2 minutes): _____ HR:_____ O_2: _____

NN CONTROL: _____

BASELINE 2 (2 minutes):_____ HR:_____ O_2: _____

WARM (5 minutes): _____

SUCROSE (2 minutes pre-procedure): _____
(0.23 mls; 6 drops)

NN CONTROL: _____

HEEL LANCE: _____ HR:_____ O_2: _____

RECORD TO RECOVERY (5 minutes minimum)

START RECOVERY: _____ HR:_____ O_2: _____

END RECOVERY:_____ HR:_____ O_2: _____

NOTES:

6.14 NET REMOVAL

As much care is needed for the infant and Net after an acquisition session as was needed prior to it to ensure infant well-being and the Net's condition:

- Completely loosen the Net's chinstraps and release center cord lock all the way to the bottom.
- Lift the Net's chinstraps up and away from the infant's face and loop them back over the head away from the infant's eyes.
- Gently remove the gauze padding if it was used under the chinstrap.
- Using a similar open, fanned hand position as you did when applying the net, work outward and upward to slowly lift Net off of infant's head, untangling hair as you go (net will be inside out when completely removed).

CARE FOR THE INFANT: Follow your facility's protocols for caring for the infant after the EEG exam including:

- Gently drying the infant's head.
- Gently cover the infant's head with an appropriate, clean, dry knit cap.

6.15 NET CLEANING

Follow these instructions to prevent skin irritation, skin infection, or net damage. In general, remember to:

- Rinse and disinfect Nets immediately after use.
- To prevent water damage to the connector, keep the connector high about the Net throughout this entire process so that no liquid or drips contact or travel to the cable sleeve or the connector (until the net is hung to dry, it is recommended a bag is kept over the connector).
- Disinfect all reusable supplies, including buckets, between patients.

1. RINSE ELECTROLYTE OUT OF NET

While keeping the connector high above the Net to protect the cable sleeve and connector from getting wet, do the following:

- Keep Net inside out.
- Fill the electrolyte/rinse bucket (the first bucket) with clean, warm (not hot) tap water.
- Immerse sensor end of the Net in the water.
- Gently, but vigorously, agitate the Net back and forth and up and down for 10-20 seconds while:
  - Keep water moving through the net and sponges for the entire 10-20 seconds (hold Net by the large cord at the base of the Net for this process and be careful not to grasp the Net in such a way that you pinch or damage any wires).
- Raise Net out of bucket.
- Drain and rinse bucket.
- Repeat the above steps until you have rinsed the Net a total of **FOUR TIMES**.

- After thoroughly rinsing the electrolyte out of the Net, gently pat the excess water out of the Net with a clean, dry towel.

⚠ **CAUTION**: protective gloves and goggles should be worn for the disinfecting portion of Net Cleaning.

2. DISINFECT THE NET

While keeping the connector high above the Net to protect the cable sleeve and connector from getting wet, do the following:

- If not already prepared, prepare 2 liters of Control III disinfectant in the disinfectant bucket (the second bucket).

INSTRUCTIONS FOR TESTING & MIXING DISINFECTANT

- Before each use, use the Control III test strips to verify the efficacy of the disinfectant:
  - If the test succeeds, proceed to the next step.
  - If the test fails, drain, rinse, and disinfect bucket (according to all instructions under "Net Cleaning"), and then prepare a fresh batch of disinfectant.
- Set timer for 10 minutes.
- Immerse the sensor end of the Net in the disinfectant
- For the first 2-3 minutes, and without submerging your hands in the disinfectant, gently and repeatedly plunge the Net up and down to ensure a thorough disinfection.
- Leave Net soaking for the remainder of the 10 minutes.
  - Keep the timer with you so that you do not leave the Net in the disinfectant for longer than that (prolonged submersion in the disinfectant may damage the Net and void the warranty).
- At 10 minutes, remove the Net from the disinfectant.

3. RINSE THE DISINFECTANT OUT OF THE NET

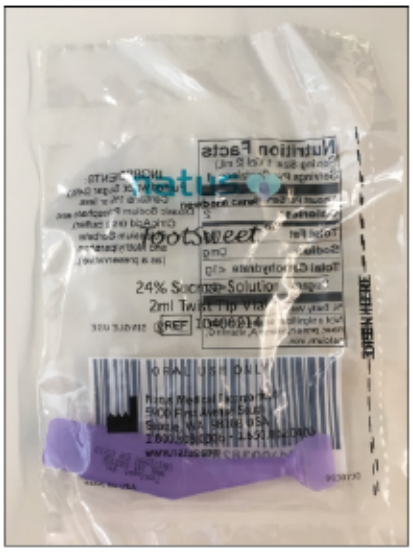
Using the disinfectant/rinse bucket (the first bucket), repeat all steps in Part 1: Rinse the electrolyte out of the Net.

Hang the Net inside out by its connector to dry and store safely until next use.

7. 24% ORAL SUCROSE

7.1 STORAGE & PREPARATION

A designated supply of 2ml 24% oral sucrose solution twist tip vials are stocked for the purpose of this study. The study sucrose should be stored at room temperature (15 to 25 degrees Celsius), no refrigeration required. No study specific preparations will be required prior to administering the sucrose.



Tootsweet 24% oral sucrose vial

Tootsweet 24% oral sucrose box

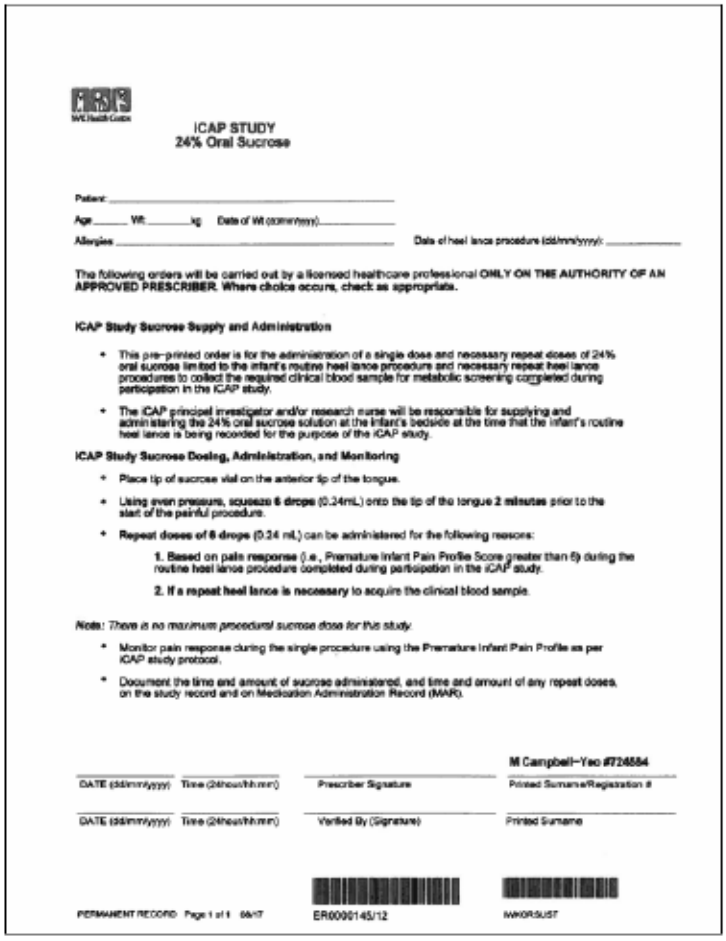
****NOTE/CAUTION**: *Current 24% oral sucrose expires* ***MARCH 2019***

7.2 INDICATION

24% oral sucrose administration is limited to the initial dose and necessary rescue doses of 24% oral sucrose limited to a single heel lance procedure and necessary repeat heel lance procedures to collect the required clinical blood sample completed during participation in the iCAP study.

7.3 ADMINISTRATION

Place tip of sucrose vial on the anterior tip of the tongue. Using even pressure, squeeze 6 drops (0.24ml) onto the tip of the tongue 2 minutes prior to the start of the painful procedure. Repeat (rescue) doses of 6 drops (0.24 ml) as needed based on pain response (i.e., Premature Infant Pain Profile Score > 6) during the single heel lance procedure completed during participation in the iCAP study. In the event that a repeat heel lance is necessary to acquire the clinical blood sample, repeat administration of 3 drops (0.24ml) onto the tip of the tongue 2 minutes prior to the start of the painful procedure ***Note: There is no maximum procedural sucrose dose for this study***. See iCAP 24% oral sucrose pre-printed order for further administration information below.



ICAP STUDY
24% Oral Sucrose

Patient: _____

Age _____ Wt: _____kg Date of Wt (dd/mm/yyyy)_____

Allergies: _____ Date of heel lance procedure (dd/mm/yyyy): _____

The following orders will be carried out by a licensed healthcare professional **ONLY ON THE AUTHORITY OF AN APPROVED PRESCRIBER. Where choice occurs, check as appropriate.**

ICAP Study Sucrose Supply and Administration

- This pre-printed order is for the administration of a single dose and necessary repeat doses of 24% oral sucrose limited to the infant's routine heel lance procedure and necessary repeat heel lance procedures to collect the required clinical blood sample for metabolic screening completed during participation in the ICAP study.
- The ICAP principal investigator and/or research nurse will be responsible for supplying and administering the 24% oral sucrose solution at the infant's bedside at the time that the infant's routine heel lance is being recorded for the purpose of the ICAP study.

ICAP Study Sucrose Dosing, Administration, and Monitoring

- Place tip of sucrose vial on the anterior tip of the tongue.
- Using **even pressure, squeeze 6 drops** (0.24mL) onto the tip of the tongue **2 minutes** prior to the start of the painful procedure.
- **Repeat doses of 6 drops** (0.24 mL) can be administered for the following reasons:
  1. **Based on pain response** (i.e., Premature Infant Pain Profile Score greater than 6) during the routine heel lance procedure completed during participation in the ICAP study.
  2. **If a repeat heel lance is necessary** to acquire the clinical blood sample.

Note: There is no maximum procedural sucrose dose for this study.

- Monitor pain response during the single procedure using the Premature Infant Pain Profile as per ICAP study protocol.
- Document the time and amount of sucrose administered, and time and amount of any repeat doses, on the study record and on Medication Administration Record (MAR).

M Campbell-Yeo #724584

DATE (dd/mm/yyyy) Time (24hour/hh:mm) Prescriber Signature Printed Surname/Registration #

DATE (dd/mm/yyyy) Time (24hour/hh:mm) Verified By (Signature) Printed Surname

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7.4 DOCUMENTATION

If the signed 24% oral sucrose pre-printed order (above) is completed and scanned down to pharmacy the day prior to collection, the medication will appear on the infants Medication Administration Record and the administration of the sucrose can be signed off on this record. If the collection occurs the day that participant is enrolled, the pre-printed order should still be scanned down to pharmacy. However, the label below should be placed on the infant Medication Administration Record in the infant chart on the Family Newborn Care Unit and the medication should be signed off on by the individual who administered it.

iCAP STUDY REB#: 1021795
PI: Britney Benoit **Site Name:** IWK Health Centre
Investigational Agent: Sucrose 24% Oral Solution 1mL Twist Tip Vial
Manufacturer: Natus Medical **Lot#:** 17889 **Expiry:** 07/2018
Store at 15-25˚C

Study dose: Dose based on infant weight:
2500 grams – 5000 grams up to 50 drops [2 mL]
> 5000 grams up to 63 drops [2.5 mL]

Peel this label off and attach to the infant Medication Administration Record

8. ADVERSE EVENTS

8.1 ANTICIPATED ADVERSE EVENTS

Monitoring for adverse events for all infants enrolled in the study will involve documentation of any incidence of

1. **Choking, apnea** (defined as unexplained cessation of breathing for 20 seconds or longer, or a shorter respiratory pause associated with bradycardia, cyanosis, pallor, and/or hypotonia; American Academy of Pediatrics Committee on Fetus and Newborn, 2003), or **bradycardia** (defined as a 30 beat per minute drop from baseline, or when the heart rate is below 100 beats per minute; Canadian Paediatric Society, 2016) following the initiation of either intervention condition (i.e., initiation of breastfeeding or administration of 24 percent oral sucrose and offered non-nutritive sucking).
2. **Need for a repeat heel lance** to allow for sufficient blood collection and administration of additional doses of "rescue" sucrose will also be recorded.
3. **Hypothermia**. As the HydroCel Geodesic Sensor Net requires that electrode sponges are soaked in a warm saline solution prior to application to the infant scalp, infant axillary temperature will be monitored every fifteen minutes throughout data collection to ensure normothermia (36.5 to 37.5 degrees centigrade). Infants will be monitored constantly during data collection in order to prevent any risk of choking or skin irritation while wearing the HydroCel Geodesic Sensor Net. The PI and/or assisting research coordinator or assistant will check for skin pressure points, overturned sensors, or redness under the chin strap every fifteen minutes to maximize infant comfort (Electrical Geodesics, Inc., 2007).

8.2 DOCUMENTING ADVERSE EVENTS & SAFETY MONITORING BOARD

The doctoral supervisory committee will serve as the safety monitoring board for this study. The study PI (Benoit) will provide monthly reports of all collected safety data to the supervisory committee for review, and suspected unexpected serious adverse reactions will be reported to the entire supervisory committee and the IWK Health Centre Research Ethics Boards.

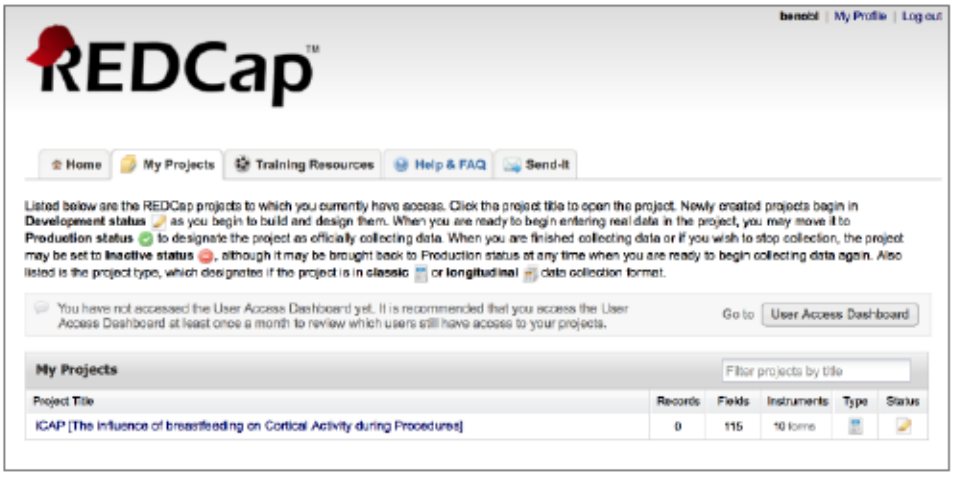
APPENDIX F – iCAP REDCap ENTRY

REDCap Login Screen accessible at: https://research-survey.nshealth.ca/redcap_v7.4.18/ProjectSetup/index.php?pid=69&msg=projectmodified

To enter study data into REDCap, complete the following steps:

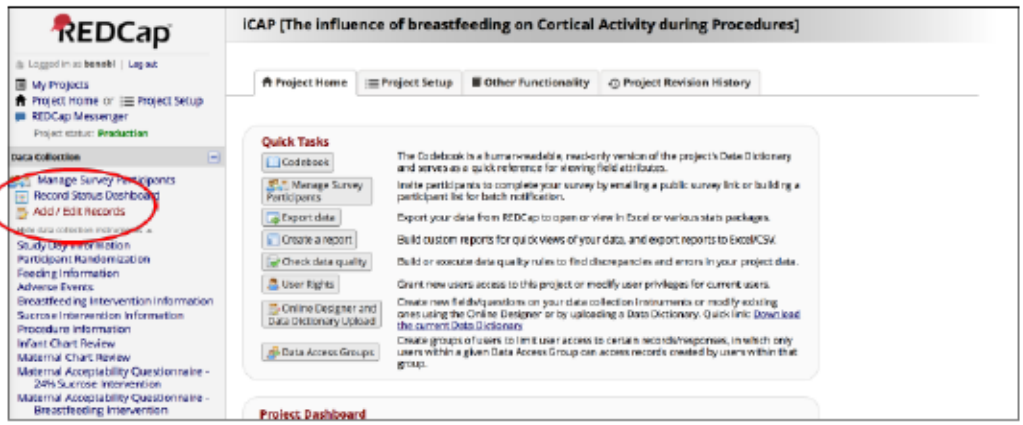
1. **Select "My Projects" tab on the main REDCap home screen**

2. **Select study iCAP [The influence of breastfeeding on Cortical Activity during Procedures]**



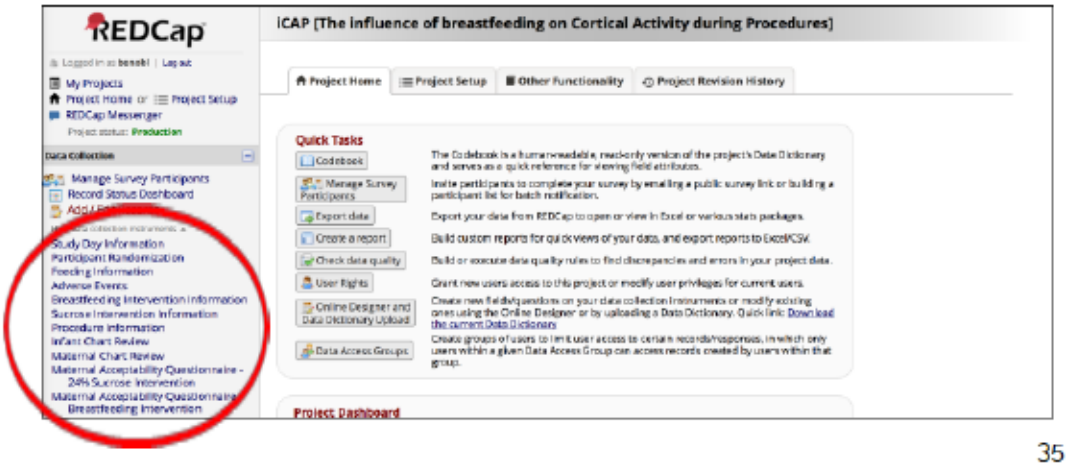
34

3. On the left navigation bar, click on "Add/Edit Records".



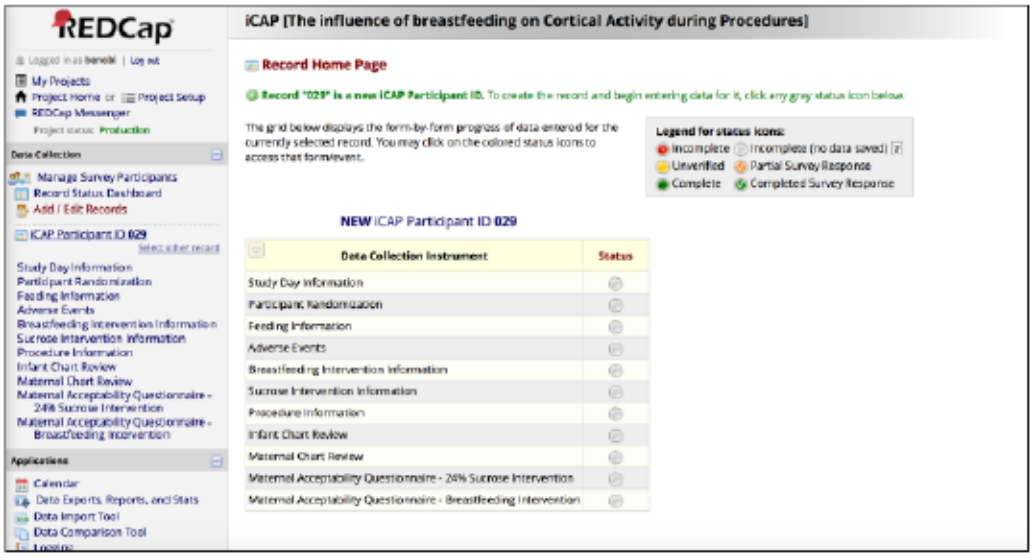
4. Enter a new iCAP Participant ID to input data for the first time. To edit data entered previously, choose an existing iCAP Participant ID.

5. In the left-hand column of the "Event Grid" under "Data Collection Instrument", there are data forms listed:



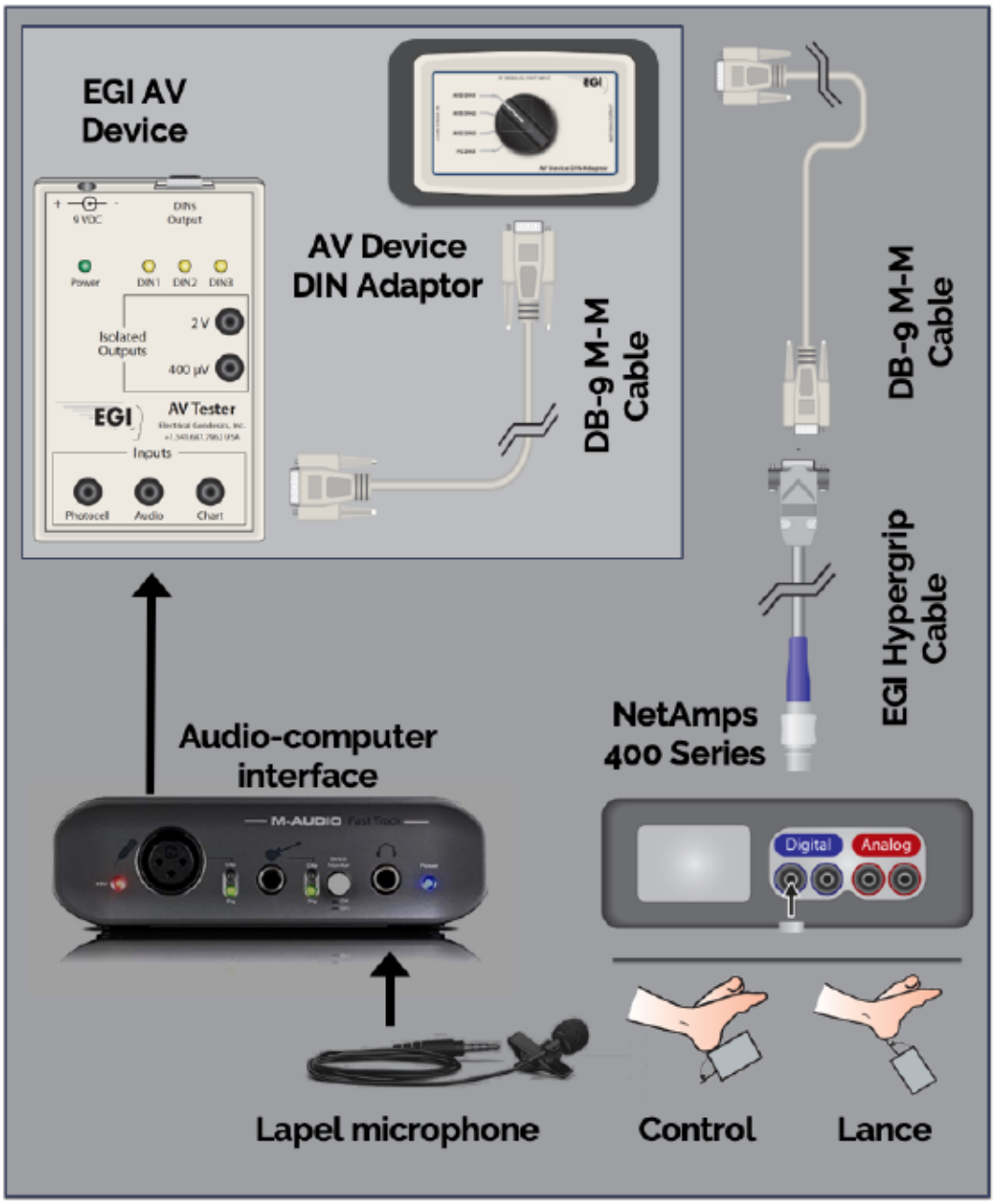
35

6. **Select the applicable circle/status icon beside one of the forms, to enter data for that form.**



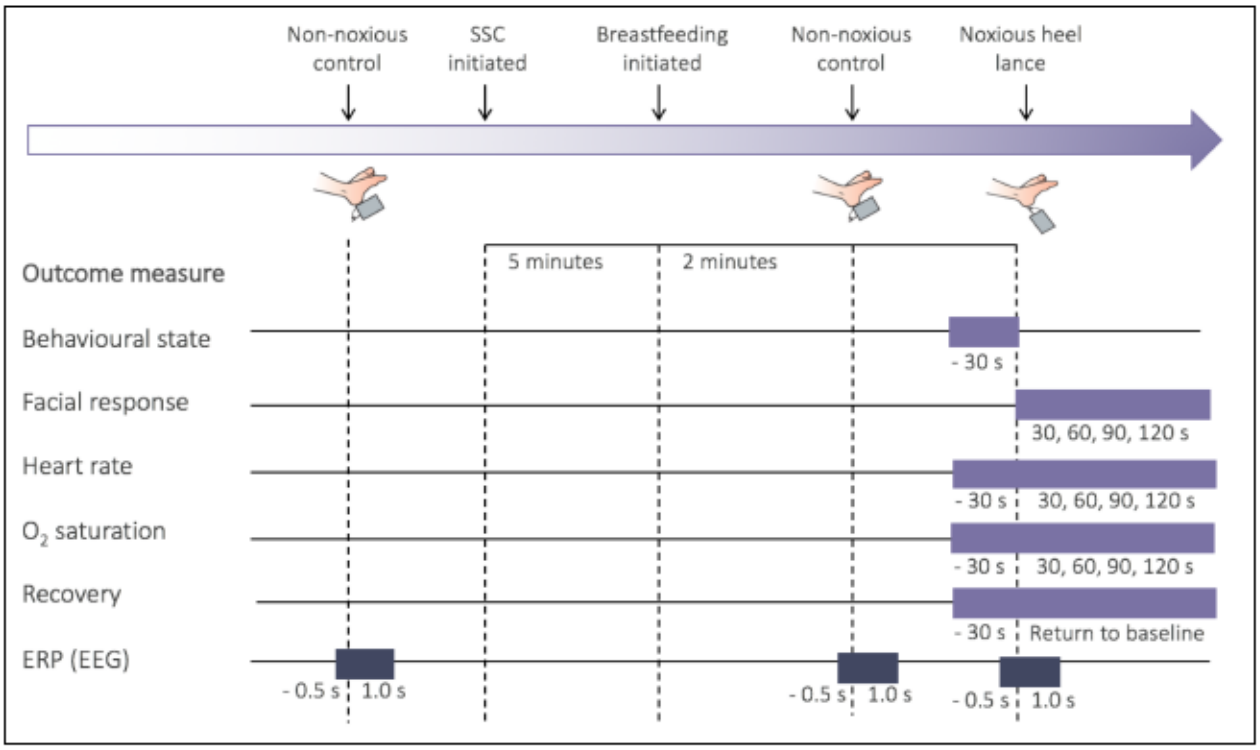
Appendix H

Equipment Set-up for Time-locking Clinically Required Heel Lance Procedure to Continuous Electroencephalogram Recording



Appendix I

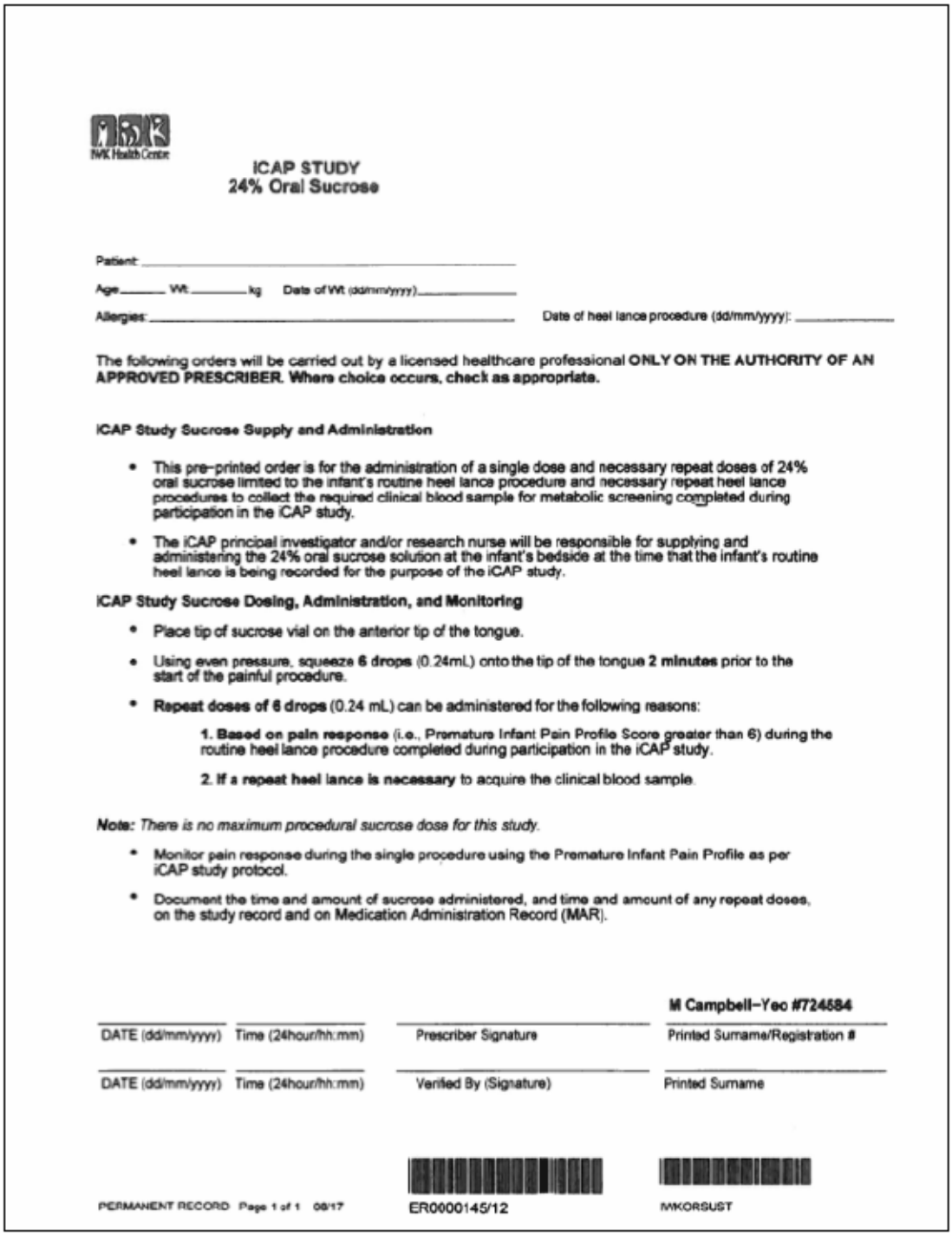
Experimental Timeline for Infants Randomized to the Breastfeeding Intervention



Note. Adapted from: Slater, R., Cornelissen, L., Fabrizi, L., Patten, D., Yoxen, J., Worley, A., . . . Fitzgerald, M. (2010). Oral sucrose as an analgesic drug for procedural pain in newborn infants: A randomized controlled trial. *Lancet, 376*(9748), 1225-1232.

Appendix J

iCAP Study Policy for Administration of 24% Oral Sucrose to Infants



IWK Health Centre

ICAP STUDY
24% Oral Sucrose

Patient: _____

Age _____ Wt: _____ kg Date of Wt (dd/mm/yyyy) _____

Allergies: _____ Date of heel lance procedure (dd/mm/yyyy): _____

The following orders will be carried out by a licensed healthcare professional **ONLY ON THE AUTHORITY OF AN APPROVED PRESCRIBER. Where choice occurs, check as appropriate.**

iCAP Study Sucrose Supply and Administration

- This pre-printed order is for the administration of a single dose and necessary repeat doses of 24% oral sucrose limited to the infant's routine heel lance procedure and necessary repeat heel lance procedures to collect the required clinical blood sample for metabolic screening completed during participation in the iCAP study.
- The iCAP principal investigator and/or research nurse will be responsible for supplying and administering the 24% oral sucrose solution at the infant's bedside at the time that the infant's routine heel lance is being recorded for the purpose of the iCAP study.

iCAP Study Sucrose Dosing, Administration, and Monitoring

- Place tip of sucrose vial on the anterior tip of the tongue.
- Using even pressure, squeeze **6 drops** (0.24mL) onto the tip of the tongue **2 minutes** prior to the start of the painful procedure.
- **Repeat doses of 6 drops** (0.24 mL) can be administered for the following reasons:

  1. Based on pain response (i.e., Premature Infant Pain Profile Score greater than 6) during the routine heel lance procedure completed during participation in the iCAP study.

  2. If a repeat heel lance is necessary to acquire the clinical blood sample.

Note: *There is no maximum procedural sucrose dose for this study.*

- Monitor pain response during the single procedure using the Premature Infant Pain Profile as per iCAP study protocol.
- Document the time and amount of sucrose administered, and time and amount of any repeat doses, on the study record and on Medication Administration Record (MAR).

M Campbell-Yeo #724584

DATE (dd/mm/yyyy) Time (24hour/hh:mm) Prescriber Signature Printed Surname/Registration #

DATE (dd/mm/yyyy) Time (24hour/hh:mm) Verified By (Signature) Printed Surname

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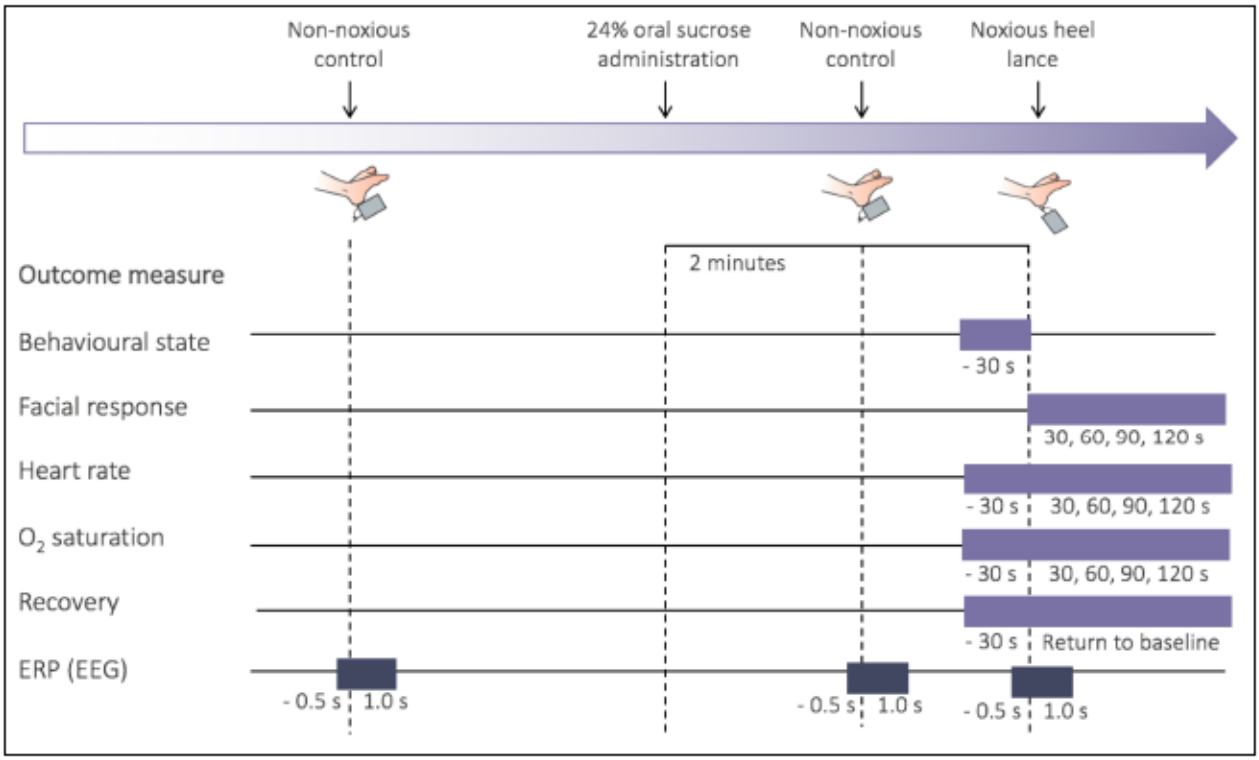
Appendix K

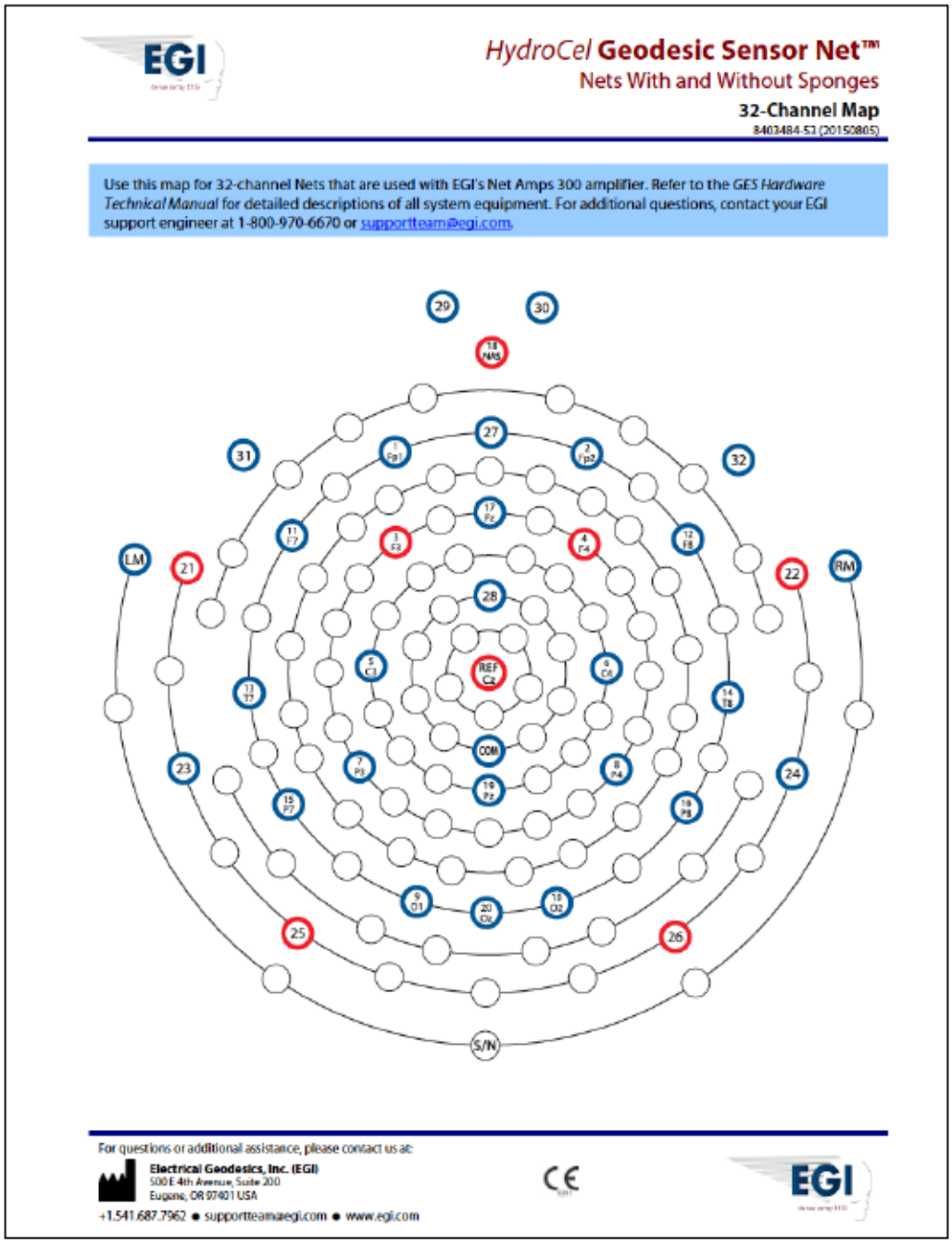
Experimental Timeline for Infants Randomized to the 24% Oral Sucrose and Offered Non-nutritive Sucking Intervention



Note. Adapted from Slater, R., Cornelissen, L., Fabrizi, L., Patten, D., Yoxen, J., Worley, A., . . . Fitzgerald, M. (2010). Oral sucrose as an analgesic drug for procedural pain in newborn infants: A randomized controlled trial. *Lancet, 376*(9748), 1225-1232.

Appendix L

Electrical Geodesic Inc. Hydrocel Geodesic Sensor Net 32-Channel Electrode Map



Appendix M

Pain Assessment in Neonates (PAiN) Software and Coding Manual

Pain Assessment in Neonates

User Manuals, updated December 2018

For more information, contact us at info@novumscientific.ca

PAiN User Manual

Getting Started

Installation:

- On macOS, click on the .dmg file to open it, then drag the *PAiN.app* file into your *Applications* folder and run the file by double clicking it.
    - You may have to enable permission to install third-party applications on your computer, which you can do by navigating to *System Preferences* > *Security & Privacy* > *Allow apps downloaded from:*, and selecting *Anywhere*.
- On Windows, double click the *PAiN.exe* file to start the program. There is no installation process.
    - You do not need administrative privileges to run PAiN on Windows. You may see a security warning pop-up, select *Run*. If you do not want to see the warning again, uncheck *Always ask before opening this file*. **Important:** You may create a shortcut to the *PAiN.exe* file in a different directory. Do not install multiple *PAiN.exe* files or copy the file to multiple directories.

Install the latest version of VLC Media Player from http://www.videolan.org/vlc/. VLC is a free cross-platform multimedia player that PAiN uses for its audio and video.

- PAiN supports the following video formats for playback: .avi, .mpg, .vob, .mp4, .m2ts, .mov, .3gp, and .mkv. If you need to convert videos to a format compatible with PAiN, HandBrake (https://handbrake.fr/) is a free tool for converting video from nearly any format. HandBrake features include batch encoding and custom conversion quality.

To use PAiN's keyboard shortcuts:

- On Windows, the shortcut key for each button will be underlined. Press 'Ctrl' + the underlined key to trigger the shortcut.
- On macOS, press 'Cmd + /' to view the shortcut key for each button. Press 'Cmd' + the key in brackets to trigger the shortcut.

Creating and editing study templates

To create a new study template, click the 'Add Study' button in PAiN's home screen. Fill out the fields in the 'Study Template' pop-up window, using the 'Add Phase' and 'Delete Phase' buttons to create the required number of phases. Detailed explanations for each field are available by hovering over the field or text in the template with your mouse. To save the study and close the window, click 'Save'.

To view and/or edit an existing study template, select the template name from the list in the top right of PAiN's home screen and click the 'View/Edit Study' button. To save changes to a template, enter a new Study Name and click 'Save as New'.

To delete a study template, open the template as if you were going to view/edit it, then click the 'Delete Study' button. **Important:** Deleting a template also deletes all of its associated settings and output data. If you need to save your data but delete the template, make sure to download the output files from the Settings window.

Video player controls

Button	Function	Shortcut
	Play/Pause	Space Bar
	Toggle fast-forward on and off (video plays at 2x speed when highlighted)	Ctrl + F (Windows) Cmd + F (macOS)
	Skip backwards half a second	Down Arrow Key
	Skip forwards half a second	Up Arrow Key
	Skip backwards one second	Left Arrow Key
	Skip forwards one second	Right Arrow Key
	Adjust volume of video	Ctrl + V (Windows) Cmd + V (macOS)

PIPP and PIPP-R coding

1. Coding for PIPP and PIPP-R is the same. In PAiN's home screen, choose the PIPP/PIPP-R study template you'd like to use and click 'Next'.

2. Click the 'Upload Video' button and select the video file for the current procedure from your computer, then click 'Next'
    a. If you would like to record that an entire procedure is missing, click the 'Missing Procedure' button and fill out the information corresponding to the missing procedure. When you click 'Next', the missing procedure data points will all be recorded in the output file as the error code '777' and you will be returned to PAiN's home screen.

3. Fill out the Infant ID, Coder Initials, and Procedure fields and click 'Next'.
    a. To record data to be used for reliability testing, click the 'Use data for reliability testing' checkbox. **Important:** Any data used for reliability testing will not be recorded in your PAiN output CSV file, but rather in a separate reliability testing CSV file. When coding for reliability, existing data will not be overwritten. You may code the same infant ID multiple times and each instance will be saved as a new line of data with a unique time stamp.

4. Use the video player controls to locate the coloured marker corresponding to the start of the phase listed in the top-right of the screen, then click the 'Set Startpoint' button. Once you have set the correct startpoint, click 'Next'. Once you have set the startpoint for a phase, PAiN will automatically navigate through the epochs according to the information inputted into the study template.
    a. If you decide to code the same procedure again, PAiN will automatically bring you to the coloured marker for each phase based on the automatically-created Startpoints CSV file.

5. If the phase you're currently recording is a baseline phase (the label at the top right of the screen says 'Record Baseline State for XXX'), click 'Start Observation', then select the appropriate baseline state from the four options given during the 20 seconds that the countdown timer runs. Once selected, click 'Record Baseline State'. Confirm that the baseline state you've selected is correct, then click 'Next'.
    a. To view a more detailed explanation for each baseline state, click 'File' > 'Help' > 'Baseline State Help'.

6. To start coding facial indicators, click 'Start Observation'. When the initial countdown timer runs out and the epoch begins, hold the spacebar during the facial indicator being coded (the instruction label will highlight blue when the facial indicator is being recorded).
    a. For more information on how to identify facial indicators, click 'File' > 'Help' > 'Facial Indicator Help'.
    b. If you enabled the 'Record Missing Epoch Time' option in your study template, hold the Option (macOS) or Ctrl (Windows) key during any time that your view of the infant is obscured during the epoch. While recording missing time, you cannot record presence of the facial indicator. If you record any missing time, you will be prompted to enter explanatory details after the coding of the epoch is complete.
    c. You can record an entire epoch as missing by clicking the 'Missing' button either before or after attempting to code the epoch, then entering the explanatory details when prompted. After clicking 'Next', you will be taken to the next epoch.

7. Once the facial indicator data has been coded in the current epoch, confirm that the recorded data is correct (if you have enabled the option to show data after each epoch in Settings) and that you want to proceed by clicking 'Next', or click 'Redo' to record the indicator for the current epoch again.

8. Repeat steps 6 and 7 until you reach the end of the current phase, at which point you will be prompted to navigate to the startpoint of the next phase.

9. Repeat steps 4 through 8 until you reach the end of the procedure, at which point you will be notified that the observation is complete. Clicking 'Home' to take you back to the home screen, where you can start an observation for the next procedure.

MBPS and FLACC coding

1. Coding for MBPS and FLACC is the same. In PAiN's home screen, choose the MBPS/FLACC study template you'd like to use and click 'Next'.

2. MBPS and FLACC coding permits the use of either one or two videos. Click the 'Upload Video 1' button and select the video file for the current procedure from your computer. To upload a second video, click 'Upload Video 2 (Optional)' and select the video file, then click 'Next'. To clear the video files and upload news ones, click the 'Clear' button.
    a. If you would like to record that an entire procedure is missing, click the 'Missing Procedure' button and fill out the information corresponding to the missing procedure. When you click 'Next', the missing procedure data points will all be recorded in the output file as the error code '777' and you will be returned to PAiN's home screen.
    b. For **dual videos:**
        i. To resize the videos, click and drag the vertical slider separating the two videos from left to right.
        ii. You can use the video control buttons on only one video at a time. When two videos are uploaded, 'Video 1' and 'Video 2' buttons appear to the bottom right of the video screens, allowing you to toggle which video is currently being controlled by the video control buttons.

3. Fill out the Infant ID, Coder Initials, and Procedure fields and click 'Next'.
    a. If you would like to record data to be used for reliability testing, click the 'Use data for reliability testing' checkbox. **Important:** Any data used for reliability testing will not be recorded in your PAiN output CSV file, but rather in a separate reliability testing CSV file. When coding for reliability, existing data will not be overwritten. You may code the same infant ID multiple times and each instance will be saved as a new line of data with a unique time stamp.

4. Use the video player controls to locate the coloured marker corresponding to the start of the needle listed in the top-right of the screen, then click the 'Set Startpoint' button. If you are using two videos, navigate to the coloured marker in both videos before clicking 'Set Startpoint'. Once you have set the startpoint, click 'Next'. Repeat this step until you have recorded the startpoints for all of the needles. PAiN will then automatically navigate through the video for the rest of the procedure.

a. If you have previously selected the startpoints for a procedure, PAiN will automatically bring you to the coloured marker for each needle based on the automatically-created Startpoints CSV file if you decide to code the procedure again.

5. To begin coding the parameters for the epoch, click 'Start'. While the video is playing, select the appropriate parameter option, and then click 'Next' to move onto the next epoch (or click 'Redo' to redo the epoch).
    a. You can record an epoch as entirely missing by clicking the 'Missing' button either before or after attempting to code the epoch, then entering the explanatory details when prompted. After clicking 'Next', you will be taken to the next epoch.
    b. If the phase you're coding is a baseline (you checked the 'Baseline' box for the phase in the study template), you can move the start of the phase back by increments of the phase's epoch length by clicking 'Move Baseline'. This should be used when your view of the infant is obstructed during the current baseline. Moving the baseline backwards will bring you back to the first epoch and first parameter of the phase you're coding.

6. Repeat step 5 until you reach the end of the procedure, at which point you will be notified that the observation is complete. Click 'Home' to take you back to PAiN's home screen, where you can start an observation for the next procedure.

Navigation and adding comments

Navigation:

- During the coding of a procedure, you can jump between phases using the 'Navigation' button. **Important:** When you jump to a different phase, you will have to start from the beginning of that phase even if you've already recorded data for it. Any existing data for that phase will be overwritten if you proceed with coding. Any existing data for other phases within the procedure will not be affected.

Adding comments:

- At any time during the coding of a procedure, you can add a comment that will be recorded in the study's output data by clicking 'Add Comment'. Comments you enter will be applied to all epochs within the phase you're currently coding.

Settings

PAiN's settings can be changed by clicking 'Settings'. This gives you the option to:

- Change PAiN's text size, by selecting an option on the scale of 1 - 5.
- Download output data files from cloud storage
- Unlock studies that another user might already coding in (only one coder is allowed into each study at a time).
- For PIPP and PIPP-R only:
    - Change the length of the countdown before coding of an epoch starts.

a. If you have previously selected the startpoints for a procedure, PAiN will automatically bring you to the coloured marker for each needle based on the automatically-created Startpoints CSV file if you decide to code the procedure again.

5. To begin coding the parameters for the epoch, click 'Start'. While the video is playing, select the appropriate parameter option, and then click 'Next' to move onto the next epoch (or click 'Redo' to redo the epoch).
    a. You can record an epoch as entirely missing by clicking the 'Missing' button either before or after attempting to code the epoch, then entering the explanatory details when prompted. After clicking 'Next', you will be taken to the next epoch.
    b. If the phase you're coding is a baseline (you checked the 'Baseline' box for the phase in the study template), you can move the start of the phase back by increments of the phase's epoch length by clicking 'Move Baseline'. This should be used when your view of the infant is obstructed during the current baseline. Moving the baseline backwards will bring you back to the first epoch and first parameter of the phase you're coding.

6. Repeat step 5 until you reach the end of the procedure, at which point you will be notified that the observation is complete. Click 'Home' to take you back to PAiN's home screen, where you can start an observation for the next procedure.

Navigation and adding comments

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PAiN Admin User Manual

Getting started

Installation:

- On macOS, click on the .dmg file to open it, then drag the *PAiN Admin.app* file into your *Applications* folder and run the file by double clicking on it.
    - You may have to enable permission to install third-party applications on your computer, which you can do by navigating to *System Preferences* > *Security & Privacy* > *Allow apps downloaded from:*, and selecting *Anywhere*.
- On Windows, double click the *PAiN Admin.exe* file to start the program. There is no installation process.
    - You do not need administrative privileges to run PAiN Admin on Windows. You may see a security warning pop-up, select *Run*. If you do not want to see the warning again, uncheck *Always ask before opening this file*. **Important:** You may create a shortcut to the *PAiN Admin.exe* file in a different directory. Do not install multiple *PAiN Admin.exe* files or copy the file to multiple directories.

Creating and editing study templates

If PAiN and PAiN Admin are installed with the same directory, a study template created in either application will appear in both. See the section on creating and editing study templates for the PAiN software for more information on templates, the same instructions apply to templates in PAiN Admin.

Formatting input data

Before calculating PIPP/PIPP-R scores it is recommended that users familiarize themselves with the required format for input data.

To access sample templates for each input data file, in PAiN Admin navigate to *File* > *Download Data File Templates* and select a directory for the template files. Templates will then be saved under the directory in a folder called *PAiN Admin Data File Templates*. Each input data file is explained below.

Input	Description
PAiN output data sheet	The coded facial indicators of pain data. The CSV output from the PAiN coding in the regular format (file **(1)** from the table here).
Gestational age data sheet	Gestational age of the infant in weeks at the time of each painful procedure. A CSV or Excel file with one column for the age at each painful procedure.

Heart rate data folder	Infant heart rate and the corresponding time during the painful procedure. Create a folder for heart rate data. Include the heart rate data for each infant as an individual .xlsx file within the folder. The file should be named using the study ID format used in the PAiN template (prefix_#, e.g. ID_2). Within each file, create a sheet for each painful procedure, labeled as the painful procedure number (e.g. 1). Each sheet should include two columns: time in seconds (hh:mm:ss) and heart rate (bpm). See sample templates for more information.
O2 saturation data folder	Infant O2 saturation (SO2) and the corresponding time during the painful procedure. Create a folder with SO2 data for each infant as an individual .xlsx file, as with the heart rate data described above. Each sheet should include two columns: time in seconds (hh:mm:ss) and SO2 (%). See sample templates for more information.
Phase marker data sheet	The time (hh:mm:ss) of the start of each phase in each procedure for each infant ID. A CSV or Excel file with one column for the start of each phase and one column denoting the procedure number.
Supplemental O2 data sheet	Optional. If supplemental oxygen (O2) was administered to the infant during a phase, mark it with a '1' in the 'Supplemental O2' column and note the procedure number in the 'Procedure' column.

Calculating PIPP and PIPP-R scores

1. Select the template name from the 'Study Template' list in PAiN Admin's home screen.

2. Select the input data sheets using the 'Upload' buttons in the 'Data File Upload' box. All input data sheets except 'Supplemental O2 Data' are required in order to calculate a PIPP/PIPP-R score.

3. In the 'Pre-Calculation Options' screen, select the column headers corresponding to the procedures and phases in the dropdown menus in the 'Gestational Age Data Column Headers' and 'Phase Marker Data Column Headers' fields. The column headers will be imported automatically from your input data sheets. Ensure the rest of the fields are filled out correctly, then click 'Calculate'.

4. Once the calculation is complete, click 'Yes' to view the output data in your file explorer.

Merging PAiN output files

In order to merge multiple PAiN output files from the same study template (files must be in the format of file **(1)** or **(2)** in the PAiN output table), click *File > Merge PAiN Output Files*. The files you are merging must be in the same format. Use the 'Upload' button and/or

drag-and-drop files directly into the listview if you are on Windows, then click 'Merge' to create the new, merged file. If this is the file you want to use in your next PIPP/PIPP-R calculation, check the 'Use merged file for PIPP calculation' button.

Settings

PAiN Admin's settings can be changed by clicking 'Settings'. This gives you the option to:

- Download output data files from cloud storage
- Change PAiN Admin's text size, by selecting an option on the scale of 1 - 5.
- Change your saved data file upload paths. These file paths point to your PAiN Admin input data files and will be automatically populated in the 'Data File Upload' section of PAiN Admin's main window so that you do not have to manually select the file paths each time you open the program.

Interpreting and managing output data

The output data from PAiN Admin is stored in Excel workbooks in a folder called *PAiN Admin Output* that is created inside the relevant study template directory in your *PAiN* folder. Each time you calculate a PIPP score, a new Excel workbook is created that notes the date and time of the calculation. Within the workbook, there are separate tabs containing the output data in both regular (grey tab colour) and database (denoted 'DB' - blue tab colour) formats. The contents of the individual tabs are explained below:

Tab Name(s)	Description
(1) 'PIPP Scores' 'PIPP Scores DB'	Contain only the PIPP/PIPP-R scores calculated by PAiN Admin. Any 'error' values can be explained by referencing the 'Error List' tab **(2)**.
(2) 'Error List' 'Error List DB'	If a PIPP/PIPP-R score could not be calculated for a certain epoch and an error value was returned for the corresponding epoch in file **(1)** or **(4)**, this file will contain an explanation for why the error occurred in the form of unique error codes. A detailed reference for each error code can be found here.
(3) 'PIPP Score Components' 'PIPP Score Components DB'	Contain a detailed breakdown of all of the scoring components of each PIPP/PIPP-R score, including values for each facial indicator, the change in HR, the change in O2 saturation and whether or not supplemental O2 was administered, among others.
(4) 'PAiN Output Data'	The original PAiN output file (file **(1)** from this table) with the PIPP/PIPP-R score cells filled in by PAiN Admin. Any PIPP/PIPP-R scores that returned an error during calculation will be left blank.

Reliability Testing

To access the reliability testing function, click File > Reliability Testing. PAiN Admin allows you to calculate both *inter*-rater reliability (correlation between observations by different raters) and *intra*-rater reliability (correlation between observations by the same rater) using files outputted from PAiN (in the format of file **(1)** in this table). Reliability is measured by six different versions of the Intraclass Correlation Coefficient, more information about which can be found here. **Important:** Reliability testing in PAiN is only relevant to PIPP and PIPP-R data, where indicators are assessed on a continuous scale from 0-1 and are not assessed categorically like in MBPS and FLACC.

The reliability function uses one CSV file as its input, the contents of which can later be organized within PAiN Admin's reliability window. To create the input file, use PAiN Admin's merge function to merge together PAiN output files including all Gold Standards (if relevant; discussed below) and observations performed by coders that you'd like to use to calculate reliability.

To calculate inter-rater reliability:

Inter-rater reliability can be calculated in two ways: against a Gold Standard (an observation considered to be accurate that acts as a benchmark for all other observations) and among different raters (to assess the consistency of different raters in assessing the same procedure).

1. Select the 'Inter-Rater Reliability' option from the top-right of the Reliability window.

2. Click the 'Load Input Data' button and select the merged data file containing all of the observations and Gold Standards (if relevant). Once selected, the data from the input file will be summarized by procedure in the 'Input List' at the top of the screen. The farthest-right column will indicate whether any data is missing from each procedure.

3. Only observations that you add to the Output List will be used in the reliability calculation. To add observations to the Output List, select them from the Input List (use Shift and Command/Control to select multiple observations) and click 'Add to Output List'. Observations can be removed from the Output List by clicking 'Remove from Output List'.

4. To assign an observation as a Gold Standard, select it from the Input List and click 'Add to Gold Standard'. Multiple Gold Standards can be used and PAiN Admin will compare all possible combinations between the procedures in the Output List and the Gold Standard list. Observations can be removed from the Gold Standard list by clicking 'Remove from Gold Standard'.

5. Check the 'Only calculate reliability against Gold Standard' box if you do not want to calculate reliability between observations in the Output List.

6. Check the 'Skip Missing Values' box if you want to ignore any blank or error values in the data. If this button is not checked, PAiN Admin will return an error for the calculation if there are any missing values.

7. Set your alpha level of significance for calculating confidence intervals in the output data. An alpha level of 5% would result in a 95% confidence interval range; an alpha of 10% would result in a 90% confidence interval range, etc.

8. Once you have selected your information and have entered your settings, click 'Calculate Reliability' at the bottom-right of the screen and select the destination for the output data on your computer.

9. Once the reliability calculation is complete, a folder will be created in the output location that you specified containing one Excel file for reliability in comparison to the Gold Standard(s), and one Excel file for the reliability among procedures in the Output List.

To calculate intra-rater reliability:

1. Select the 'Intra-Rater Reliability' option from the top-right of the Reliability window.

2. Click the 'Load Input Data' button and select the merged data file containing all of the observations. Once selected, the data from the input file will be summarized by procedure in the 'Input List' at the top of the screen. The farthest-right column will indicate whether any data is missing from each procedure.

3. Only observations that you add to the Output List will be used in the reliability calculation. To add observations to the Output List, select them from the Input List (use Shift and Command/Control to select multiple observations) and click 'Add to Output List'. Observations can be removed from the Output List by clicking 'Remove from Output List'.

4. Check the 'Skip Missing Values' box if you want to ignore any blank or error values in the data. If this button is not checked, PAiN Admin will return an error for the calculation if there are any missing values.

5. Set your alpha level of significance for calculating confidence intervals in the output data. An alpha level of 5% would result in a 95% confidence interval range; an alpha of 10% would result in a 90% confidence interval range, etc.

6. Once you have selected your information and have entered your settings, click 'Calculate Reliability' at the bottom-right of the screen and select the output destination for the results.

7. Once the reliability calculation is complete, an Excel file will be created in the output location that you specified containing intra-rater reliability calculations for all possible combinations of the procedures in the Output List.

Error Codes

Error Code	Description	Details
999	Technical problem with video - entire epoch missing	In PAiN, the user has marked the entire epoch missing or unusable due to a technical problem with the corresponding section of the uploaded video.
998	View obstructed by medical equipment - entire epoch missing	In PAiN, the user has marked the entire epoch missing or unusable due to their view being obstructed by medical equipment in the corresponding section of the uploaded video.
997	View obstructed by person - entire epoch missing	In PAiN, the user has marked the entire epoch missing or unusable due to their view being obstructed by a person in the corresponding section of the uploaded video.
996	Other - entire epoch missing	In PAiN, the user has marked the entire epoch missing or unusable due to an issue with the corresponding section of the uploaded video that is not described above.
899	Technical problem with video - epoch partially missing	In PAiN, the user has marked a portion of the epoch missing or unusable due to a technical problem with the corresponding section of the uploaded video. The proportion of the epoch that is missing is greater than the acceptable threshold set by the administrator in PAiN Admin.
898	View obstructed by medical equipment - epoch partially missing	In PAiN, the user has marked a portion of the epoch missing or unusable due to their view being obstructed by medical equipment in the corresponding section of the uploaded video. The proportion of the epoch that is missing is greater than the acceptable threshold set by the administrator in PAiN Admin.
897	View obstructed by person - epoch partially missing	In PAiN, the user has marked a portion of the epoch missing or unusable due to their view being obstructed by a person in the corresponding section of the uploaded video. The proportion of the epoch that is missing is greater than the acceptable threshold set by the administrator in PAiN Admin.
896	Other - epoch partially missing	In PAiN, the user has marked a portion of the epoch missing or unusable due to an issue with the corresponding section of the uploaded video that is not described above. The proportion of the epoch that is missing is greater than the acceptable threshold set by the administrator in PAiN Admin.

777	Entire procedure missing	In PAIN, the user has marked the entire procedure as missing. There is no data in the procedure that can be used to calculate a pain measure.
776	Epoch cut off from end of video	The observation time exceeded the length of the video being coded, resulting in one or more epochs being cut off and excluded.

699	Heart rate data completely missing	PAiN Admin could not find any heart rate data for this epoch. The data could be either (1) missing entirely from the input data sheets, or (2) not correctly formatted and causing an error during calculation of the pain measure. For the correct data format, refer to the downloadable data templates in PAiN Admin (File > Download Data Templates).
698	Heart rate data partially missing	The heart rate data for this epoch is incomplete and is below the required threshold set by the administrator in PAiN Admin. Some of the data points may be formatted incorrectly. For the correct data format, refer to the downloadable data templates in PAiN Admin (File > Download Data Templates).
697	O2 data completely missing	PAiN Admin could not find any O2 saturation data for this epoch. The data could be either (1) missing entirely from the input data sheets, or (2) not correctly formatted and causing an error during calculation of the pain measure. For the correct data format, refer to the downloadable data templates in PAiN Admin (File > Download Data Templates).
696	O2 data partially missing	The O2 saturation data for this epoch is incomplete and is below the required threshold set by the administrator in PAiN Admin. Some of the data points may be formatted incorrectly. For the correct data format, refer to the downloadable data templates in PAiN Admin (File > Download Data Templates).
695	Heart rate data point outside of normal range	One or more of the heart rate data points is below 65 beats per minute or above 150 beats per minute, which indicates an error in the collection or entry of the heart rate data.
694	O2 data point outside of normal range	One or more of the O2 saturation data points is below 65% or above 100%, which indicates an error in the collection or entry of the O2 saturation data.

599	Facial indicator data missing	The facial indicator data for the epoch could not be found in the PAiN output data. The epoch may not have been recorded

		properly or there is a formatting error in the PAiN output data file.
598	Facial indicator data error	The facial indicator data in the PAiN output data file is causing an error in PAiN Admin. The epoch may have been modified or not have been recorded properly.

499	Gestational age data missing	There is no gestational age data for this epoch in the input data sheet. The data could be either (1) missing entirely from the input data sheets, or (2) not correctly formatted and causing an error during calculation of the pain measure. For the correct data format, refer to the downloadable data templates in PAiN Admin (File > Download Data Templates).
498	Gestational age data error	The gestational age data sheet is causing an error in PAiN Admin. The data may have been modified or not be correctly formatted. For the correct data format, refer to the downloadable data templates in PAiN Admin (File > Download Data Templates).
497	Baseline state data missing	There is no baseline state data for this epoch in the input data sheet. The data could be either (1) missing entirely from the input data sheets, or (2) not correctly formatted and causing an error during calculation of the pain measure.
496	Baseline state data error	The baseline state data sheet is causing an error in PAiN Admin. The data may have been modified or not be correctly formatted.

399	Baseline heart rate data completely missing	PAiN Admin could not find any heart rate data for this baseline epoch. The data could be either (1) missing entirely from the input data sheets, or (2) not correctly formatted and causing an error during calculation of the pain measure. For the correct data format, refer to the downloadable data templates in PAiN Admin (File > Download Data Templates).
398	Baseline heart rate data partially missing	The heart rate data for this baseline epoch is incomplete and is below the required threshold set by the administrator in PAiN Admin. Some of the data points may be formatted incorrectly. For the correct data format, refer to the downloadable data templates in PAiN Admin (File > Download Data Templates).
397	Baseline O2 data completely missing	PAiN Admin could not find any O2 saturation data for this baseline epoch. The data could be either (1) missing entirely from the input data sheets, or (2) not correctly formatted and causing an error during calculation of the pain measure. For the

		correct data format, refer to the downloadable data templates in PAiN Admin (File > Download Data Templates).
396	Baseline O2 data partially missing	The O2 saturation data for this baseline epoch is incomplete and is below the required threshold set by the administrator in PAiN Admin. Some of the data points may be formatted incorrectly. For the correct data format, refer to the downloadable data templates in PAiN Admin (File > Download Data Templates).
395	Baseline heart rate data point outside of normal range	One or more of the baseline heart rate data points is below 65 beats per minute or above 150 beats per minute, which indicates an error in the collection or entry of the heart rate data for this baseline epoch.
394	Baseline O2 data point outside of normal range	One or more of the baseline O2 saturation data points is below 65% or above 100%, which indicates an error in the collection or entry of the O2 saturation data for this baseline epoch

Appendix N

iCAP Study Information, Consent, and Authorization Form

Information, Authorization and Consent Form

RESEARCH TITLE: The Influence of Breastfeeding on Pain-specific Event-related Potentials and Bio-behavioural Indicators of Procedural Pain in Newborns: A Randomized Controlled Trial (iCAP Trial)

RESEARCHERS:
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Ruth Martin-Misener, PhD NP RN, School of Nursing, Dalhousie University
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Margot Latimer, PhD NP RN, School of Nursing, Dalhousie University, Centre for Pediatric Pain Research, IWK Health Centre

FUNDING: Dalhousie University School of Nursing Research Fund, IWK Health Centre Category A Research Grant

RESEARCH COORINATOR/ASSISTANT: Sarah Foye BN RN, Kim Caddell BN RN

INTRODUCTION: You are being invited to take part in the research study named above. This form provides information about the study. Before you decide if you want to take part, it is important that you understand the purpose of the study, the risks and benefits, and what you will be asked to do. You do not have to take part in this study. Taking part is entirely voluntary (your choice). Informed consent starts with the initial contact about the study and continues until the end of the study. A staff member of the research team will be available to answer any questions you have. You may decide not to take part or you may withdraw from the study at any time. This will not affect the care you or your family members will receive from the IWK Health Centre in any way.

WHY ARE THE RESEARCHERS DOING THE STUDY? Even healthy babies are exposed to painful procedures as part of routine medical care. In addition to causing suffering, unmanaged early pain is associated with negative consequences, such as feeling increased pain during later procedures. Current evidence has led to

recommendations that oral sucrose (sweet tasting liquid placed on the tongue) and breastfeeding are equally effective in reducing bio-behavioural pain scores (e.g., infant facial responses and physiologic responses to pain) for full term infants undergoing needle related medical procedures. However, there is some evidence that suggests that sucrose does not reduce pain response in the newborn brain. Research shows that breastfeeding during painful procedures is effective in reducing pain in newborns. However, no studies have examined the influence of breastfeeding on pain response in the newborn brain.

The proposed study will address this knowledge gap by examining to what extent breastfeeding impacts on pain response in the infant brain during a heel stick procedure in comparison to 24% oral sucrose (the most commonly used sweet tasting solution). In light of the negative outcomes of unmanaged pain in infants, it is important that this research is conducted so that we know the most effective pain relieving interventions to use for newborns. The findings of this study will be important for helping develop best practices for pain assessment and management in newborns.

HOW WILL THE RESEARCHERS DO THE STUDY? This study is being conducted on the Family Newborn Care Unit of the IWK Health Centre. We plan to enroll 126 healthy full term infants born at greater than 37 0/7 weeks' gestational age over a one-year period.

WHAT WILL I BE ASKED TO DO? If you choose to allow us to include your baby in this study, he/she will be randomly assigned (like rolling a dice) to either have their routine metabolic screening blood work completed while you are breastfeeding them or they will be assigned to remain in their infant cot for the blood work with the use of sucrose and potentially sucking on a soother or a finger for comfort. The heel stick that they have for the blood work is a procedure that they will be having as part of routine hospital care and no painful procedures will be completed only for the purpose of this study. All babies will be closely watched before, during, and after the heel stick procedure.

> **BREASTFEEDING:** If you and your baby are assigned to be breastfeeding during the heel stick procedure, a nurse will assist you to place your baby in skin-to-skin contact and latch your baby to the breast during the procedure. If your baby appears to still have pain during the procedure, he/she will be offered sucrose in addition to breastfeeding.

SUCROSE: If your baby is assigned to receive sucrose during the heel stick procedure, they will be offered a small amount of sucrose on to the tip of their tongue. In addition, if your baby is receiving sucrose, they will also be offered a soother or gloved finger (based on your preference) to suck on during the heel stick for comfort. If your baby appears to still have pain during the procedure, he/she will be offered another dose of sucrose.

If your baby is in this study his/her face will be videotaped during the procedure to see his/her facial actions during the procedure. The video will be close up of the baby and mothers face will not be visible in the video recording. While we will make all attempts to support the infants sustained latch to the breast to minimize exposure of the mothers' breast in the video, it is possible that the mothers breast will be recorded on video if the infants latch to the breast is interrupted. Trained coders and/or the use of a coding program will be observing only your baby's face response on these videos. In addition, normal body functions (heart rate, oxygen levels, and brain activity) will be recorded before, during, and after the heel stick procedure. To measure heart rate and oxygen levels, one saturation probe will be placed on your baby's hand or foot. These saturation probes are made specifically for infants and are routinely used in the hospital. To record brain activity, an electroencephalogram (EEG) recording will be done. To do this EEG recording, an EEG net will be placed on your baby's head. This net, which is specifically designed for use with infants, has small electrodes that would equally spread across your baby's head and a strap that goes under your baby's chin. The EEG system that we are using to record brain activity is certified with Health Canada, is approved for use in the IWK Health Centre, and is ideal for use with infants. We will monitor your baby continuously while they are having their brain activity recorded to ensure that they are comfortable. Data collection will begin by securing the saturation probe on your baby's foot, then the EEG net will be placed on your baby's head. We will then start video recording your baby's face while they are in their cot and will continue to video record their face when they are breastfeeding if they are in the breastfeeding intervention group. It is anticipated that this part of study participation will take approximately one hour.

Figure 1. Newborn infants wearing EEG net as part of the research study

Regardless of the intervention (breastfeeding, sucrose) your baby receives during this study, you will be asked to complete a short maternal questionnaire on the day that your baby has their heel stick completed. This questionnaire should take about five to ten minutes and will ask questions about how you felt about your baby's pain relief and your experience taking part in the study.

Finally, if you decide to take part in this study, we will review your health record and your baby's health record on the day of the heel stick procedure to collect information about your baby's birth history, breastfeeding experience, medications, normal body functioning, and the number of procedures your baby has had during his/her hospital stay. Information collected from your chart will include obstetrical, medical, and medication history, as well as information about your family arrangement and education.

If your baby is in the study, no changes to standard care, other than the ones listed above, will be made. You will receive a copy of the consent form to keep. Below, we will ask your permission to use the video-tape for teaching and demonstration purposes. You do not have to agree to this. If you prefer that your baby's video not be used for these other purposes, you and your baby can still take part in this study.

WHAT ARE THE BURDENS, HARMS, AND POTENTIAL HARMS? There are no known risks for babies participating in this study. Both breastfeeding and sucrose are known to provide pain relief for newborns, so your baby will receive an effective pain reducing intervention regardless of which group they are assigned to. The EEG net used to measure your baby's brain activity in this study needs to be soaked in a warm salt-water (saline) solution before it is placed on your baby's

head. No other researchers using this net have had issues with this causing baby's in studies to get cold. However, to ensure that your baby's temperature is stable, we will monitor your baby's temperature (by placing a thermometer probe under their arm) every fifteen minutes during the study. If your baby develops any unexpected side effects, such as unstable heart rate, oxygen levels, or temperature, the nurse and physician/midwife caring for your baby will be notified immediately and the study session will be stopped.

WHAT ARE THE POSSIBLE BENEFITS? Taking part in this study may be of no help to you personally. It is hoped that what is learned will benefit other full term infants undergoing painful procedures. Regardless of your baby's group assignment, he/she will receive at least one form of a known effective pain relieving intervention.

WHAT ALTERNATIVES TO PARTICIPATION DO I HAVE? You may decide not to enroll your baby or you may withdraw him/her from the study at any time. This will not affect your baby's care at the IWK Health Centre in any way. Breastfeeding and sucrose during a heel stick is not considered part of routine care on the Family Newborn Care Unit. If the baby does not participate in the study, the heel stick will likely be carried out in the standard manner. This includes performing the heel stick with your baby on his/her back or side in his/her infant cot. Comfort measures such as swaddling may be provided as necessary by the laboratory staff.

CAN I WITHDRAW FROM THE STUDY? The participation of you and your baby in the study is voluntary. Consent may be withdrawn and all data and videotapes destroyed at any time with no change in care given to your baby at the IWK Health Centre.

WILL THE STUDY COST ME ANYTHING AND, IF SO, HOW WILL I BE REIMBURSED? Participation in this study will not result in any expenses to you or your baby. All data will be collected while you and your baby are admitted to the IWK Health Centre during your post-partum stay. Therefore, you will not be expected to travel to the hospital to participate in the study.

ARE THERE ANY CONFLICTS OF INTEREST? The research investigators state that they have no conflicts of interest or have anything to disclose.

WHAT ABOUT POSSIBLE PROFIT FROM COMMERCIALIZATION OF THE STUDY RESULTS? There is no potential for profit from commercialization of the study results.

HOW WILL I BE INFORMED OF STUDY RESULTS? Overall research study results will be made available to you at the completion of the study. Please indicate in the box following your signature if you wish to have a copy of the results.

HOW WILL MY PRIVACY BE PROTECTED? Your baby will not be identified as a study participant in any reports or publications of this research. If you give permission for your baby's videotapes, heart rate, oxygen saturation, and EEG recording to be used, no other information will be shared outside the research team. Your baby's study data, videotapes, and EEG recording will be kept in a locked file cabinet in a locked office at the IWK Health Centre. All data will have your baby's name removed and will be identified only by a code. The code will be kept in a locked file cabinet at the IWK Health Centre. All information collected from you and your baby for the purpose of the study will stay at the IWK Health Centre. No information will be transferred to other sites. Study data will be stored and/or shared with members of the study team using secure and encrypted methods.

All study files will be kept for a minimum of 25 years. Only the staff involved in the research will see them. Members of the IWK Research Ethics Audit Committee may look at the records to ensure the study is being conducted properly. Your baby's confidentiality will be maintained.

WHAT ARE MY RESEARCH RIGHTS? Your signature on this form only indicates that you have understood to your satisfaction the information regarding participation in the study and agree for you and your baby to participate as subjects. In no way does this waive your legal rights nor release the research team, the study funder(s) or involved institutions from their legal and professional responsibilities. If you or your baby become ill or injured as a direct result of participating in this study, necessary medical treatment will be available at no additional cost to you. You and your baby are free to withdraw from the study at any time without jeopardizing the health care you are entitled to receive.

In order to verify the ethical management of the research project, it is possible that a member of the research ethics committee may review the research data and your baby's chart.

If you have any questions at any time during or after the study about research in general you may contact the Research Office of the IWK Health Centre at (902) 470-8765, Monday to Friday between 9a.m. and 5p.m.

PERMISSION TO CONTACT TO FUTURE RESEARCH You will be asked if you are willing to be contacted for future studies related to this one. If you wish to be contacted, please initial the signature page to indicate this and include a contact phone number and/or e-mail address. You may change your mind in the future and choose not to participate in any further studies. This will not impact the care of you or your baby in any way.

WHAT IF I HAVE STUDY QUESTIONS OR PROBLEMS? The study principle investigator (Britney Benoit) will carry a pager at all times. If you have any questions or concerns following your enrolment, you may call the IWK Health Centre at 470-8888 and ask for Britney Benoit at pager number #3244 or you may call her office at (902) 470-7103 from Monday-Friday between 9 am and 5 pm.

AUTHORIZATION AND CONSENT FORM

RESEARCH TITLE: The Influence of Breastfeeding on Pain-specific Event-related Potentials and Bio-behavioural Indicators of Procedural Pain in Newborns: A Randomized Controlled Trial

PARTICIPANT ID: _____

PARTICIPANT CONSENT *I have read or had read to me this authorization and consent form and have had the chance to ask questions which have been answered to my satisfaction before signing my name. I understand the nature of the study and I understand the potential risks. I understand that I have the right to withdraw my child from this study at any time without affecting my child's care in any way. I have received a copy of the Authorization and Consent form for future reference. I freely agree to have my child participate in this research study.*

Name of Participant: (Print)_____
Name of Mother (Print)_____
Signature of Mother _____
Date: _____ Time: _____

I wish to have a copy of the results sent to me (initial): Yes_____ No_____
I agree to be contacted about related studies in the future (initial) Yes_____ No_____

Mailing/email address/phone number
_____
_____
_____

In addition, I agree or consent for my videotape(s) to be used for (initial as appropriate):

1. Secondary analyses of the data collected to answer research questions similar to this study that will be subjected to the same ethical review by the IWK Research Ethics Board as the original study_____
2. Teaching and demonstration at the IWK_____
3. Teaching and demonstration at meetings outside the IWK_____
4. Not to be used for anything else _____

STATEMENT BY PERSON PROVIDING INFORMATION ON STUDY

I have explained the nature and the demands of the research study and judge that the person authorized named above understands the nature and demands of the study.

Name: (Print)_____
Signature:_____

Position:_____ Date: _____ Time: _____

STATEMENT BY PERSON OBTAINING CONSENT

I have explained the nature of the consent process to the participant and judge that they understand that participation is voluntary and that they may withdraw at any time from participating.

Name: (Print)_____
Signature:_____

Position:_____ Date: _____ Time: _____

Appendix O

Geodesic EEG System™ 400 Series Health Canada Medical Device License

Santé Health Canada Canada

LN/NH: 91684

Therapeutic Products Directorate
Medical Devices Bureau
Direction des produits thérapeutiques
Bureau des matériels médicaux

Medical Device Licence — **Homologation d'un instrument médical**

* AMENDED * — * MODIFIÉE *

Licence Number:	91684	No d'homologation:
First Issue Date:	2013/07/16	Première date de délivrance:
Amended Date:	2014/03/11	Date de modification:

Device Class/Classe de l'instrument: 2

This Licence is issued in accordance with the Medical Devices Regulations, Section 36, for the following medical device:

La présente homologation est délivrée en vertu de l'article 36 du Règlement sur les instruments médicaux pour l'instrument médical suivant:

Licence Name/Nom de l'homologation:

GES 400 SERIES EEG SYSTEMS

Licence Type/Type d'homologation:

Group Family / Famille de groupes

Reason for Amendment/Raison de la modification

CHANGE IN MANUFACTURER'S ADDRESS

Manufacturer Name & Address/Nom du fabricant & adresse

ELECTRICAL GEODESICS, INC

500 EAST 4TH AVE
SUITE 200
EUGENE, OREGON
UNITED STATES
97401

Application Number: / Numéro de la demande: 222995

Manufacturer ID: / Identificateur du fabricant: 116384

Components/Parts/Accessories/Devices for this Licence
Les composantes, parties, accessoires et instruments médicaux pour cette homologation

GES EEG SYSTEM

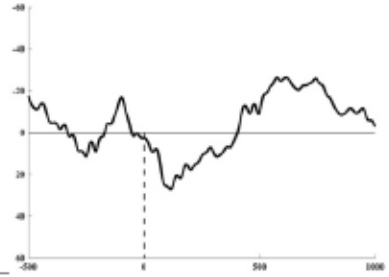
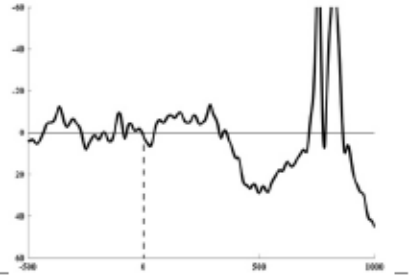
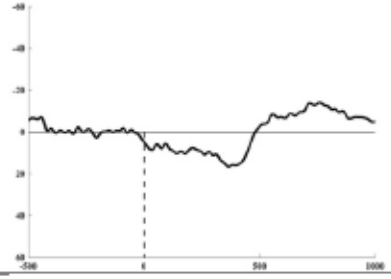
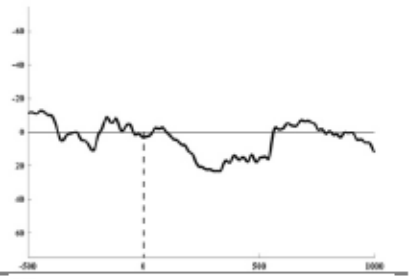
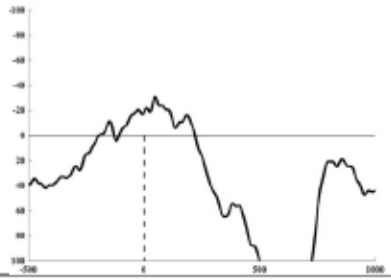
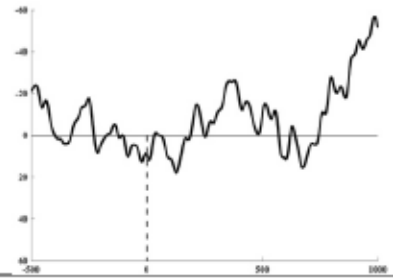
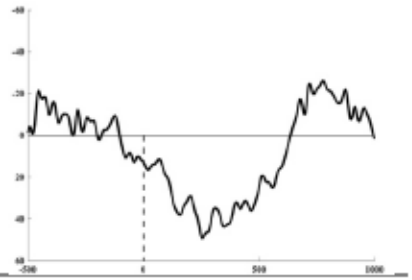
Device ID/No de l'instrument: 591850

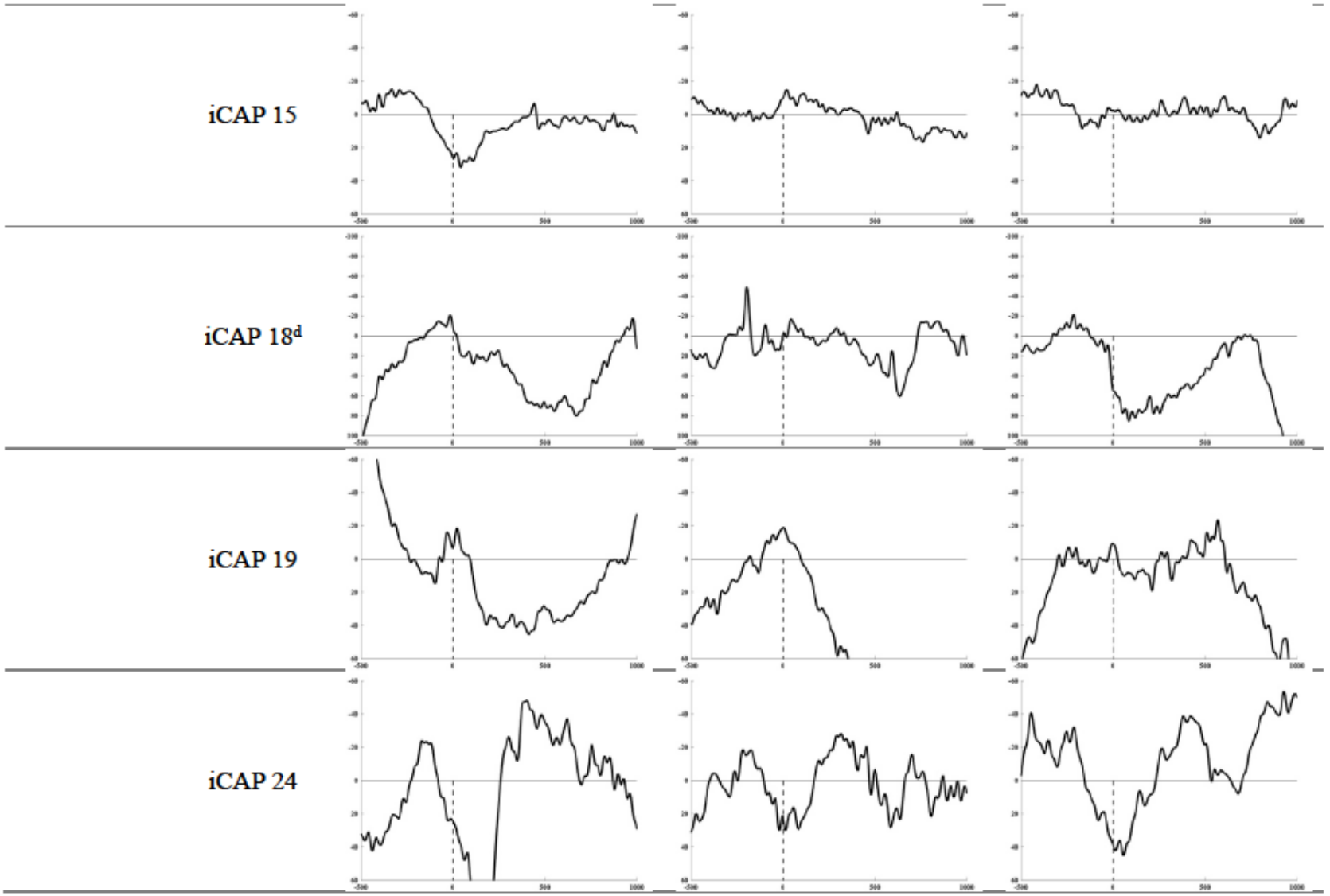
Device Identifier / Identificateur de l'instrument (Model/Catalog Detail/No de modèle/Catalogue):

GES 400
GES 405
GES 410

Appendix P

Individual Participant Average EEG Waveforms in Response to Non-noxious Control Stimuli and Noxious Heel Lance

Intervention condition	Participant	Non-noxious control 1	Non-noxious control 2	Noxious heel lance
24% oral sucrose and offered non-nutritive sucking	iCAP 04[a]		No data available	
	iCAP 08[b]		No data available	
	iCAP 14[c]			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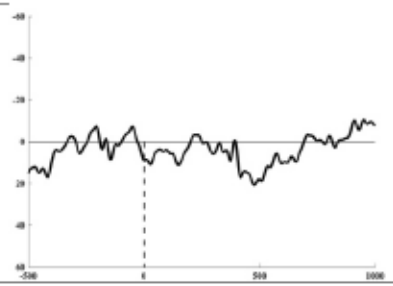
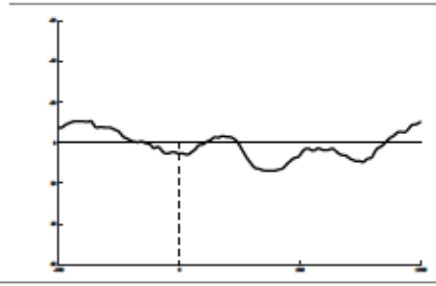
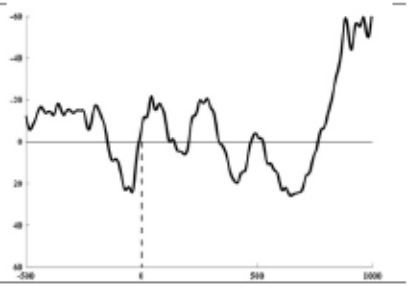
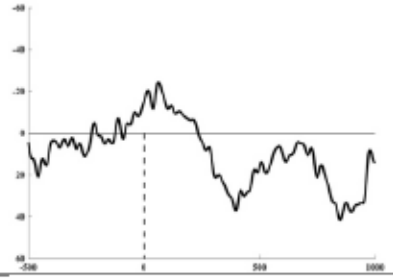
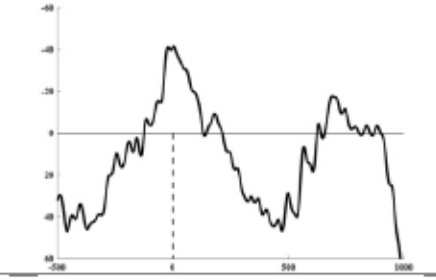
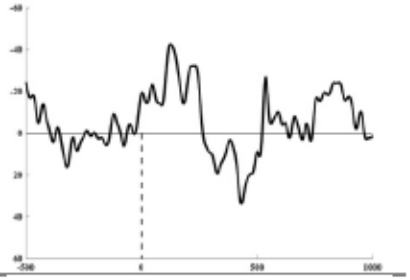
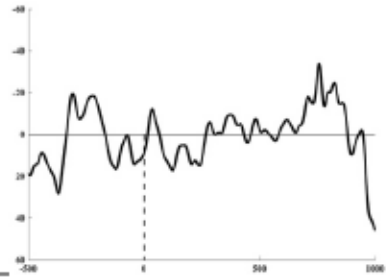
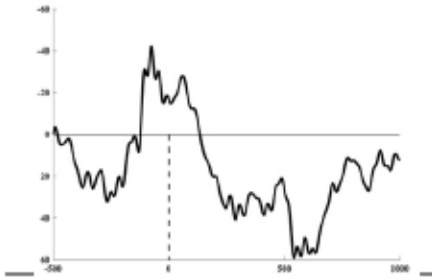
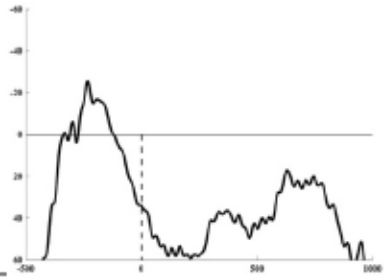
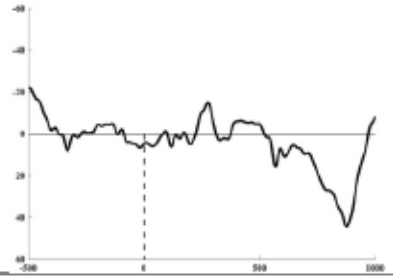
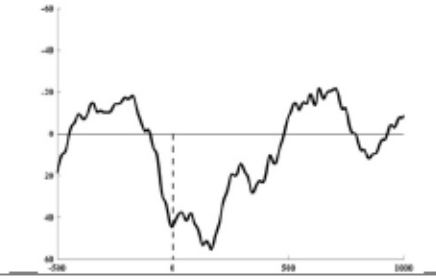
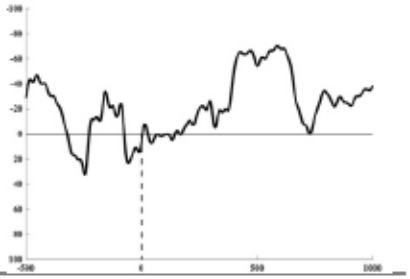
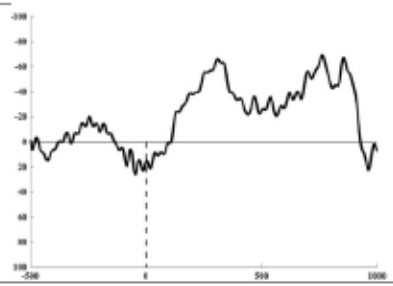
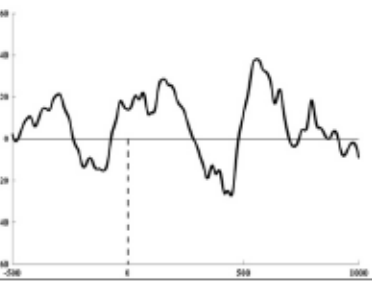
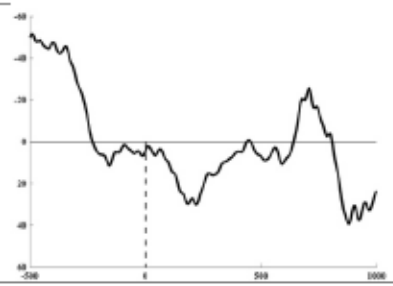
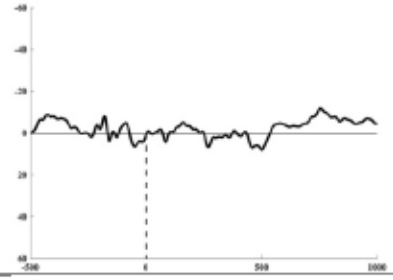
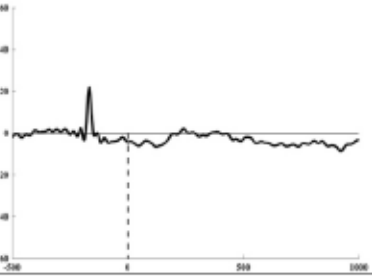
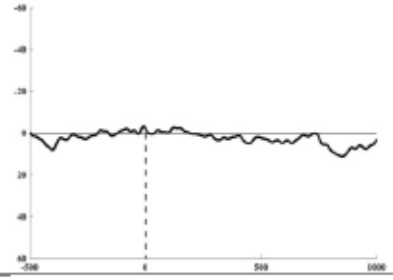
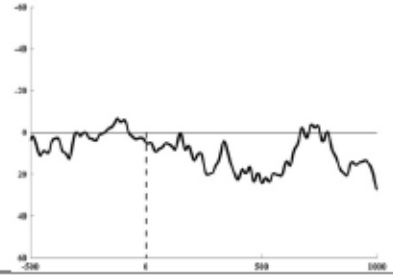
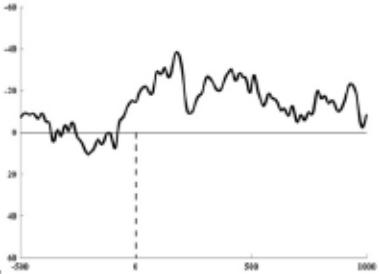
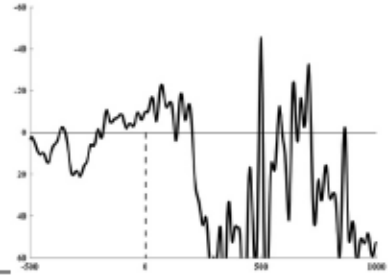
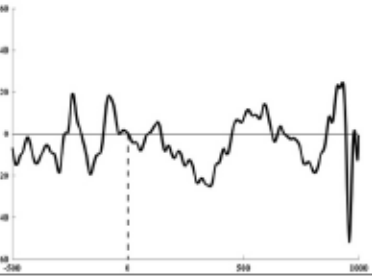
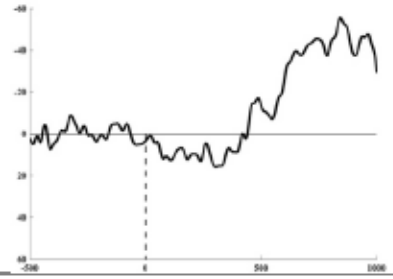
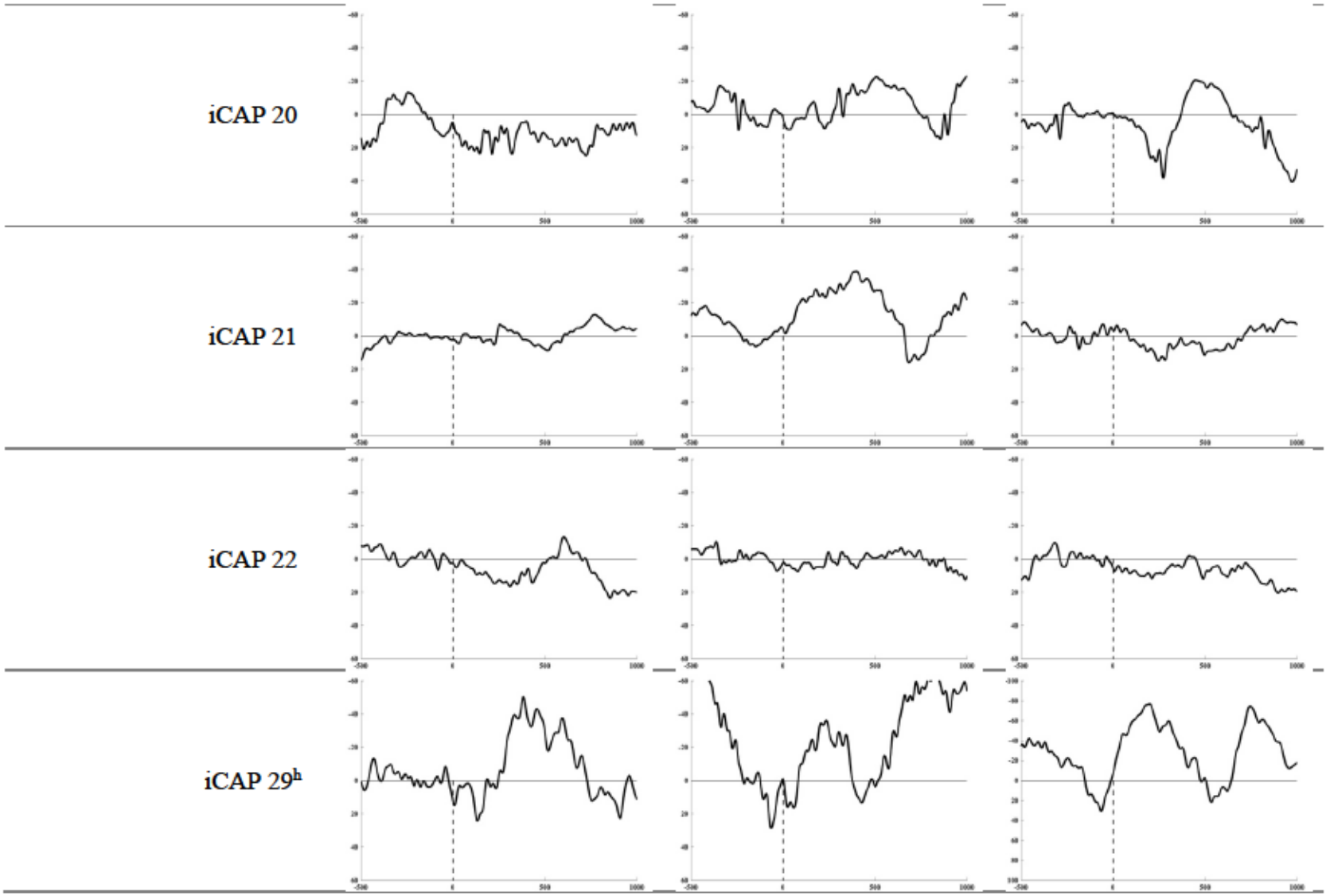
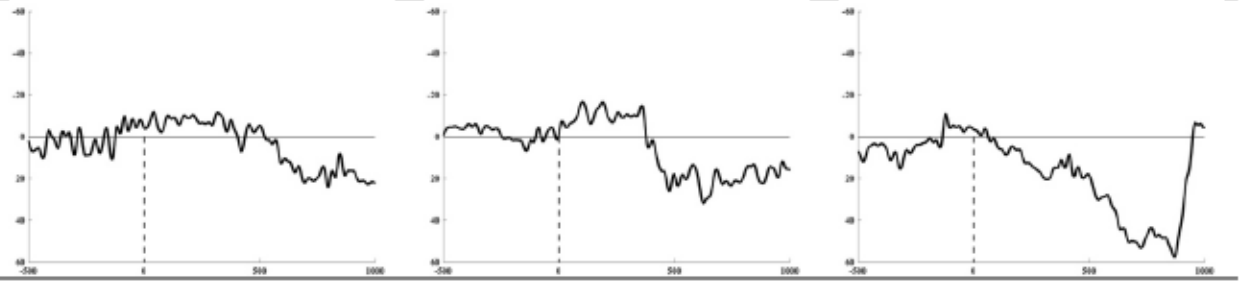
iCAP 18d
iCAP 19
iCAP 24

	iCAP 25			
	iCAP 33			
	iCAP 36			
Breastfeeding	iCAP 06[e]			

iCAP 07[f]			
iCAP 12			
iCAP 13			
iCAP 17[g]	No data available		


iCAP 20
iCAP 21
iCAP 22
iCAP 29h

iCAP 38	

Note. Table presents individual participant average waveforms following application of non-noxious control stimulus 1 (NN1 [applied prior to initiation of assigned intervention]), non-noxious control stimulus 2 (NN2 [applied after the initiation of assigned intervention]), and noxious heel lance (NHL) completed in assigned intervention condition. Individual waveforms represent the pain-related waveform at vertex electrode E19. Vertical dashed line marks the onset of the respective stimulus. The stimuli were applied at time 0, epoch includes 500-milliseconds prior to stimulus and 1000-milliseconds following stimulus. Plots scaled from -60 to 60 μV. Negative is plotted upwards.

[a]Failed time-locking of NN2 stimulus to EEG recording.

[b]Failed time-locking of NN2 stimulus to EEG recording.

[c]Plot for NN1 scaled from -100 to 100 μV.

[d]Plot plot for NN1, NN2, NHL scaled from -100 to 100 μV.

[e]Plot for NHL scaled from -100 to 100 μV.

[f]Plot for NN1 scaled from -100 to 100 μV.

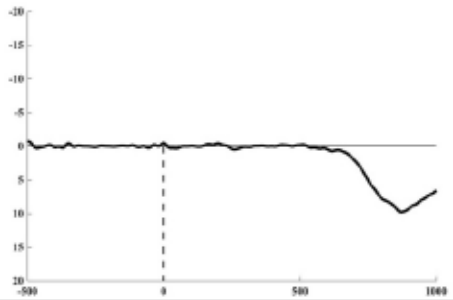
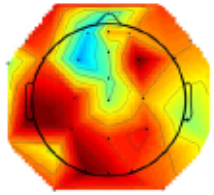
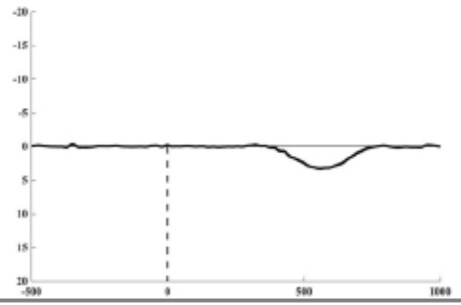
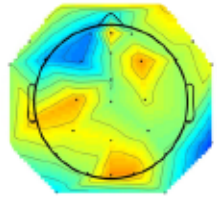
[g]NN1 not completed as baby fussy, therefore, transferred to skin-to-skin contact (SSC) with mother immediately.

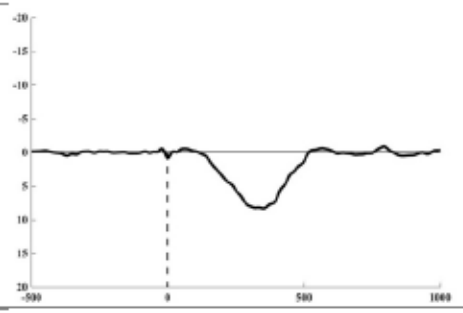
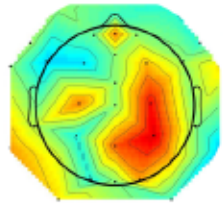
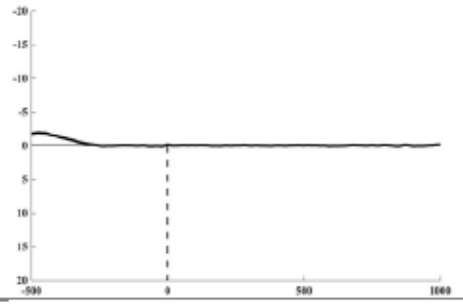
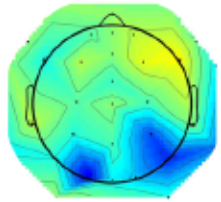
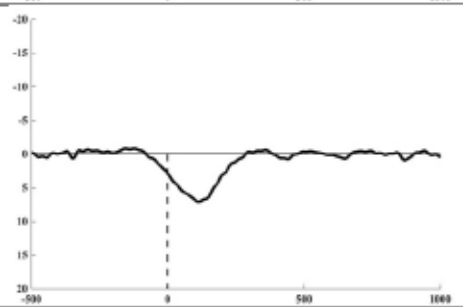
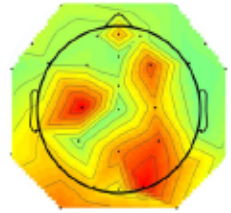
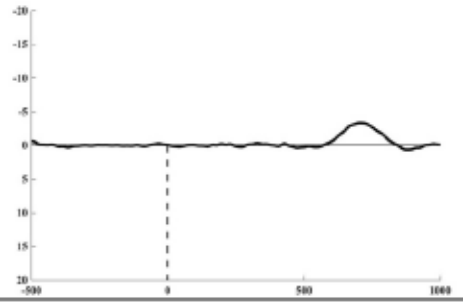
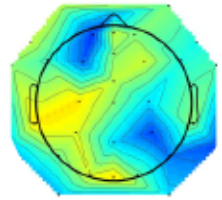
[h]Plot for NHL scaled from - 100 to 100 μV.

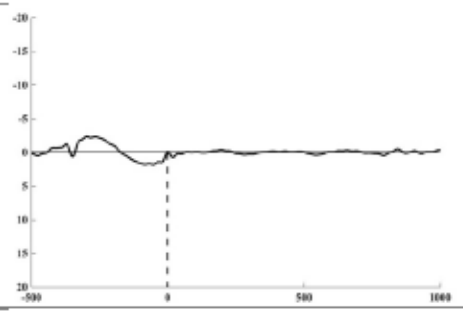
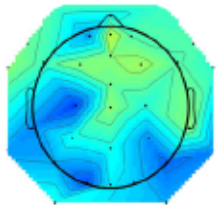
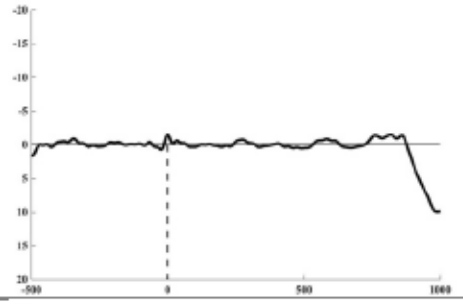
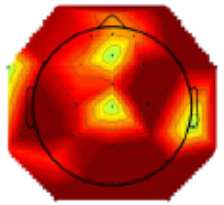
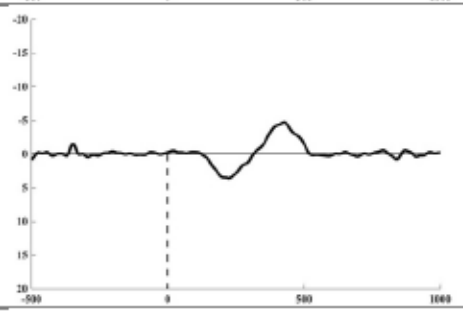
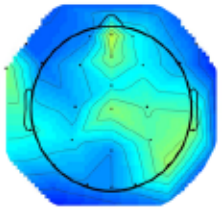
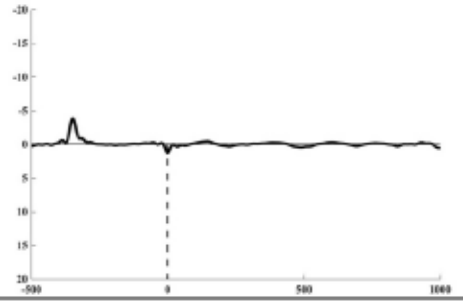
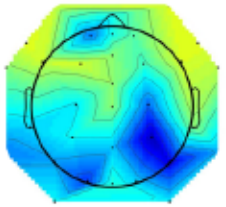
Appendix Q

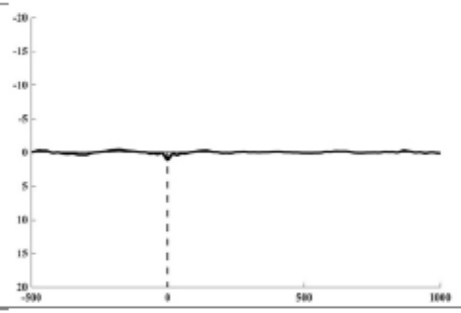
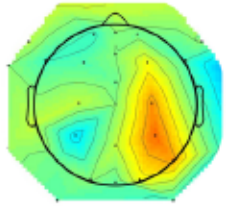
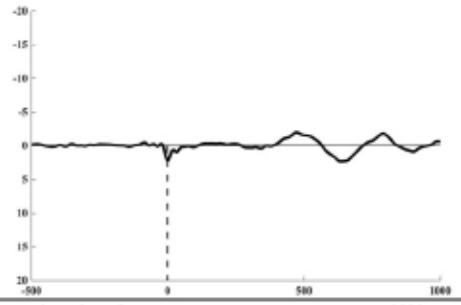
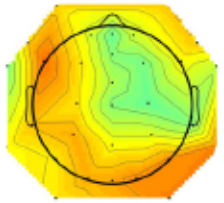
PCA Factors of Temporal Principal Component Analysis of Subject Data Following Noxious Heel Lance

Temporal principal component plots following noxious heel lance

Factor number		Variance (%)	Peak latency (ms)	Peak electrode		Spatial topographic plot
				+	-	
PC1		23.35%	872	E8	E2	
PC2		18.50%	559	E22	E6	

PC3		14.77%	351	E20	E20	
PC4		8.93%	-475	E8	E19	
PC5		8.44%	114	E29	E13	
PC6		6.76%	706	E16	E22	

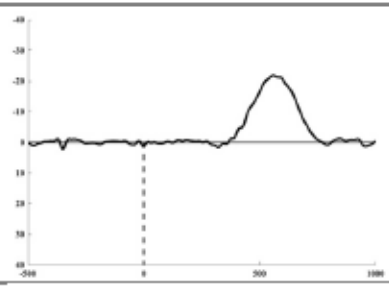
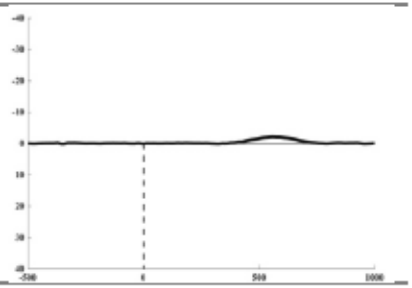
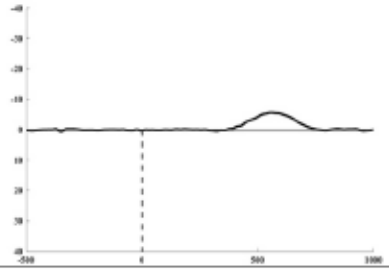
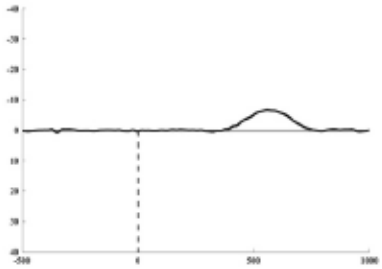
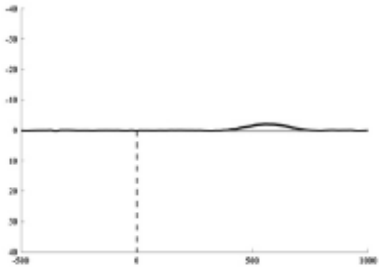
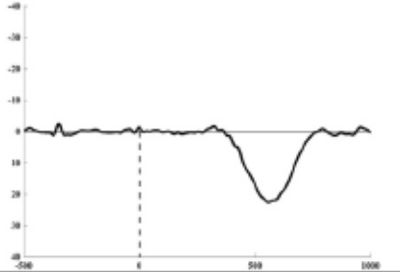
PC7		3.31%	-297	E22	E1	
PC8		3.28%	991	E24	E13	
PC9		2.25%	427	E30	E16	
PC10		2.22%	-348	E3	E5	

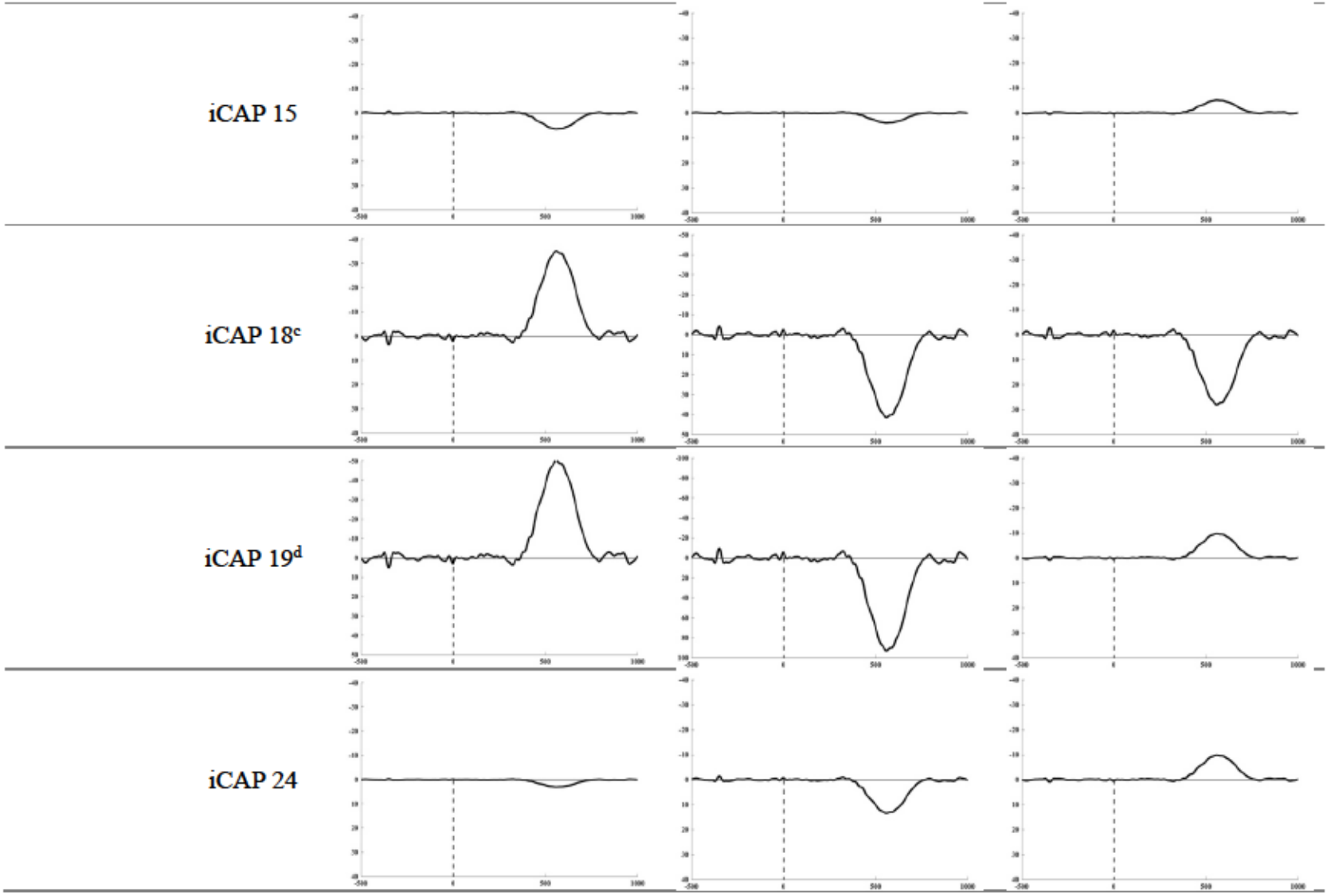
PC11		1.47%	635	E8	E13	
PC12		1.42%	-472	E8	E8	

Note. $n = 20$; PC = principal component; ms = milliseconds. Temporal PCA with promax rotation and Kaiser loading weighting on the 1500-millisecond epoch surrounding the noxious heel lance and the non-noxious control stimuli. Time points were considered as variables and electrode sites and subjects were considered observations. The covariance matrix was selected as the association matrix. Contribution of individual component variance represents individual component contribution to total variance in average waveform in 1500-millisecond epoch following application of tactile noxious and non-noxious stimuli. Total variance represented in 12-factor structure PCA = 94.7%. Topographic spectral plot of power presented at point of maximum amplitude of the PC waveform.

Appendix R

Individual Participant Principal Component Waveforms in Response to Non-noxious Control Stimuli and Noxious Heel Lance

Intervention condition	Participant	Non-noxious control 1	Non-noxious control 2	Noxious heel lance
24% oral sucrose and offered non-nutritive sucking	iCAP 04[a]		No data available	
	iCAP 08[b]		No data available	Automatically excluded from the PCA due to poor data quality
	iCAP 14			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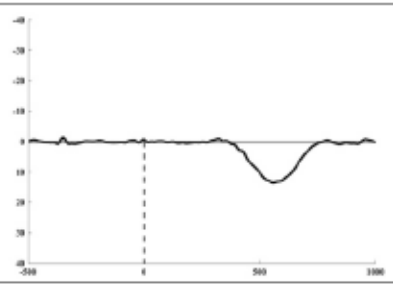
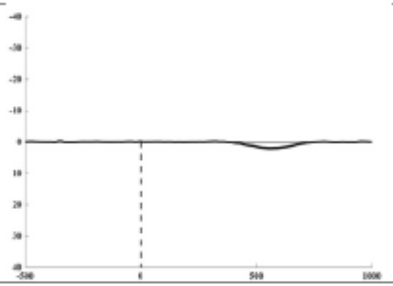
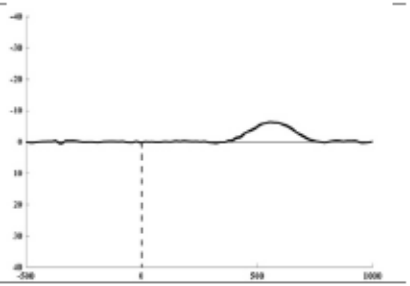
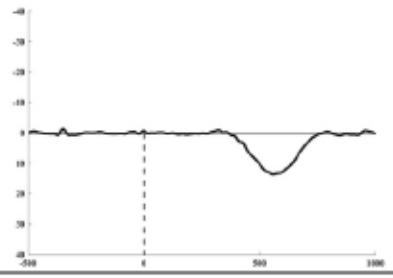
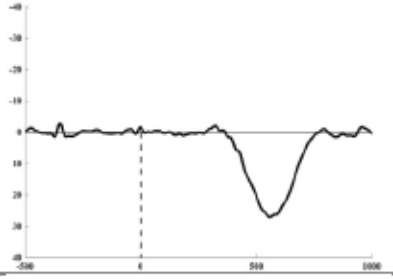
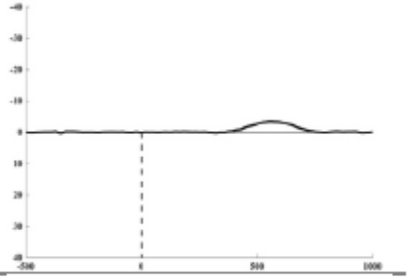
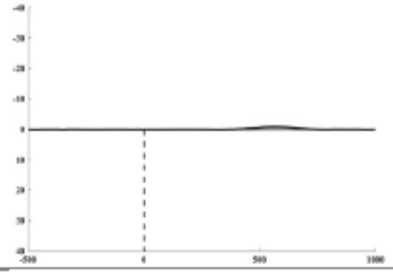
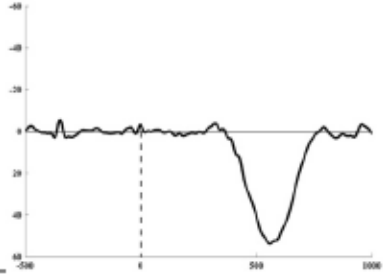
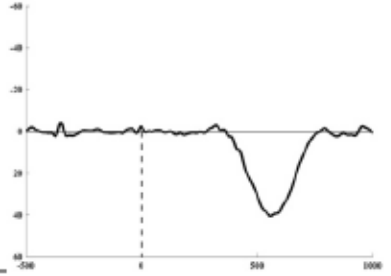
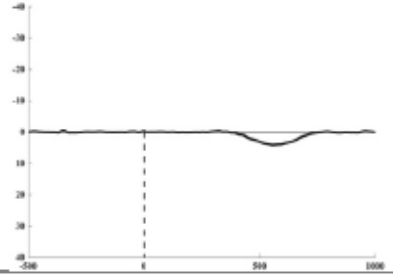
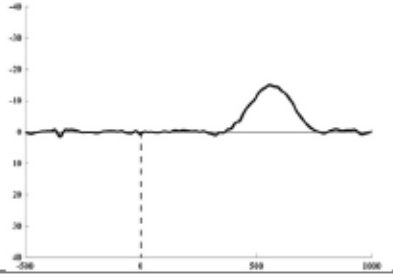
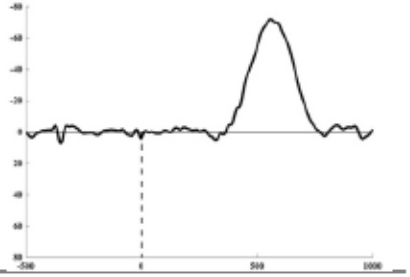
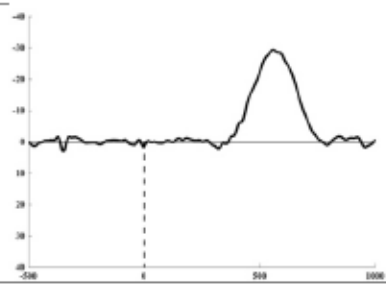
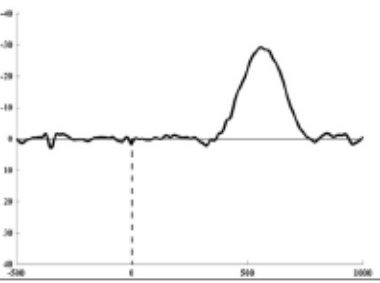
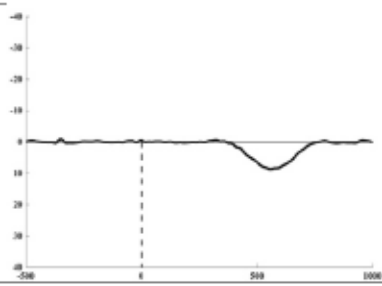
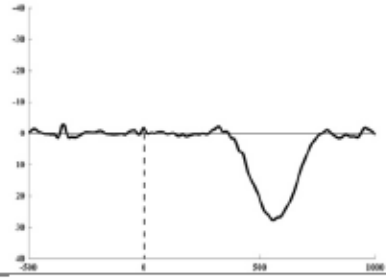
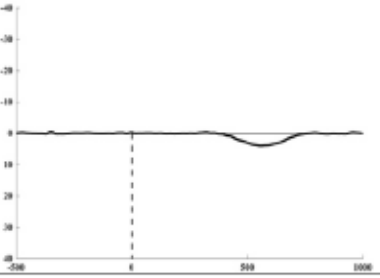
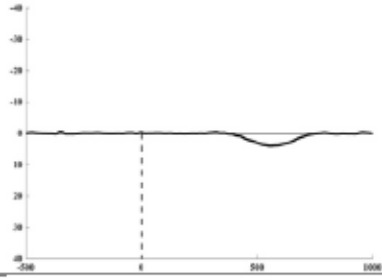
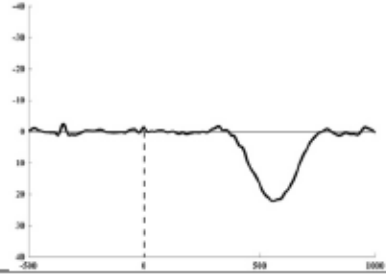
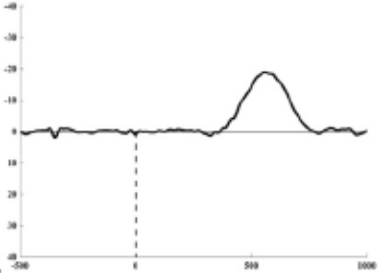
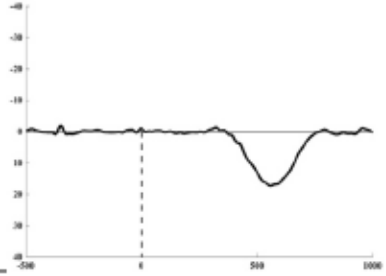
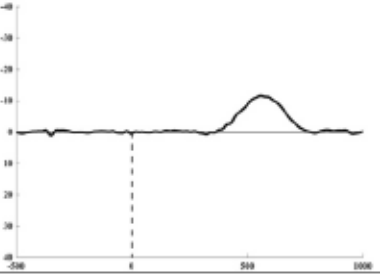
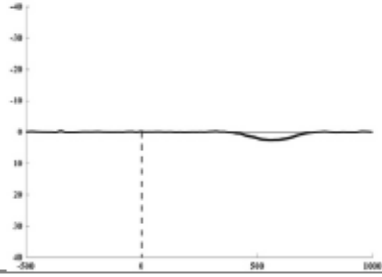
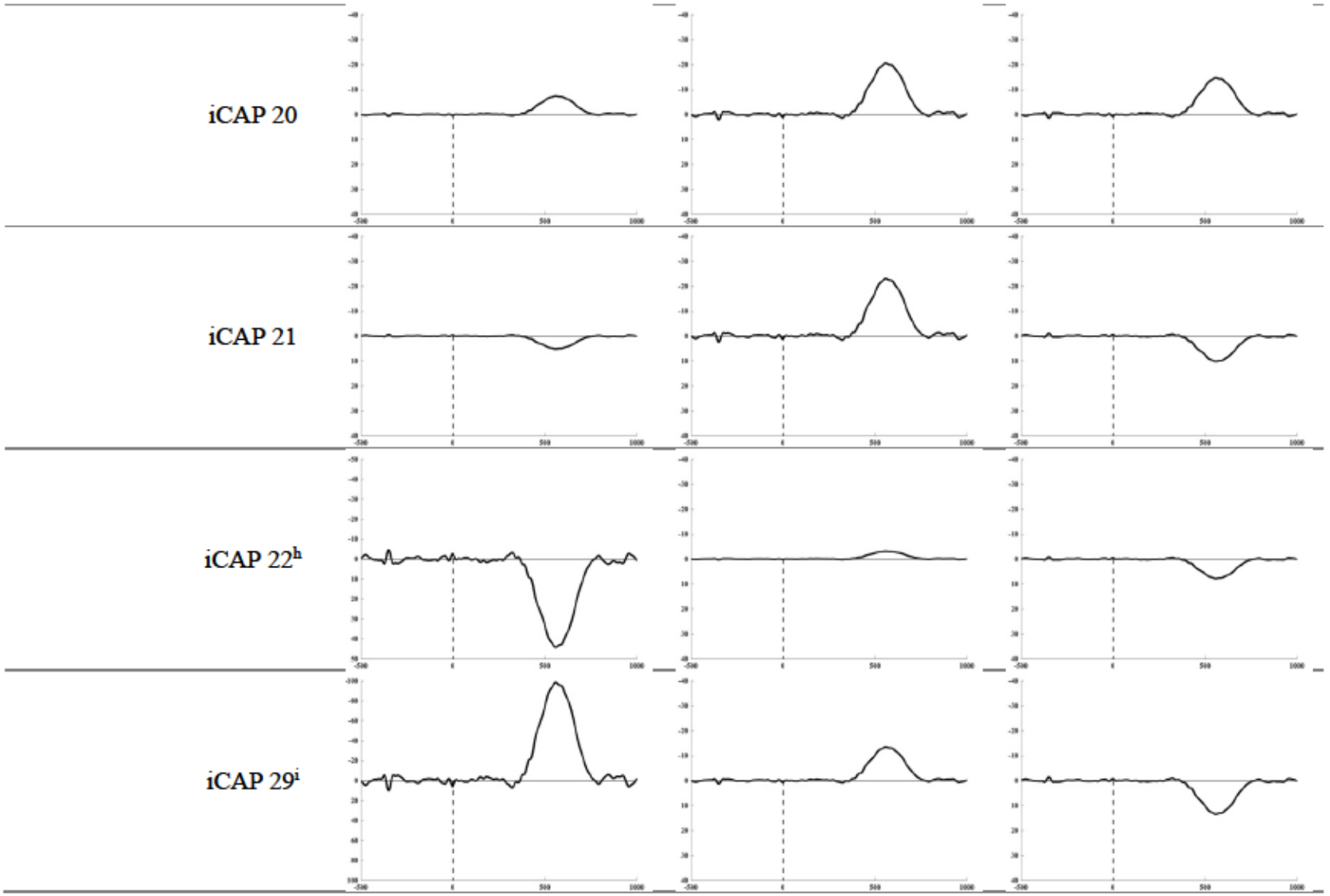
iCAP 18c
iCAP 19d
iCAP 24

	iCAP 25			
	iCAP 33			
	iCAP 36[e]			
Breastfeeding	iCAP 06[f]			

iCAP 07				
iCAP 12				
iCAP 13				
iCAP 17[g]	No data available			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iCAP 21
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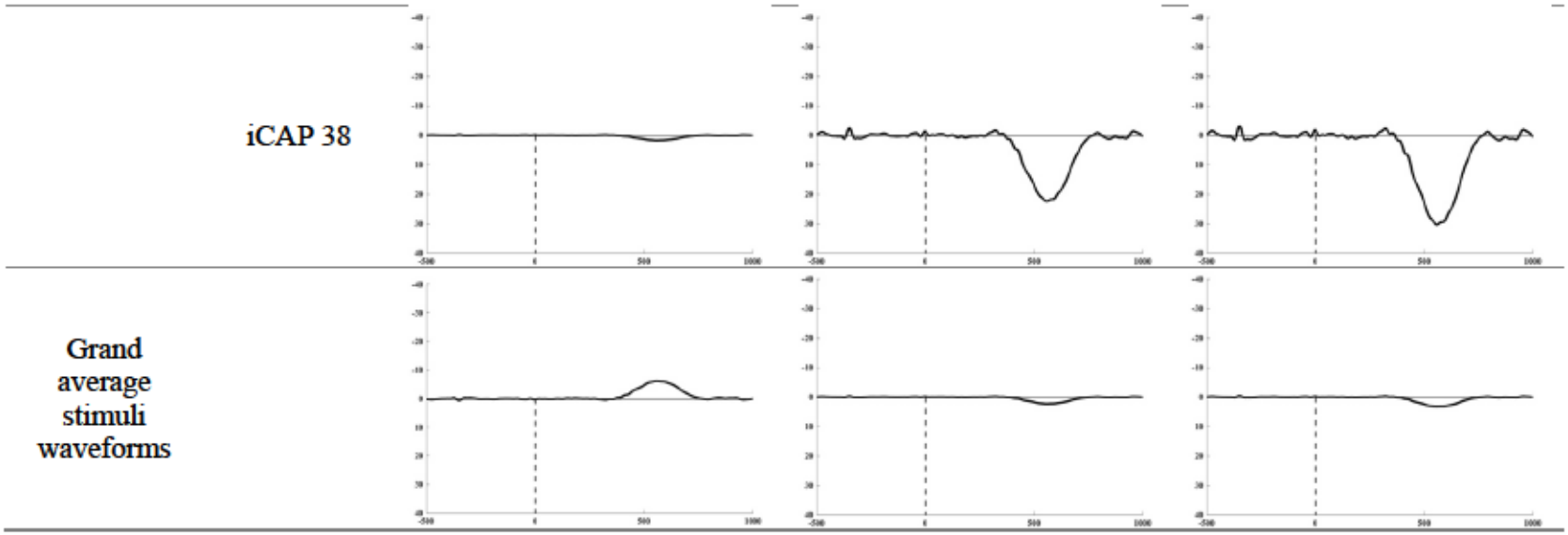
iCAP 29i



Note. Table presents individual participant principal component waveforms following application of non-noxious control stimulus 1 (NN1 [applied prior to initiation of assigned intervention]), non-noxious control stimulus 2 (NN2 [applied after the initiation of assigned intervention]), and noxious heel lance (NHL) completed in assigned intervention condition. Individual principal components represent the pain-related principal component at vertex electrode E19. Vertical dashed line marks the onset of the respective stimulus. The stimuli were applied at time 0, epoch includes 500-milliseconds prior to stimulus and 1000-milliseconds following stimulus. Plots scaled from -40 to 40 μV. Negative is plotted upwards.

[a]Failed time-locking of NN2 stimulus to EEG recording.

[b]Failed time-locking of NN2 stimulus to EEG recording.

[c]Plot for NN2 scaled from -50 to 50 μV.

[d]Plot plot for NN1 scaled from -50 to 50 μV, NN2 from -100 to 100 μV.

[e]Plot for NN2 scaled from -60 to 60 μV, NHL from -60 to 60 μV.

[f]Plot for NHL scaled from -80 to 80 μV.

[g]Plot for NN2 scaled from -60 to 60 μV. NN1 not completed as baby fussy, therefore, transferred to skin-to-skin contact (SSC) with mother immediately.

[h]Plot for NN1 scaled from -50 to 50 μV.

[i]Plot for NN1 scaled from -100 to 100 μV.