NON-PHARMACOLOGICAL PAIN RELIEF INTERVENTIONS AND CONTEXTUAL FACTORS INFLUENCING PAIN RESPONSE IN PRETERM INFANTS: ARE WE MEASURING WHAT WE INTEND TO MEASURE?

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Gila Sellam

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auf Antrag von:

Fakultätsverantwortliche:	Prof. Dr. S. De Geest
Dissertationsleitung:	Dr. E. Cignacco
Externe-Dissertationsleitung:	Prof. Dr. S. Engberg
Externe-Referat:	Prof. Dr. A. Grob

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Dekan Prof. Dr. med. Christoph Beglinger

Layout: Arlette T. Bernasconi

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

BPSN	Bernese Pain Scale for Neonates
B-BPSN	Behavioral BPSN scores
CFs	Contextual Factors
CONSORT	Consolidated Standards of Reporting Trials
CPAP	Continuous Positive Airway Pressure
df	degrees of freedom
FT	Facilitated Tucking
GA	Gestational Age
HPA	Hypothalamic-Pituitary-Adrenocortical
NFCS	Neonatal Facial Coding System
NICU	Neonatal Intensive Care Unit
NIPS	Neonatal Infant Pain Scale
NIRS	Near Infrared Spectroscopy
NIDCAP	Neonatal Individualized Developmental Care Assessment
NPIs	Non-Pharmacological Interventions
PAMINA	PAin Management In NeonAtes
P-BPSN	Physiological BPSN scores
PIPP	Premature Infant Pain Profile
PMA	Post-Menstrual Age
PNA	Post-Natal Age
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RCT	Randomized Controlled Trial
SD	Standard Deviation
T-BPSN	Total BPSN scores

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SUMMARY

SUMMARY

Preterm birth is one of the most significant worldwide problems in perinatology. The limited viability of preterm infants have been advanced thanks to the ongoing development of neonatal intensive medicine, although the rates of mortality and morbidity vary according to gestational age. Beside mortality the challenges of preterm birth are associated with short-term morbidity during the neonatal period and moderate to severe long-term morbidity, such as childhood disabilities [1,2] and high financial burdens for the society [1]. Preterm infants are spending the first period of their life in a neonatal intensive care unit (NICU), which is critical for their survival. However, this period is characterized by repeated pain exposure, which is occurring during the critical window of central nervous system development [3,4]. Recurrent pain exposure during this critical time is associated with permanent changes in peripheral, spinal and supraspinal pain processing, neuroendocrine function and neurologic development [5,6]. Furthermore, these changes can be manifested by alterations in pain thresholds, stress responses, cognitive function, behavioral disorders, and long-term disabilities in learning and dampened behavioral pain response [4,7-19]. This information brought the pain expert community to recognition that efficient pain management in this population is critical for their future development.

The average number of painful procedures preterm infants are exposed to in a NICU stands on ±14 procedures per day [20-22], while most of these procedures being associated with minor to moderate pain. While pharmacological pain relief agents are appropriate for severe pain management, they are not adequate to manage minor and routine painful procedures such as heel-stick [20]. Furthermore, most of pain medications used for preterm infants in NICUs are off-label or

unlicensed for use in this population [23]. Therefore, non-pharmacological pain relief interventions are proposed to overcome these challenges. These interventions include methods that involve reducing the sensitivity of the neonates during and after minor painful procedures (e.g. sucrose, non-nutritive sucking, kangaroo care, and facilitated tucking) [24,25]. A number of studies have reported the efficacy of non-pharmacological pain relief interventions. Most of these studies, however, examined single painful events. Information is lacking regarding the comparative effectiveness of these interventions and their efficacy over time. In order to provide a basis for efficient pain management, comprehensive pain assessment is required.

Pain assessment has gone through major development in the past decades [26], with more than two-dozen pain assessment instruments developed and evaluated. The recommendation is for comprehensive multidimensional assessment method, which include both behavioral (cry, facial expression) and physiological (heart rate, oxygen saturation) measures [27]. Despite the major advances in pain assessment in neonates, challenges in understanding the behavior of pain in preterm infants remain. Pain response in preterm infants is variable within and between infants [22,28], and weak correlations are repeatedly reported between behavioral and physiological responses [28-30], which makes clinical interpretation of pain scores difficult. These phenomenon reinforce the belief that pain response in these vulnerable infants seems to involve more than the invasive procedure itself but is further influenced by demographic and medical contextual factors [20,22-24,45]. In the past years the scientific pain expert community has widely recognized the issue of contextual factors associated with pain response. The results in the existing literature indicate that the contextual factors consistently associated most with pain responses of preterm infants are age related factors [31-40], previous pain exposure [15,31,34-36,39,41,42], and severity of illness [15,30,34-36,41-43]. However, findings even in relation to these contextual factors are not consistent across studies. One explanation for this inconsistency is the varying methodological approaches used in these studies. Therefore, further research is needed to determine which contextual factors are most strongly associated with pain response and to progress one step further with more comprehensive pain assessment instruments.

The overall purpose of this project was to compare the effectiveness of two non-pharmacological pain relief interventions over time, and to explore the association between medical and demographic contextual factors and pain response of preterm infants under the impact of non-pharmacological pain relief interventions during repeated routine heel-stick procedures.

This thesis includes 7 chapters:

Chapter I presents a comprehensive introduction into the relevance of pain in neonates, particularly in preterm infants. The chapter gives an overview of the problem of premature neonatal pain within the context of neurologic development in preterm infants. This leads to the issue of the serious short and long-term consequences of high pain exposure during the neonatal period. The challenges in pain assessment are described within the complexity of the neonatal pain experience in the NICU, leading to the importance of pain management with non-pharmacological pain relief interventions. The last part of the introduction presents the theoretical framework this study was based on. **Chapter II** describes the specific aims of the dissertation.

Chapter III presents a publication of the results of the parent study PAMINA (PAin Management In NeonAtes). PAMINA is multicenter randomized control trial (RCT) that aimed to compare the effectiveness of non-pharmacological pain relief interventions; oral sucrose and facilitated tucking, across 5 heel-stick procedures in preterm infants aged between 24 and 32 weeks of gestation. Seventy-one infants were randomly allocated to one of three interventions: sucrose, facilitated tucking, or the combination of both interventions. Four experienced nurses, blinded to the phase of the heel-stick (baseline, heel-stick, and recovery) assessed pain with the Bernese Pain Scale for Neonates (BPSN). The results show that sucrose with and without facilitated tucking had pain-relieving effects even in preterm infants younger than 32 weeks of gestation and remained effective across time.

Chapter IV presents the publication of a commentary article about the intervention of facilitated tucking. In this manuscript we raise the question of the clinical feasibility of facilitated tucking, which requires additional manpower. In light of economic restraints of the health care system, and the lower effectiveness of facilitated tucking compared to sucrose, this commentary encourages re-evaluating the recommendations regarding methods such as facilitated tucking and further recommending for comparative effectiveness studies of non-pharmacological pain relief interventions.

Chapter V presents the results of a systematic review, which examined studies investigating the impact of contextual factors on pain response of heel-stick procedures in preterm infants. A total of 23 studies meeting inclusion criteria were included in the review. The studies varied relative to their design, sample, analysis procedures, and variables examined. Six categories of contextual factors emerged: age, pain exposure, health status, therapeutic interventions, behavioral status, and demographic factors. The review supports the influence of some contextual factors on pain response with the factors most consistently related to pain response being age related factors, previous pain exposure and severity of illness. The examined contextual factors varied in the strength of their association with pain response, and none were consistently related, as evidenced by contradictory findings. In some cases the inconsistencies appeared attributable to the methodological heterogeneity of the studies included in the review. The results of the review also support the low correlation between behavioral and physiological pain responses, and the need for further investigation of contextual factors, to better understand their influence on pain response.

Chapter VI presents the results manuscript of the exploratory sub analysis of the PAMINA study. This study aimed to explore the association of contextual factors with pain response of preterm infants receiving non-pharmacological interventions for repeated heel-stick procedures. In total 10 demographic and medical CFs were extracted from medical charts over the first 14 days of life. In this study we confirmed the low correlation between behavioral and physiologic pain scores in preterm infants. The results of the study emphasize that higher exposure to

painful procedures; male infants and having CPAP or mechanical ventilation were the contextual factors associated with physiological responses**.** The only variables that were significantly associated with the behavioral scores of the Bernese Pain Scale for Neonates, were Apgar scores at 1 and 5 minutes, however these relationships were inconsistent. In this study we examined a variety of contextual factors that previous studies have suggested may influence preterm infants' pain responses. The use of multivariate analysis while controlling for potential confounders allowed us to examine the independent contribution of each examined contextual factor in explaining pain responses. Furthermore, we utilized a pain assessment instrument that allowed us to examine the impact of the contextual factors on both behavioral and physiologic pain responses. Our findings also add to the growing body of research that suggests the need to considering contextual factors when assessing pain in this population. However, given that findings about the impact of CFs are mixed across studies, additional multicenter research including large sample is needed to determine the contextual factors that need to be incorporated into pain assessment instruments.

Finally in **Chapter VII** the results of all study parts are synthesized and discussed, followed by suggestions for further research and clinical practice development. Pain assessment and management remains a major challenge in preterm infants. The findings of this dissertation support the efficacy of sucrose over time and recommend it over facilitated tucking. While our findings support the importance of considering contextual factors as influencing pain responses in this vulnerable population, the specific contextual factors that need to be incorporated into pain assessment scales remains unclear. Our findings raise important methodological issues that need to be considered as future studies are designed to examine the impact of contextual factors on pain responses of preterm infants.

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CHAPTER I

INTRODUCTION

1. INTRODUCTION

1.1 PROBLEM STATEMENT

Preterm birth is defined as birth occurring before completing 37 weeks of gestation [1]. Preterm birth rates have increased since the early 1980s both worldwide (9.6%) and in industrialized countries including the USA (12-13%) and European countries (5-9%) with Switzerland having a prematurity incidence rate of 7.1% [2-6]. Preterm birth is one of the most significant problems in perinatology. These infants require neonatal intensive care in order to ensure their survival and improve their outcomes. After neonatal intensive care unit (NICU) admission, preterm infants often face increased medical challenges and are neurodevelopmentally less prepared to cope with multiple stimuli such as recurrent procedural pain.

Preterm infants, particularly the youngest and most vulnerable ones with extremely low birth weight are exposed to both major and minor painful procedures [7,8]. Findings from recent studies suggest that neonatal exposure to pain is extremely high during their hospitalization in the NICU and that the pain relieving methods utilized are often inadequate [7,9-11]. Although the number of painful procedures that a neonate in the NICU is exposed to has decreased during the past decades and use of analgesic has increased [12], pain management during NICU procedures still generally falls short of current pain management guidelines [12,13]. Some NICUs still have no standard protocols of pain management during routine minor painful procedures such as heel-sticks [14]. Inadequate pain management is known to have negative effects on the motor and cognitive development of this patient population [15,16]. Preterm infants' vulnerability related to inadequate pain management is associated with their neurologic immaturity and the high plasticity of their central nervous system. Repeated pain exposures during the critical windows

of central nervous system development can lead to chronic changes of the cytoarchitecture of the brain. These changes can be manifested in later childhood by alterations of cognitive function, behavioral disorders and long-term disabilities [17-19].

1.2 NEUROLOGIC DEVELOPMENT OF PRETERM INFANTS

Pain as an unpleasant sensorial experience has been scientifically recognized in neonates only since the mid 80ies [20]; this recognition led to a paradigmatic change in the perception of pain in neonates. Since then, it is widely accepted that fetal pain perception begins in the third trimester of pregnancy [21-24]. Behavioral pain expression is seen in preterm infants as young as 24 weeks gestation [25,26]. It has also become clear that the nervous system undergoes extensive postnatal development [27] and there is increasing evidence that repeated pain exposure as a result of common therapeutic or diagnostic procedures in the NICU setting may lead to serious alterations during the sensitive developmental phase of the central nervous system, as demonstrated in many human and animal studies [28-39].

Nociceptive neural circuits are formed during the embryonic and the early postnatal period, a time when painful events are normally absent or limited. In a series of animal experiments, Ruda and colleagues [29] found that peripheral localized pain and inflammation experienced during the neonatal period has an impact on the nociceptive neural circuitry development, which is responsible of pain processing in the spinal dorsal horn. Furthermore, peripheral tissue injury can lead to a state of hyperalgesia and allodynia in which noxious responses are enhanced [40] and general sensitivity to subsequent pain is altered [21].

One of the reasons for the extreme vulnerability of the developing premature brain is its neuroplasticity. Neuroplasticity can be defined as the property the brain has in terms of the extent to which nerve cells and neural networks are able to modify their structure, network connectivity and/or operating mode in response to changes in intrinsic (genetic or lesions) or extrinsic (environmental changes) events. This malleability of the brain structure and

function is mainly attributed to the developing brain in premature newborns [41]. The developing nervous system is in a critical vulnerable period of growth from the 20th week of gestation through the first 18-24 month of life. This period is characterized by dendritic arborization, axonal growth, myelination, peak synaptogenesis, gliogenesis, and maturation of the mechanisms and structures involved in synapsis neurotransmission [42,43].

There is some evidence to suggest that preterm neonates may be more sensitive to pain than more mature infants. They show a lower tactile threshold than term infants with additional decreases in the threshold after repeated exposure to painful stimuli [44,45]. Furthermore, there is increasing evidence that repeated exposure to pain may lead to serious changes during the sensitive developmental phase of the central nervous system, as demonstrated in several human and animal studies [28-39] and that tissue injury, e.g. related to therapeutic or diagnostic procedures, in the early neonatal period can cause profound and long-lasting changes in the pain thresholds and subsequent patterns of pain processing [17-19]. The frequency of exposure to pain and the adequacy of pain management are, therefore, critical issues in the care of preterm infants both in terms of their comfort and motor and cognitive development.

1.3 THE INFLUENCES OF PAIN ON THE DEVELOPING BRAIN AND ON BEHAVIORAL PATTERNS OF PREMATURE INFANTS

1.3.1 Short term effects of pain exposure on physiologic and behavioral changes

Studies in animals and humans have shown a number of negative short-term consequences of painful procedures during the premature neonatal phase. These consequences include decreased levels of plasma cortisol [35]; increased levels of catecholamines, aldosterone, glucagon, and growth hormone [21,46]; decreased oxygen saturation [47-49]; increase in heart rate [47,49-52]; higher sensitivity to cutaneous stimuli [44]; and transient (5-60 seconds) decreases in cerebral blood flow of 20% to 50% [36]. Despite the negative effects of exposure to repeated noxious

procedures, studies have shown that preterm infants' behavior pain responses are often blunted. Johnston and Stevens [49] reported that infants who had undergone frequent invasive procedures during their hospitalization displayed less intense facial responses to heel-stick procedures when compared to infants who had undergone fewer procedures. A possible explanation for this change of behavioral patterns is the synactive theory proposed by Als [53-55]. The theory assumes that preterm infants' behavior is their primary route for communicating stress. However, during their hospitalization in NICU preterm infants' behavior becomes disorganized under the extreme stress of preterm birth followed by intensive care treatment. As a consequence of the exhaustion resulting from extreme stress and repeated painful procedures, they are not able to respond coherently to noxious stimuli. Fitzgerald [40] provided explanation for this altered pain reactivity in premature neonates based on understandings of the physiological processes, which shape the newborn pain response. Animal and human studies have demonstrated that reflex thresholds are low in newborns and there is an absence of the normal inhibitory control that mature brain structures would exert. As a result, premature neonates display more diffuse responses to noxious stimuli rather than more complex affective reactions [27]. Further evidence from both animal and human studies suggest that the early and repetitive pain experiences of premature newborns may affect future pain processing by altering nociceptive circuitry in the CNS [40]. A study by Grunau et al. [56] examined demographic and therapeutic determinants of pain responses in 136 preterm infants between 23 to 32 weeks of gestation. They showed that diminished behavioral and autonomic pain responses were primarily a function of high exposure to previous invasive procedures and gestational age at birth. The authors further elucidated how much pain experience is required to shift the subsequent response. Exposure to 20 invasive procedures may be enough at this stage of development to convert an infant from a stimulus-naïve responder to a stimulus non-naïve responder. Cignacco et al. [9] examined the number of procedures ventilated preterm infants were exposed to during their first 14 days of life. They found that the infants were exposed to an average of 23 procedures a day of which 17 were associated with pain. Based on these findings

and those of Grunau et al. [56] it can be assumed that infants are converted from stimulus-naïve responder to a stimulus non-naïve responder within the first 2 days of NICU hospitalization and are, therefore, at high risk for negative consequences.

1.3.2 Long lasting effects of pain exposure on ex-premature infants

The long-term consequences of pain for preterm infants are mainly related to the high plasticity of the developing premature brain [40,57]. Evidence suggests that early experiences with pain are associated with altered pain responses later in infancy [28,30,31,37,38,58,59]. Furthermore, animal research suggests that some of these responses may last into adulthood [40] due to the influence of pain on the nociceptive pathways [60,61]. The type and effect of the alteration in pain responses depend on the developmental maturity of the infant at the time the pain occurred, clinical factors such as the length and extent of exposure to pain, and other contextual factors (medical and environmental) during the time of pain exposure [62]. In studies of newborn animals, skin wounds triggering pain had prolonged nociceptive effects. Wounds remained hypersensitive long after they healed [63], and the increases in the size of the dorsal horn receptive field were present for about six weeks after the injury [64]. In studies of rats, repeated skin breaking and heat injuries lead to generalized hypoalgesia in adulthood [65,66]. In other studies, this hypoalgesia effect was only seen when the mechanical and thermal injury occurred within the first 10 days of life [39,40]. In a human study by Andrews and Fitzgerald it was found that receptive fields are wider the younger the gestational age (GA) of the infant is, which results in a more widespread sensitivity [44].

Preterm birth results in long hospitalization of the infant who is physiologically unprepared for stress exposure outside the protective intrauterine environment [67]. Hermann and colleagues [58] compared 19 former preterms (GA $\leq$31 weeks) to 20 full-term children (both groups undergone NICU hospitalization), and 20 healthy full-term control children (at the time of the study all children were 9-14 years old). They found that the NICU groups had significant enhanced sensitization to painful thermal stimulation compared to the term control group. Repeated pain experience during the neonatal period may induce changes in the functioning of pain pathways

that persist way beyond infancy. Former preterm infants had significantly more tender points and lower tender point thresholds at 12 – 18 years of age than former full-term infants [59]. There is evidence to suggest that repeated pain exposure during the neonatal period may lead to cognitive limitations in learning as well as behavioral consequences (more fear during surgery in childhood as well as signs of post-traumatic stress disorders) [16]. Other studies reported subtle alterations in parasympathetic, sympathetic and initial behavioral reactions to acute pain in former low birth weight infants [31,37,38]. Grunau et al. [15] proposed mechanisms by which perinatal pain in preterm infants may lead to a long-term negative impact on normal brain development. One of these mechanisms is that excitoxic damage may lead to altered apoptosis (programmed cell death) and neuronal survival. Bhutta et al [68] conducted a meta-analysis of 15 case-control studies examining cognitive and/or behavioral data of children born prematurely and evaluated after their fifth birthday. Children born prematurely had significantly lower cognitive test scores ($p<.001$) than control children and there was a significant correlation between test scores and birth weight (R^2 = 0.52, $p < 0.001$) and GA (R^2 = 0.49, $p < 0.001$). In addition, children born preterm had a 2.64 increased risk of developing ADHD (attention deficit hyperactivity disorders) compared to the control children and manifested internalizing (e.g. anxiety, depression) or externalizing (e.g. hyperactivity, delinquency) behaviors more often than control children. Although these studies did not specifically examine the impact of prenatal pain on later cognitive and behavioral function, it may be one of the multiple components of the NICU experience (pain, severity of illness, medication exposure, etc.) that cumulatively have a negative impact on later function [15].

To conclude, pain exposure during this vulnerable period of central nervous system development is critical and needs to be addressed by comprehensive pain management strategies (which includes assessment and treatment). Pain management in preterm infants has been, and still is, a major challenge as it is lacking with a systematic approach despite the existing recommendations. Furthermore, due to the extreme immaturity of this population, pain response is highly variable and may involve many external factors, which hamper (effective) pain assessment and treatment.

1.4 PAIN MANAGEMENT

1.4.1 Challenges of pain assessment in preterm infants

A new conceptualization of pain by McCaffery [69] about 40 years ago made patients the experts in their pain by defining self-report as the pain assessment gold standard; "Pain is what the person says it is and exists whenever he or she say it does". However, in a non-verbal population such as neonates, this definition cannot be applied. As a result, neonates are highly depending on pain assessment performed by health care providers.

Many pain assessment instruments have been created to measure pain in this population. The purpose of the measurement of pain is to discriminate between pain and no pain, and to provide a picture of experienced pain that is as complete as possible and include some quantification of the pain. Measurement and assessment of pain are crucial in protecting the neonate from harm and deleterious consequences of the many painful diagnostic and therapeutic procedures in the clinical settings. Unfortunately, to-date none of the existing instrument have been designated as the gold standard. Despite tremendous advances in the development of pain assessment instruments for neonates, the challenge of measuring pain in preterms remains as a result of infants' inability to verbally report their level of pain [70] and their responses being less vigorous, more variable and less consistent than term infants [71]. In light of the infants' inability of self-report, pain assessment instruments must be multidimensional incorporating both physiological and behavioral indicators [70] which will provide maximal information [72]. A multidimensional measurement approach can be accomplished by simultaneously employing both subjective (e.g. assessment of facial activity) and objective (e.g. heart rate) data and by utilizing multiple dimensions within a particular measurement domain[73]. Behavioral responses to pain can be measured with crying, facial activity and body movements. Common physiological responses to pain are assessed through changes in heart rate, respiratory rate, blood pressure, oxygen saturation, and palmar sweating [74]. Johnston and Stevens [49] reported that while preterm infants hospitalized in NICU demonstrated physiological

changes such as decreases in oxygen saturation and increases in heart rate in response to pain, their behavioral responsiveness was blunted. The behavioral responses of the preterm infants in the Johnson and Stevens's study [49] were associated with the amount of exposure to pain, whereas the physiological responses were associated with the GA at birth and postnatal age (PNA). Many studies have explored the influence of contextual factors (CFs) such as GA, severity of illness related factors, behavioral state, and demographic factors on pain responses [71,72,74,75].

1.4.2 Contextual factors associated with pain response of preterm infants

Studies support the influence of CFs on pain responses in preterm infants. Several studies suggest that both GA and PNA affect behavioral pain response. Studies have reported that as GA increases, preterm infants' responsiveness to pain increases as well [49,52,75,76]. Gibbins et al. [48] compared pain responses in infants of different GAs. They collected physiological and behavioral data during a routine heel-stick from four age groups (<27 6/7 weeks; 28-31 6/7 weeks; 32-35 6/7 weeks and >36 weeks). All infants showed behavioral (facial) activities. The magnitude of response was proportional to the GA with the youngest group showing the least amount of changes. Decreases in oxygen saturation and increased heart rate were observed during the acute phase of the heel-stick with no statistically significant differences across age groups. These findings are supported by other studies, suggesting that the cephalocaudal development of facial musculature might influence the magnitude of facial activity as evidenced by the fact that preterm infant have less muscular strength, posture, tone, and body movement compared to term infants [35,47,77]. Both Johnston and Stevens [49], & Anand and Scalzo [78] found that the number of invasive procedures an infant had undergone was the greatest predictor of decreased behavioral response to pain. Johnston and Stevens compared two groups of preterm infants, one born at 28 weeks of gestation and assessed at 32 weeks the second born at 32 weeks and assessed within 4 days. The first group had an increased physiological response and a decrease behavioral response to pain. The primary factor that explained the results was former pain experiences. The Apgar score was the secondary factor related to pain response [49]. Reduced pain response based on number of painful procedures has a variety of explanations. In another study

of Johnston et al. [75] they stated that one of the explanations for the reduced response to pain, might be the proximity to the last painful procedure. They hypothesized that the production of endorphins in response to the first procedure might have protected the infant from the pain of the following painful procedure. Infants with a very low GA (24-28 weeks) were found to have a higher procedural exposure due to their general immaturity and need for a higher degree of intensive care interventions [9]. According to Johnston and colleges [75] the blunted pain responses in very preterm neonates may also be sign of exhaustion from all of the procedures and handling that the preterm infant experiences during hospitalization in the NICU. These CFs and others are suggested as explanatory factors for the variability in pain response between and within preterm infants and the low correlation between behavioral and physiologic responses [49,52,79]. Although there is a wide range of validated pain assessment instruments for neonates, pain assessment in preterm infants remains a challenge. Cignacco et al. [80] reported high intra-infant variability in pain responses across repeated heel-sticks, which make pain assessment even more challenging. One possible explanation for this variability is in variations of external factors that affect the behavioral and physiological parameters of pain. Another important issue to be considered in pain assessment is the findings of either no or weak correlations between behavioral and physiologic pain responses [70,76,79,81]. A low correlation was also reported between behavioral measures and cortisol [82]. This phenomenon of variability in pain responses may also be explained by the contextual factors as mentioned above.

1.4.3 Further pain assessment techniques

In addition to evaluating pain using observational pain instruments, other bio-physiologic methods have been proposed to measure pain responses. One of the methods proposed is cortisol measurement. Cortisol can be measured as a biomarker for pain-related stress response. In humans, cortisol is the primary glucocorticoid of the hypothalamic-pituitary-adrenocortical (HPA) system. The primary effect of cortisol is to stimulate hepatic gluconeogenesis, which increases the available energy [83]. Another effect is to stimulate brain tissue in the regions where cortisol and its precursors are located, which may in turn, influence emotions and learning [84].

Studies examining cortisol responses to pain in preterm infants are inconsistent with some studies reporting higher levels and others reporting lower levels. An additional method for physiological pain assessment is the near infrared spectroscopy (NIRS). NIRS is a non-invasive technique that can detect subtle changes in oxygenated (HbO_2) and de-oxygenated (HbH) hemoglobin. Studies comparing NIRS to functional magnetic resonance imaging or positron emission scans demonstrate that it is a reliable method of monitoring cortical activation during functional studies [89]. Bartocci et al. [85] used NIRS to determine whether acute pain activated the somatosensory cortex in preterm neonates. The painful procedure used during this study was a venipuncture. They demonstrated increases in HbO_2 concentrations in both hemispheres during the painful stimulation. However this technique is not well validated as a measure of pain in preterm infants and required more research.

To conclude, secondary to the complex nature of the pain response in preterm infants and consistent reports of low correlations between behavioral and physiological dimensions, one must use a comprehensive pain assessment approach that includes multiple dimensions. Multidimensional approaches to pain assessment may increase the probability of detecting pain in preterm infants, which is critical to effective pain management [70].

1.4.4 Pain treatment

Pain management is a basic human right [86], however, in infants is viewed as inadequate in the context of acute diagnostic and therapeutic procedures in the NICU [87,88]. Approaches to pain treatment in preterm infants include both pharmacological and non-pharmacological methods.

1.4.5 Pharmacological pain relieving interventions

Most of the pain medications used for preterm infants in NICUs are off-label or unlicensed for use in this population [89]. The pharmacological methods, which are mainly used for major procedural pain (e.g. surgery) in NICUs, include Morphine, Fentanyl, and Paracetamol. Morphine is the most widely used pharmacological agent in the NICU [9]. However, the efficacy of Morphine in managing

pain in preterm infants is unclear and can have many negative adverse effects. In a randomized controlled trial (RCT) [11] focusing on pain relief in ventilated preterm infants during endotracheal suction, Morphine was not associated with pain relief as measured by three pain assessment scales. Another RCT comparing placebo to Morphine for analgesia in ventilated preterm neonates failed to show any beneficial effect of Morphine infusion compared to placebo. In an open-label study, Morphine was associated with a significant increase in illness severity and a longer length of ventilation and NICU hospitalization compared to the placebo group [90]. While pharmacological interventions are considered appropriate for severe pain, they are too strong and not recommended for the treatment of minor and routine painful procedures (e.g. heel-stick) [7].

There are recurrent reports that an average preterm infant hospitalized in NICU undergoes a mean of 14 painful and uncomfortable interventions per day [8,10,79,91]. Recent studies report that pain is still poorly managed in NICUs [7,12] despite evidences about the negative short and long term consequences of pain. There is also a growing body of research that suggest that simple non-pharmacological methods provide effective pain relief during many routine NICU procedures [92,93].

1.4.6 Non-pharmacological pain relieving interventions

Non-pharmacological interventions (NPIs) for pain prevention and relief are increasingly recommended for routine minor painful procedures in the NICU. These interventions include methods that involve reducing the sensitivity of the neonates during and after minor painful procedures[92,93]. Many NPIs have been proposed to control pain in infants including sucrose breast-feeding, non-nutritive sucking, kangaroo care, facilitated tucking (FT), music, positioning, and swaddling. As our research compared sucrose and FT, we will focus on those two NPIs. The hypothesized mechanism underling the effectiveness of oral sucrose is that the sweet taste induces the release of endogenous opioids [94-98]. FT is gentle positioning of the infant's arms and legs in a flexed midline position (see figure 1 and 2 in chapter 4). This technique provides the infant with support and the chance to control his/her own body [99]. In a resent Cochrane review, Stevens et al.

[93] examined evidence about the efficacy of oral sucrose. Their findings from 41 RCTs showed that oral sucrose reduced pain associated with single minor painful procedure. In another Cochrane review of 51 RCTs examining the efficacy of NPIs (non-nutritive sucking related interventions, kangaroo care, swaddling, and FT), Pillai Riddell et al. [92] reported sufficient significant evidences supporting the effectiveness of these NPIs. However, most of studies on the effectiveness of NPIs in relieving pain have examined them for single painful procedure. There is little evidence about the effectiveness of NPIs over time.

To summarize, preterm infants are exposed to a very high number of painful procedures during their stay in NICU. A large body of evidence point to a short and long-term negative effects of this exposure. These findings beg for appropriate pain management of this vulnerable population. While NPIs such as oral sucrose and FT has been extensively evaluated and shown to be effective and safe for pain relief during single painful procedures [9,47,91,96,99-111], there is a lack of evidences regarding the effectiveness of these interventions over time. Moreover, pain measurement is a complex issue and there is growing evidence that pain response in preterm infants is variable and may be influenced by many external medical and demographic factors, so called Contextual Factors (CFs). The CFs mentioned the most in the current literature are GA and PNA [112], and the number of previous painful procedures that the infant undergoes across the time of hospitalization in the NICU [113]. Findings in relation to the impact of CFs on pain responses, are however inconsistent and requires further research.

There is a need for a deeper understanding of the factors that influence the response, assessment and treatment of pain in preterm infants. External factors are important in pain response as well as its assessment and treatment. In order to recognize the complexity of pain management, we embedded our research within a conceptual framework and choose to use the Socio Communication Model of Infants' Pain [114].

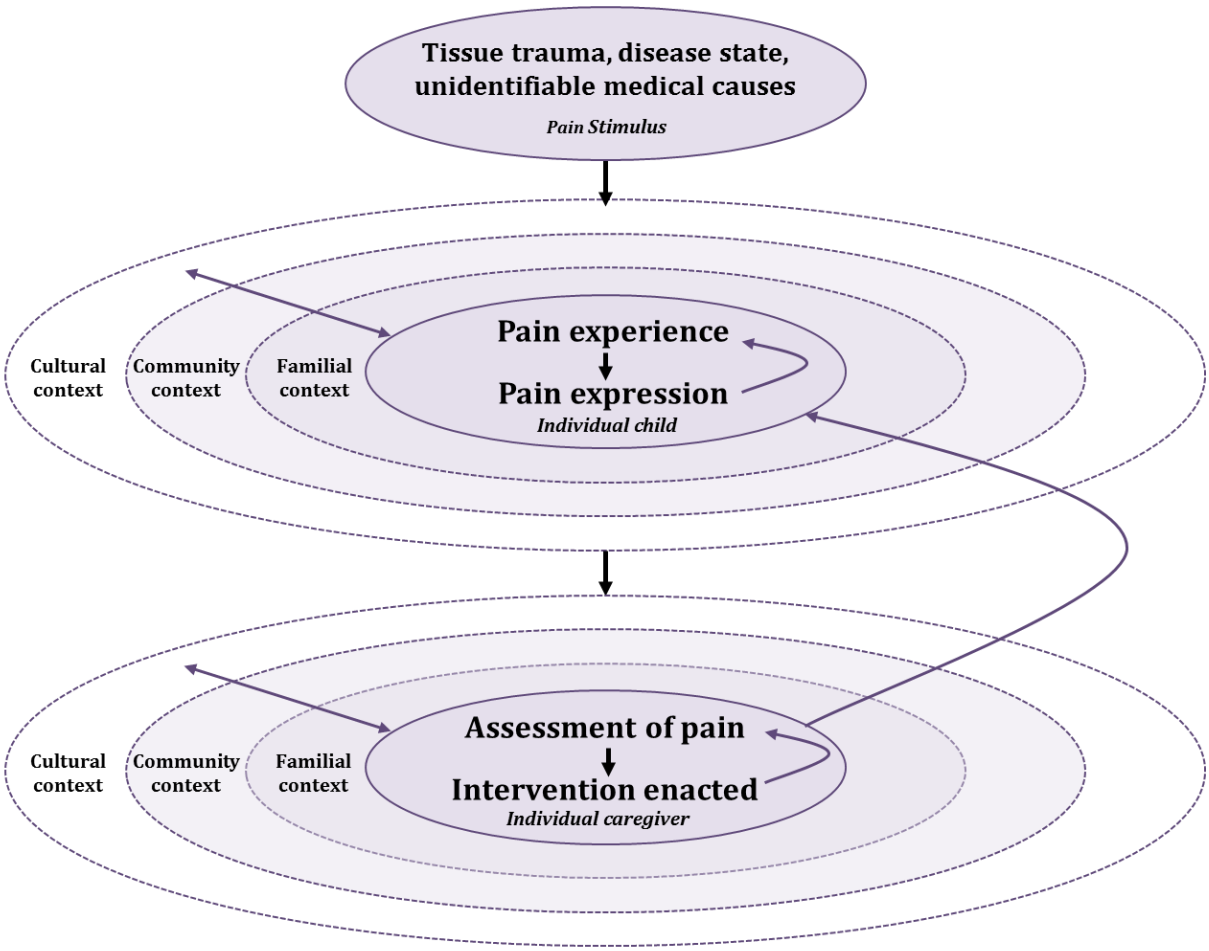
1.5 CONCEPTUAL FRAMEWORK: THE SOCIO COMMUNICATION MODEL OF INFANT'S PAIN

Craig and Pillai Riddell [114] provide a basis for conceptualizing infant pain within a socio communication model. In humans, pain rarely relates to the individual only, and is always embedded in a complex social context. From an anthropologic perspective, the individual's ecologic system is embedded in a set of constructs, which are nested each inside the next, like a set of Russian dolls. The inner most level is the individual person. Each level represents a system that plays an important role in the individual's life. These systems interact with each other with some having more direct influence and some being more distal [115]. These levels can be represented by the family, ethnic identity, religion, community, culture, and race. The individual experience of pain is usually of profound importance to others, and has automatic transparent features (e.g. cry, speech) that serve social communication functions [116,117]. Humans are relatively unique in their ability to express pain through a combination of language skills, facial expression and body movements. The interaction between an individual in pain and an observer during an acute painful event can have important impact on the outcomes for the person in pain [114], especially during infancy and childhood, when children face challenges of establishing secure attachments to primary caregivers [118,119]. During this critical phase of development, recurrent and prolonged pain could influence bonding, feelings of security, and trust in others. Furthermore, persons suffering from persistent pain at any age may experience social isolation, diminished opportunities to interact with others, loss of skills, and even be at a risk of becoming stigmatized [114,120]. However, the external social parameters of pain are more meaningful when pain is considered in older infants and young children.

In their model (Figure 1), Craig and Pillai Riddell suggest that pain should be viewed as a dynamic and interactive process between the infant and caregiver, which is also influenced by the social context. In this model, there are three central features: the painful event, the child, and the caregiver. These central features are influenced by the familial, community, and cultural

environments. Each layer influences every other layer directly or indirectly (familial, community and cultural contexts). In addition to influencing each other, these contexts also influences the caregiver and child experiencing and expressing the painful event.

Figure 1: The Socio-Communication Model of Infants' Pain



Craig & Pillai Riddell (2003)

In the model, the solid lines around the child and the caregiver represent a synthesis of the social influences, which are created in all individuals because of their biological/personal factors and their position in their family, community, and culture. Furthermore, when the model refers to culture, it is considered in a broad perspective. In an era of globalization and fluid immigration between countries and continents, culture not only refers to the culture heritage of the individual, but also to the complexity of integrating one's culture in another environmental culture.

In light of the complexity of premature infants' pain, and considering that these infants are hospitalized in a NICU setting from birth, as well as the importance of CFs influencing pain response, the model was modified for the purpose of this thesis. We adapted the model to a NICU environment where the infant's primary care givers are not the parents and the family, but rather the health care providers. We elaborated the model, by changing the layers surrounding the two core features, the infant and the caregiver, by placing the health care team and unit culture in the first circle. Furthermore, we added the immediate influence of medical and demographic factors on the infant's pain. The modified model is presented in chapter 5.

This dissertation will address gaps in the literature by:

- Presenting results of the randomized controlled parent study examining the comparative effectiveness of two non-pharmacological pain relief interventions: oral sucrose and facilitated tucking alone, and in combination for repeated pain exposure in preterm infants (chapter 3).
- Providing a commentary point of view regarding the clinical feasibility of facilitated tucking (chapter 4).
- Presenting a systematic review of evidence describing the association between medical and demographic contextual factors and pain response in preterm infants during heel-stick procedures (chapter 5).
- Examining the association between contextual factors and pain responses in preterm infants under the therapeutic effect of the non-pharmacological interventions (examined in chapter 3) (chapter 6).

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CHAPTER II

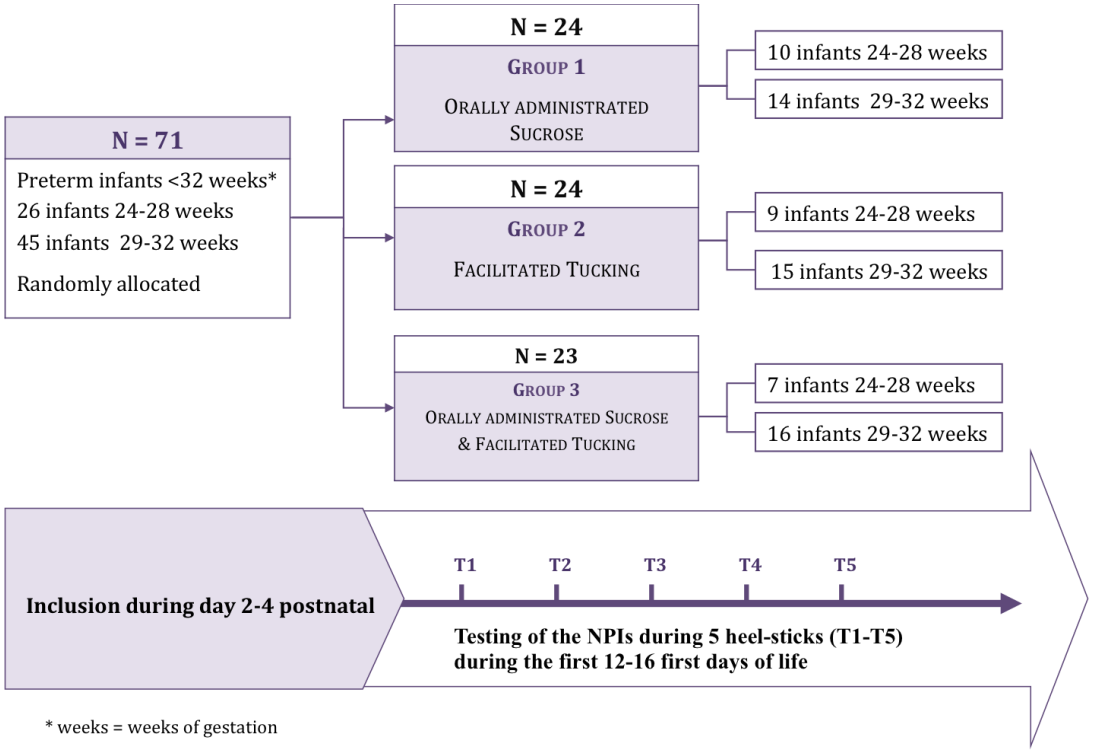
STUDY AIMS

2. STUDY AIMS

2.1 PARENT STUDY (CHAPTER III)

Preterm infants are exposed to numerous minor and moderated painful procedures on a daily basis. This exposure may cause short and long lasting negative effects on the developing nervous system. To address this problem non-pharmacological pain relieving interventions (NPIs) are recommended to prevent and treat pain. Despite studies supporting the efficacy of NPIs for single painful procedures, there is a lack of evidence regarding the effectiveness of NPIs over repeated pain exposure. The aim of this study, therefore, was to test the comparative effectiveness of two non-pharmacological pain relieving interventions, sucrose and facilitated tucking administered alone or in combination across 5 repeated heel-sticks in preterm infants aged between 24 and 32 weeks of gestation.

Figure 1: Flow chart for recruitment and randomization



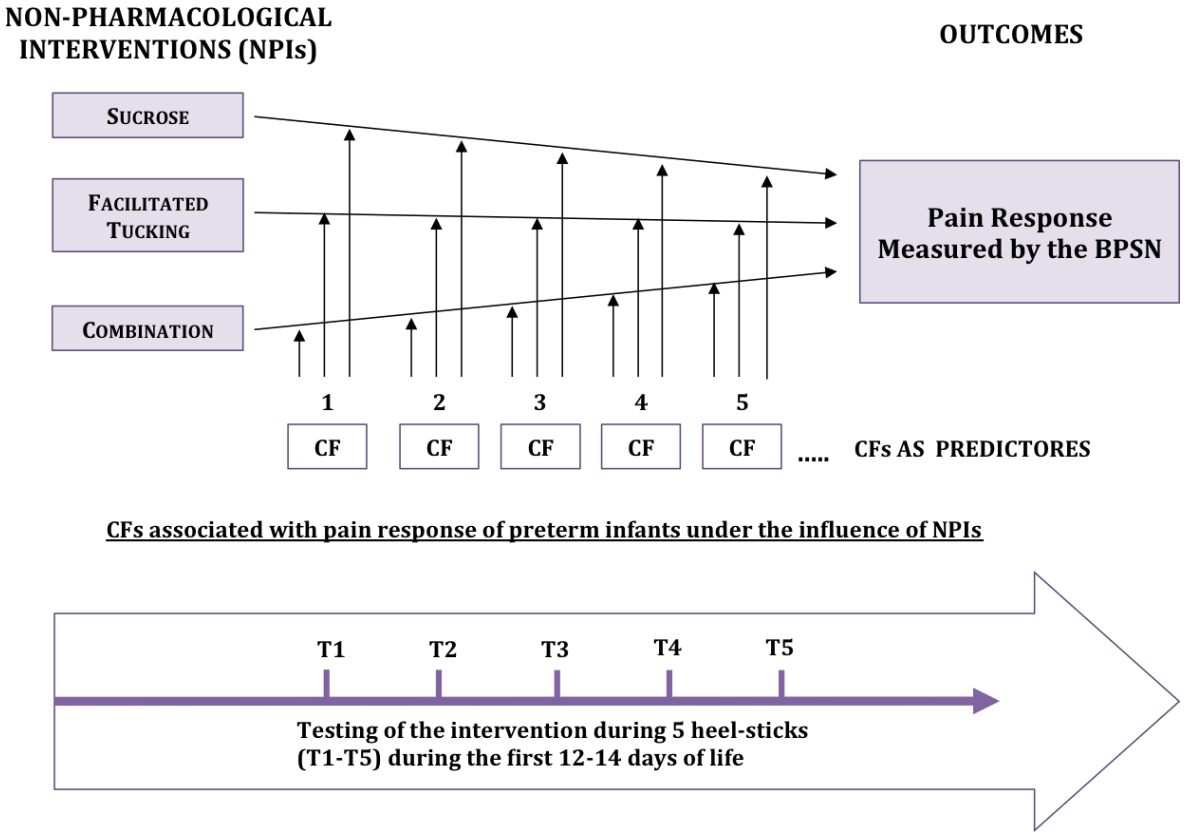
2.2 SYSTEMATIC REVIEW (CHAPTER V)

The importance of medical and demographic contextual factors (CFs) associated with pain response of preterm infants has been recognized and explored by the neonatal pain researchers and are of great concern for accurate pain measurement and assessment. However, the influence of CFs related to medical and demographic determinants remains unclear to date. The aim of this study was to systematically examine, identify, and summarize the impact of medical and demographic CFs on pain response in preterm infants exposed to heel-stick procedures.

2.3 SUB STUDY (CHAPTER VI)

Given the present challenges of pain assessment and treatment in preterm infant population as presented in the introduction chapter, there is a need to address the phenomenon of CFs in pain management research in this vulnerable population. CFs were studied however so far the results remains unclear. The aim of this study was to explore the association between CFs and pain response of preterm infants age 24-32 weeks of gestation being treated with NPIs. This is the first study to examine the association between a wide range of medical and demographic CFs and multidimensional pain responses.

Figure 2: Contextual factors influencing pain response under the therapeutic effect of non-pharmacological pain relief intervention.



CHAPTER III

ORAL SUCROSE AND FACILITATED TUCKING FOR REPEATED PAIN RELIEF IN PRETERMS: A RANDOMIZED CONTROLLED TRIAL

Cignacco EL, Sellam G, Stoffel L, Gerull R, Nelle M, Anand KJS, Engberg S

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ABSTRACT

Objectives: To test the comparative effectiveness of two non-pharmacological pain-relieving interventions administered alone or in combination across time for repeated heel-sticks in preterm infants.

Patients and Methods: A multicenter randomized controlled trial in three NICU's in Switzerland compared the effectiveness of oral sucrose, facilitated tucking (FT), and a combination of both interventions in preterm infants between 24 and 32 weeks of gestation. Data were collected during the first 14 days of their NICU stay. Three phases (baseline, heel-stick, recovery) of 5 heel-stick procedures were videotaped for each infant. Four independent experienced nurses blinded to the heel-stick phase rated 1055 video sequences presented in random order utilizing the Bernese Pain Scale for Neonates (BPSN), a validated pain assessment instrument.

Results: Seventy-one infants were included in the study. Inter-rater reliability was high for the total BPSN score (Cronbach's alpha: α = 0.90-0.95). FT alone was significantly less effective in relieving repeated procedural pain (p<0.002) than sucrose (0.2ml/kg). FT in combination with sucrose seemed to have added value in the recovery phase with lower pain scores (p=0.003) compared to both single treatment groups. There were no significant differences in pain responses across gestational ages.

Conclusions: Sucrose with and without FT had pain-relieving effects even in preterms less than 32 weeks of gestation having repeated pain exposures. These interventions remained effective during repeated heel-sticks across time. FT was not as effective and cannot be recommended as a non-pharmacological pain relief intervention for repeated pain exposure.

3.1 BACKGROUND

The survival of preterm infants is dependent on highly sophisticated intensive care associated with an exceedingly high number of painful procedures [1]. This is particularly true for infants with extremely low gestational ages (GA) who also receive less analgesia [2-5].

Repeated pain exposures during critical windows of central nervous system development are associated with permanent changes in peripheral, spinal and supraspinal pain processing; neuroendocrine function and neurologic development [6,7]. These changes can be manifested by alterations in pain thresholds, stresses responses, cognitive function, behavioral disorders and long-term disabilities [7,8]. Despite this knowledge, many painful procedures in neonatal intensive care units (NICU) are performed without pharmacologic or non-pharmacological analgesia [1-3,9]. Disadvantages of pharmacological analgesia include side effects, questionable efficacy and possible negative impact on neonatal outcomes [10-12]. As an alternative approach, non-pharmacological interventions (NPIs) are recommended for pain management [13-15].

NPIs (e.g. oral sucrose, breastfeeding, non-nutritive sucking, facilitated tucking (FT), kangaroo care, swaddling) effectively reduce pain for minor to moderately painful procedures [15-17]. They promote self-regulation of the infant and provide oro-tactile, oro-gustatory and tactile stimulation, capable of reducing infants' pain responses during most painful procedures [15,18-21]. Sucrose is recommended extensively for pain relief in preterm infants [22-24] and shown to be highly effective and safe for single procedures by Stevens and collegues [17]. Sweet taste solutions seem to trigger endogenous opioids and non-opioids pathways [25,26]. FT is described as holding the infant by placing a hand on his hands and feet and by positioning the infant in a flexed midline position whiles in either a side-lying, supine, or prone position [27,28]. This technique provides the infant with support

and the chance to control his/her own body [16]. Several studies reported that FT stabilizes behavioral and physiological states, during single heel-sticks and endotracheal suctioning, reducing the infant's stress in coping with pain [16,27-31].

Although current evidence supports the effectiveness of NPIs for a single painful procedure, there is little research examining their effectiveness across repeated painful procedures. To date, few studies have evaluated the effectiveness of sucrose over time [32-36], and none have evaluated FT across time. The combination of two NPIs (e.g. oral sucrose and FT) may have additive effects by stimulating infants in a multi-sensorial way to cope with the painful experience [18,37, 38].

This study compared the impact of sucrose and FT alone and in combination on pain reactivity across multiple painful procedures. Randomized groups received oral sucrose, FT, and a combination of both strategies in order to evaluate possible additive effects. The primary outcome was pain response measured by the "Bernese Pain Scale for Neonates" (BPSN) total and component scores. The secondary outcome was the impact of gestational groups (24 0/7 - 27 6/7 and 28 0/7 - 32 0/7 gestational weeks) on the effectiveness of these interventions.

3.2 METHODS

3.2.1 Setting and sample

This randomized controlled trial was carried out in NICUs of three University Hospitals in Switzerland from January 12th to December 31st 2009. Infants admitted to the NICU during this period were assessed for eligibility according to the following inclusion criteria: born between 24 0/7 and 32 0/7 weeks of gestation and anticipated clinical need for at least 5 routine capillary blood samples within two weeks after birth. Infants were excluded if they had sever intra-ventricular hemorrhage (grade III and IV), life-threatening malformations or disorders affecting brain circulation or the cardiovascular system, undergone a surgical procedure, a pH<7.00, or any problem that could impair pain expression.

3.2.2 Sample size calculation

Using sucrose only, we performed a feasibility study to calculate a preliminary power-analysis [39]. We formulated our calculations based on the assumptions that sucrose and FT will have equivalent effects, which will sustain over time. According to this analysis, a group size of n=24 for each intervention group (n=72 total sample size) provided adequate power to detect a pain reduction of 33% for the combination group relative to the two single intervention groups with a power of 80%.

3.2.3 Data collection and management

Data were collected during five nonconsecutive routine heel-sticks (T1-T5) between postnatal days 2 and 16, with the first heel-stick performed no later than day 4. For other painful procedures including heel-sticks where data were not being collected the infants were provided with sucrose 20%, which was the standard of care in all-participating NICUs. Because the timing of blood sampling was determined by clinical considerations, there were no fixed time points for data collection. Demographic data were collected from medical records.

Data collection occurred during: (1) baseline (before any manipulation), (2) heel-stick (skin preparation, heel-stick, and hemostasis after blood was drawn), and (3) recovery (3 minutes after the heel-stick). Most heel-sticks took place in the morning and each infant was undisturbed for at least 30 minutes prior to data collection. Phases were videotaped (Panasonic high definition camcorder, model HDC-HS9., Osaka, Japan) for at least 3 minutes by trained study nurse using a standardized procedure. No recording occurred during heel warming (2-3 minutes) between the first and second phases. The infant's nurse performed the heel-stick. Sucrose was administrated by the nurse, while the FT was performed by a second nurse or trained study nurse. The exact time of the videotaping of each phase was documented as well as the duration of heel-sticks. Fifteen videotape segments were produced per infant (3 sequences per procedure x 5 heel-sticks = a total of 1065 video sequences for the study).

Each video segment was checked for quality and digitally edited by trained study nurses using the Final Cut Express software (version 4.0.1.® 2002-2008 Apple Inc., California, USA) to eliminate any information that would have indicated the heel-stick phase. Video recordings of poor quality were discarded (n=10). The final sample of 1055 sequences was assigned in random order in relation to the number (T1 – T5) and the phase (baseline, heel-stick, recovery) of the heel-stick being recorded. All digital records were provided to 4 nurses for assessment of pain responses during each sequence. The videotaping procedure was designed to ensure that the raters could not see if the heel-stick procedure was being performed.

The NPI groups were: (1) oral sucrose 20% (0.2ml/kg) (2) FT and (3) a combination of both interventions. Sucrose was administrated orally about two minutes prior to the heel-stick. If the infant seemed to be in pain during the heel-stick phase, up to two additional doses of sucrose were administrated and noted in the study chart. FT was started at the beginning of the baseline phase, and the infant was "tucked" through all three phases. In the combination group, the FT was started at the beginning of the baseline phase and sucrose was given two minutes before the heel-stick.

3.2.4 Variables and measures

Information about GA, method of delivery, gender, parity, birth weight, Apgar scores, mechanical ventilation or continuous positive airway pressure (CPAP) during the heel-stick, and number of painful procedures each day was collected.

Pain response, the dependent variable, was measured using the BPSN total and component scores. The BPSN contains 9 items, three physiologic (heart rate, respiratory rate, and oxygen saturation) and six behavioral (grimacing, body movements, crying, skin color, sleeping patterns, consolation) items. Physiological data (heart rate and oxygen saturation) synchronized with the three phases of data collection were downloaded from the clinical monitoring database for the BPSN. Raters counted the breathing rate while viewing the video sequences. Raters scored behavioral items and breathing only; heart rate and oxygen saturation were scored using

physiological data collected during each phase. Each item was scored on a 3-point scale (0-3 points). Higher scores for the behavioral items and greater changes in the physiological items indicated increased pain, while a total score of ≤ 11 was considered non-painful [40]. The neonatal nursing experts who rated the video sequences attended a standardized instruction session about how to perform the rating and they rated the sequences independently.

Initial psychometric testing of the BPSN demonstrated good construct validity with differentiation between painful and non-painful procedures ($F = 41.27$, $p \leq 0.0001$) and intra-rater and inter-rater reliability correlation coefficients of $r = 0.98$-0.99 and $r = 0.86$-0.97 respectively. In a recent revalidation of the BPSN, a cut-off- score of ≤ 11 was considered non-painful (sensitivity of 100 % and specificity of 89.4%) [41]. For this study, three BPSN scores were calculated: the total (T-BPSN), behavioral (B-BPSN), and physiological (P-BPSN) BPSN scores.

3.2.5 Inter-Rater-Agreement

Inter-rater reliability for the total BPSN scores in this study averaged 99.2% for the 5 heel-sticks (range: 98.8% for heel-stick 1 and 99.8% for heel-stick 5). Since the inter-rater reliability was very high, we used the average raters' BPSN scores within infants over time (5 heel-sticks). Within-infant variability in total BPSN scores across time was high (86.3%, $p < 0.0001$). Inter-rater reliability for behavioral BPSN scores was 98.8%. Inter-rater reliability, measured by Cronbach's alpha coefficient, ranged between 0.90 and 0.95 for the different phases.

3.2.6 Randomization

To assure equal balance of the intervention group per site, block randomization using SPSS® (version 16) was performed. For each site, 8 infants were randomly allocated to each of the three interventions (24 infants per site, and 24 infants per intervention group for the entire study sample). For each site, group assignments were sealed in opaque envelopes and consecutively numbered. When parents consented to participation, the envelope was opened by

a study nurse and the intervention group was revealed. Envelopes were prepared by a study nurse not involved in the data collection process.

During the study period, 201 infants less than 32 weeks of gestation were assessed for eligibility to participate in the study. In each site a study nurse called the referring NICU daily and asked if any new infants were eligible to participate in the study.

3.2.7 Ethical Consideration

The study was approved by the ethical boards of the Cantons of Basel, Bern and Zürich. Written informed consent was obtained from a parent. The study design did not include a no-intervention control group based on the evidence that exposure of preterm infants to pain procedures without treatment is harmful [14].

3.2.8 Data analysis procedures

All data were analyzed using IBM SPSS statistics software® (version 19) and SAS (version 9.1). Data entry quality was controlled by double-entry procedures and an error rate of < 1% was detected. Descriptive statistics were utilized to describe the demographic and medical characteristics of the infants while the chi-square test (χ^2), and Kruskal-Wallis or Mann-Whitney U tests were utilized for comparisons between the three intervention groups and the two GA groups. The mean number of painful procedures was compared per site per infant per day, utilizing one-way ANOVA and post hoc Tukey test. Correlations between physiological and behavioral items of the BPSN were calculated with the Pearson coefficient. Clinical site, GA group, number of painful procedures and heel-stick duration were examined as possible confounders on the impact of the NPIs on BPSN scores; none had a confound effect. The primary hypothesis was tested using a repeated measure analysis. We used a random slopes regression model, which allowed each subject to have its own regression over the five heel-sticks. Due to high inter-rater reliability, scores of the four raters were averaged and transformed logarithmically to satisfy the assumption

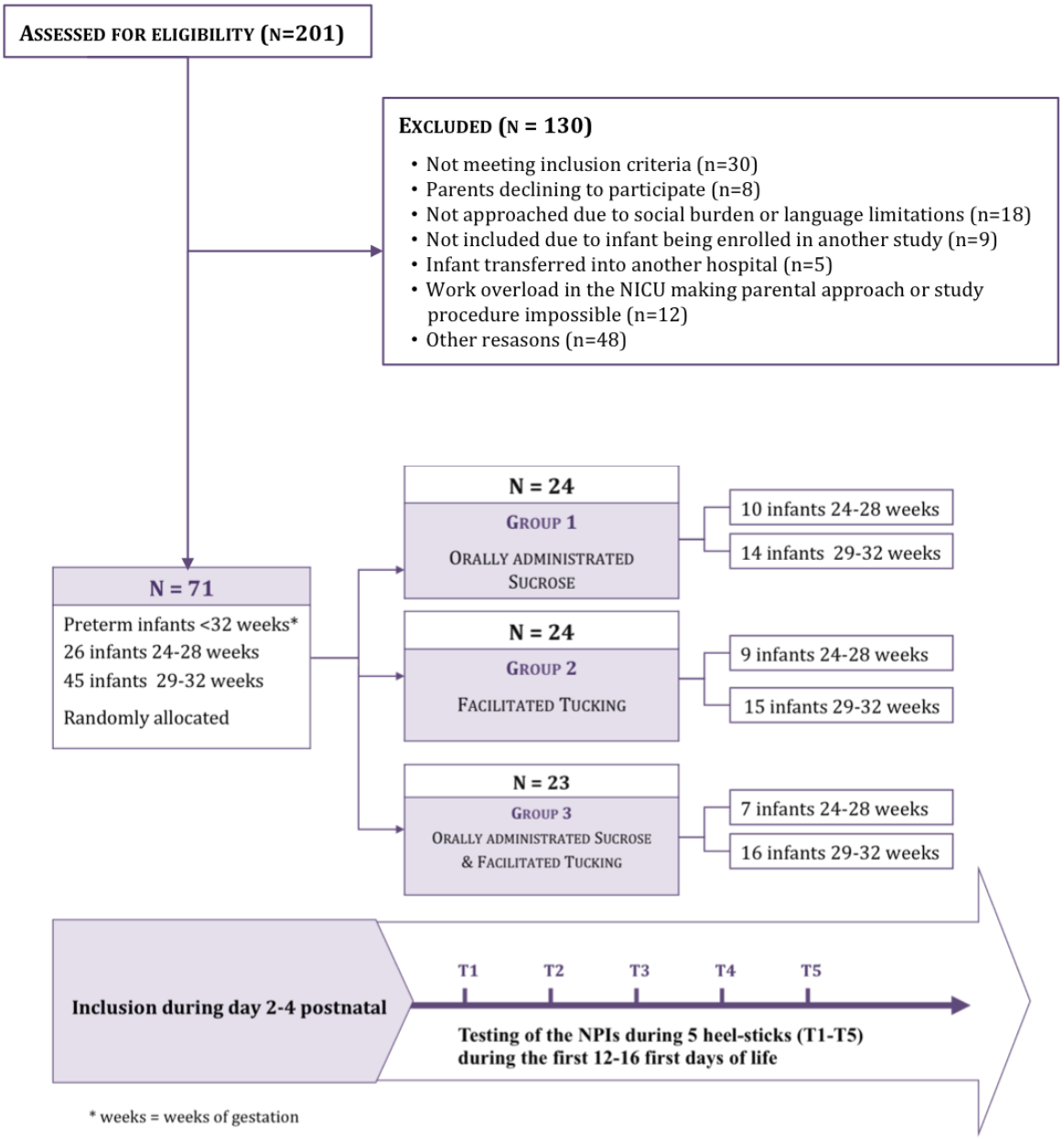
of normally distributed residuals. For comparisons between the three phases, scores across the five heel-sticks were also averaged. An alpha of 0.05 was considered significant.

3.3 RESULTS

3.3.1 Sample characteristics and number of daily procedures

Seventy-one infants were enrolled in the study and all but one had complete data. The fifth heel-stick was missed for one infant. Figure 1 presents the flow diagram of the recruitment and randomization process based the Consolidated Standards of Reporting Trials (CONSORT) guidelines [42].

Figure 1: Flow diagram of the recruitment and randomization process



The mean GA of the participating infants at birth was 29.2 (SD ±1.8) weeks, mean birth weight was 1174 grams (SD ±337), and mean number of painful procedures during 0-14 days was 201 (SD ±104). Sample characteristics are summarized in Table 1.

Table 1: Demographic and medical characteristics of the sample

	Total Sample n (%)	Non-pharmacological intervention group			χ^2
		Sucrose n (%)	FT n (%)	Combination n (%)	
Sample	**71(100)**	**24 (33.8)**	**24 (33.8)**	**23 (32.4)**	**p=0.986**
Gender					**p=0.983**
Female	32 (45.0)	11(45.8)	11 (45.8)	10 (43.5)	
Male	39 (55.0)	13 (54.2)	13 (54.2)	13 (56.5)	
Way of delivery					**p=0.805**
Normal birth	6 (8.5)	3 (12.5)	1 (4.2)	2 (8.7)	
Planned C-section	16 (22.5)	4 (16.7)	6 (25.0)	6 (26.1)	
Emergency C-section	49 (69.0)	17 (70.8)	17 (70.8)	15 (65.2)	
Parity					**p=0.462**
Single	50 (70.4)	19 (79.2)	14 (58.3)	17 (73.9)	
One of twins	14 (19.7)	3 (12.5)	8 (33.3)	3 (13.0)	
One of triplet	5 (7.0)	2 (8.3)	1 (4.2)	2 (8.7)	
One of quadruplet	2 (2.8)	0 (0)	1 (4.2)	1 (4.3)	
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Kruskal-Wallis**
GA at birth (weeks and days)	**29.24 (1.8)**	**28.95 (1.7)**	**29.20 (1.7)**	**29.5 (2)**	**p=0.389**
Duration of heel-stick (min)					
HS 1	5.04 (5.2)	4.38 (3.8)	4.12 (3.6)	6.7 (7.4)	**p=0.687**
HS 2	4.75 (4.3)	4.54 (4.5)	4.67 (3.5)	5.04 (5.0)	**p=0.851**
HS 3	5.01 (4.8)	4.46 (3.1)	4.95 (4.7)	5.65 (6.2)	**p=0.878**
HS 4	4.73 (3.7)	5 (3.9)	5.33 (4.0)	3.83 (3)	**p=0.305**
HS 5	4.77 (3.6)	4.65 (3.7)	5.38 (4.1)	4.26 (2.9)	**p=0.751**
Birth weight (gr)	1174.44	1080 (286)	1228 (397)	1217 (309)	**p=0.308**
APGAR scores					
1 min	5.97 (2.1)	5.65 (2.3)	5.46 (2.3)	6.83 (1.6)	p=0.070
5 min	7.58 (1.6)	7.21 (1.4)	7.29 (1.8)	8.26 (1.2)	p=0.030*
Number of painful procedures per day	**14.38 (7.4)**	**15 (10)**	**15 (9)**	**13 (9)**	**p=0.012***
Number of heel-sticks per infants per day	**1.07 (1.2)**	**1 (1)**	**1 (1)**	**1 (1)**	**p=0.539**

*The significance level is 0.05

FT= facilitated tucking **GA**=gestational age **SD**= standard deviation

3.3.2 Testing the effectiveness of the non-pharmacological interventions

We compared the effectiveness of sucrose, FT and their combination in reducing pain responses during heel-stick procedures. Table 2 presents the mean scores of the total, behavioral and physiological BPSN scores for the three intervention groups.

Table 2: Mean pain scores for all raters across all heel-sticks measured by the Bernese Pain Scale for Neonates (BPSN)

		Non-pharmacological intervention group					
		Sucrose		FT		Combination	
Score	Phase	Mean	SD	Mean	SD	Mean	SD
T-BPSN	Baseline	4.03	2.08	4.99	3.24	4.62	2.88
	Heel-stick	7.48	3.64	9.75	4.73	7.53	3.75
	Recovery	4.87	2.04	5.18	2.87	4.23	2.68
B-BPSN	Baseline	4.02	2.08	4.97	3.25	4.62	2.88
	Heel-stick	5.58	2.95	7.01	3.59	5.49	2.95
	Recovery	3.66	1.71	3.9	2.47	3.18	2.24
P-BPSN	Baseline	0	0	0.04	0.22	0	0.03
	Heel-stick	1.89	1.79	2.72	1.98	2.03	1.73
	Recovery	1.23	1.35	1.28	1.31	1.05	1.23

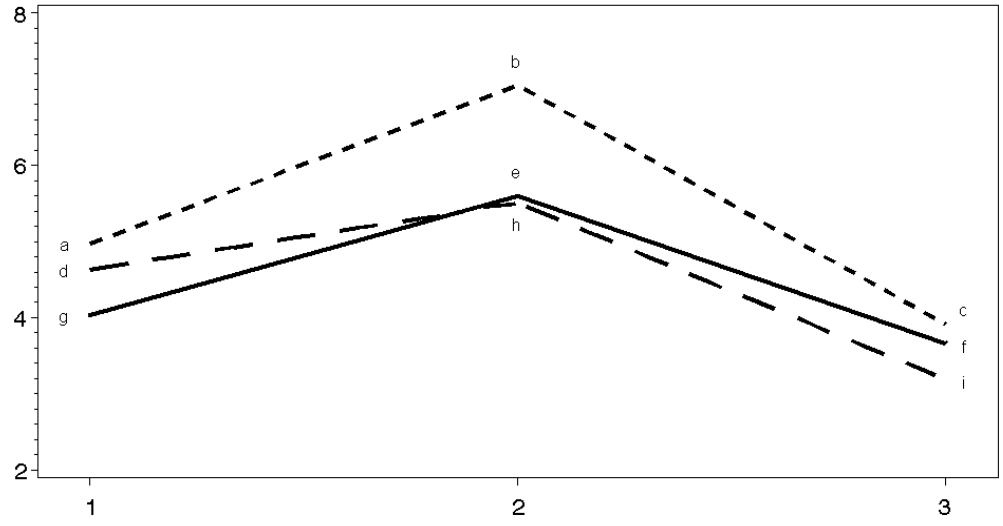
T-BPSN= total BPSN scores **B-BPSN**= behavioral BPSN scores **P-BPSN**= physiological BPSN scores
FT= Facilitated tucking **SD**= standard deviation

The correlation between the infants' mean behavioral and physiological pain scores across the raters and phases of the heel-stick was low (r = 0.19). Effectiveness of the interventions was examined by comparing mean pain responses over all heel-sticks. Figure 2 presents the mean behavioral and physiological pain scores over all 5 heel-sticks as predicted by the regression analysis. During heel-stick phase, the FT group had significantly higher B-BPSN (p = 0.01; 0.007) and P-BPSN (p = 0.0002; 0.003) scores than the sucrose and combination groups. During the recovery phase, there were no significant differences in P-BPSN scores, but the combination group had significantly lower B-BPSN scores than both the other groups (p = 0.006; 0.008).

Figure 3 shows how the B-BPSN and P-BPSN of heel-stick phase scores for each group changes across the five heel-sticks. P-BPSN scores for the FT group increased significantly from heel-stick 1 to 5 (p = 0.01), while there were no significant changes for the sucrose (p = 0.08) and

combination (p = 0.43) groups. Overall B-BPSN scores showed no significant changes over time, but the slope between heel-stick 1 and 2 decreased significantly (p = 0.01) for the FT group and the slope between heel-stick 4 and 5 increased significantly (p = 0.03) for the sucrose group.

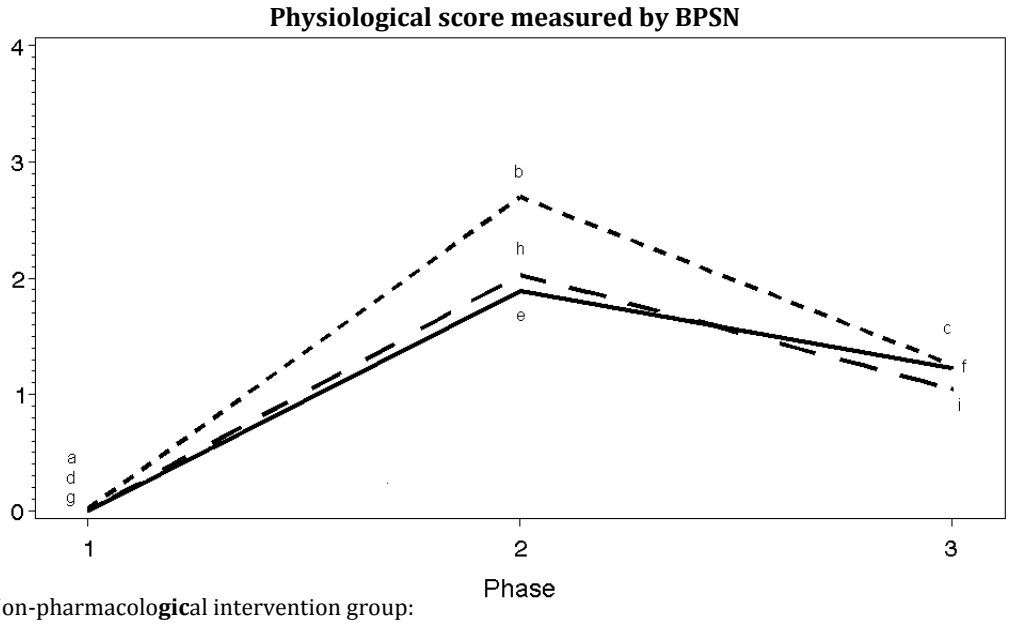
Figure 2: Slope testing-non-pharmacological intervention groups and phases



Non-pharmacological intervention group:
_____: Sucrose .....: Facilitated tucking _ _ _ _: Combination

	Physiological BPSN scores		Estimate	SE	df	t-value	*p*-value
Phase 1 to 2	Facilitated tucking (FT)	Point a versus b	-0.3644	0.06915	905	-5.27	<.0001*
	Sucrose	Point g versus h	-0.2882	0.06858	905	-4.2	<.0001*
	Combination	Point d versus e	-0.1753	0.06947	905	-2.52	0.0118*
Phase 1	FT – Sucrose	Point a versus g	-0.1478	0.08839	905	-1.67	0.0948
	FT – Combination	Point a versus d	-0.05171	0.08911	905	-0.58	0.5618
	Sucrose – Combination	Point d versus g	0.09612	0.0891	905	1.08	0.281
Phase 2	FT – Sucrose	Point b versus e	-0.224	0.08873	905	-2.53	0.0117*
	Sucrose – Combination	Point h versus e	0.01679	0.08907	905	0.19	0.8506
	FT – Combination	Point b versus h	0.2408	0.08947	905	2.69	0.0072*
Phase 3	Sucrose – Combination	Point f versus i	0.2433	0.08896	905	2.73	0.0064*
	FT – Combination	Point c versus i	0.2356	0.08886	905	2.65	0.0082*
	FT – Sucrose	Point c versus f	0.007718	0.08801	905	0.09	0.9301

*The significance level is 0.05
Phase 1= baseline **Phase 2**= heel-stick **Phase 3**= recovery **SE**= Standard error **df**= degrees of freedom



Non-pharmacological intervention group:

_____: Sucrose ..... : Facilitated tucking _ _ _ _: Combination

	Physiological BPSN scores		Estimate	SE	df	t-value	*p*-value
Phase 1 to 2	Facilitated tucking (FT)	Point a versus b	-1.1101	0.06304	905	-17.61	<.0001*
	Sucrose	Point g versus h	-0.8767	0.06258	905	-14.01	<.0001*
	Combination	Point d versus e	-0.9265	0.06339	905	-14.62	<.0001*
Phase 1	FT – Sucrose	Point a versus g	-0.02477	0.06867	905	-0.36	0.7184
	FT – Combination	Point a versus d	-0.02193	0.06916	905	-0.32	0.7512
	Sucrose – Combination	Point d versus g	0.002840	0.06916	905	0.04	0.9673
Phase 2	FT – Sucrose	Point b versus e	-0.2581	0.06905	905	-3.74	0.0002*
	Sucrose – Combination	Point h versus e	-0.05262	0.06915	905	-0.76	0.4468
	FT – Combination	Point b versus h	0.2055	0.06955	905	2.95	0.0032*
Phase 3	Sucrose – Combination	Point f versus i	0.08641	0.06903	905	1.25	0.2110
	FT – Combination	Point c versus i	0.1068	0.06891	905	1.55	0.1214
	FT – Sucrose	Point c versus f	-0.02042	0.06829	905	-0.30	0.7650

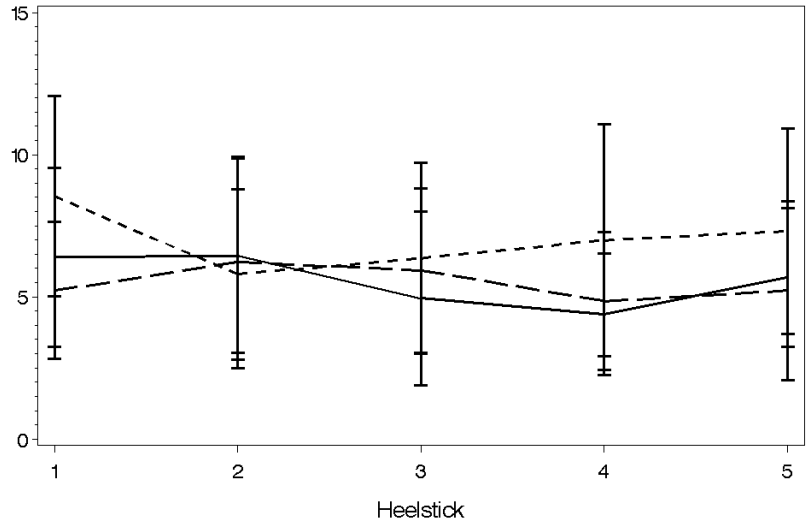
The significance level is 0.05*

Phase 1= baseline **Phase 2**= heel-stick **Phase 3**= recovery **SE**= Standard error **df**= degrees of freedom

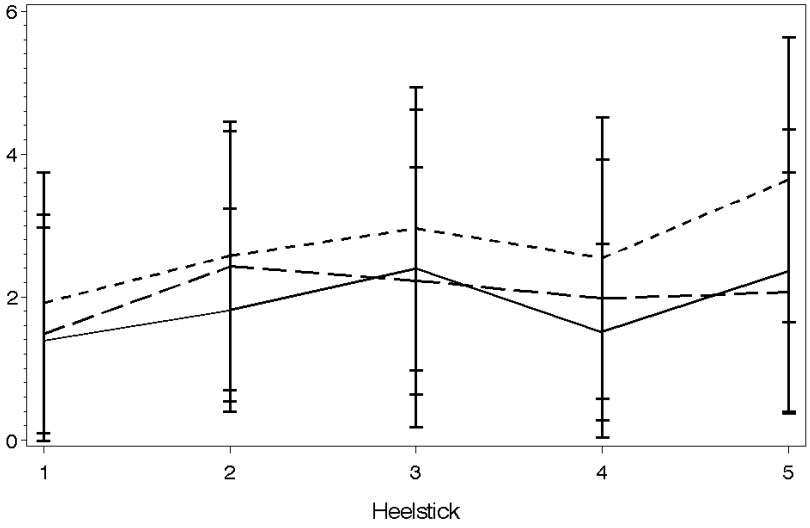
In the sucrose and combination groups (n = 47), 21 infants (44.7%) received additional doses of sucrose. During heel-stick phase, infants who received additional doses of sucrose had significantly higher B-BPSN scores than those who did not receive additional doses (p = 0.02), while their P-BPSN scores were not significantly different (p = 0.50). There were no significant differences in the recovery phase behavioral (p = 0.85) or physiological (p = 0.26) pain scores of infants who did or did not receive additional doses of sucrose.

Figure 3: Pain scores over the heel-sticks for phase 2 (heel-stick) measured by the Bernese Pain Scale for Neonates (BPSN)

Behavioral score measured by the BPSN



Physiological score measured by BPSN



Non-pharmacological intervention group:

_____: Sucrose .....: Facilitated tucking _ _ _ _: Combination

3.4 DISCUSSION

The findings of this study show that either sucrose alone or sucrose in combination with FT remains effective across five heel-sticks in preterm infants under 32 weeks of gestation, while FT alone appears to be less effective. Furthermore, the results indicate that the combination of sucrose and FT may have additive pain relieving effects during the recovery phase.

Our findings are consistent with previous studies regarding the efficacy of sucrose over time [32-35]. No sign of tolerance to the analgesic effects of sucrose was observed. It is important to note that FT was not lacking efficacy but rather was less effective than the two other interventions. The mean T-BPSN and B-BPSN scores during all phases were less than 10 points, even for infants in the FT group. Previous data suggest that a T-BPSN score ≤11 points or a B-BPSN score <8 points is considered as "no pain". The efficacy of FT has been described in several studies [16,27-29,43], although these studies did not compare FT with other NPIs. Our study is the first to compare the efficacy of FT or sucrose across time, to its efficacy when used in combination with sucrose.

Infants in the sucrose and combination groups who received additional doses of sucrose had significantly higher behavioral pain scores across all 5 heel-sticks. Regardless these findings, there were no differences in recovery phase between infants who did or did not receive additional doses of sucrose. Our findings correspond to those of Johnston and colleagues [44], who examined the effects of repeated doses of sucrose in preterm infants receiving sucrose solution or sterile water either 2 minutes before, just before, or 2 minutes after the heel-stick. Their results showed that repeated doses of sucrose, at 2 minutes intervals increases the analgesic effect in preterm infants.

Another previous randomized trial questioned the analgesic efficacy of sucrose, based on EEG and EMG recordings in healthy term newborns (37–43 weeks), receiving sucrose or sterile water 2 minutes prior to a heel-stick. Slater et al. [45] found no differences in nociceptive brain activity or in the magnitude or latency of the spinal nociceptive reflexes after the heel-stick,

between infants who received sucrose and those who received water, although Premature Infant Pain Profile (PIPP) scores and pain-related facial expressions were significantly reduced in the sucrose-treated infants. These findings contradict data from a large body of literature supporting the analgesic efficacy of sucrose or other sweet solutions. Moreover, there are several methodological concerns related to the Slater et al. study that make it seem premature to conclude that sucrose is ineffective based only on its findings [46].

Although the differences between the sucrose and combination groups in behavioral and physiological scores during the recovery phase were statistically significant, the magnitude of these differences is probably not clinically meaningful. This poses a critical question related to the cost-effectiveness of this intervention. FT is a time consuming intervention, which could be used as a procedure to enhance parenting or bonding, but needs to be questioned as a nursing intervention. Although there are promising findings regarding FT as an effective NPI [30], the challenges of this specific intervention in nursing practice need to be re-considered in an environment characterized by economic constraints [47].

The methodological strengths of this study are the strategies undertaken to enhance the internal validity of these results. The use of 4 trained and experienced nurses to perform pain assessment, the randomization of the sequences in order to blind the raters to the phase of the procedure, and the thorough elaboration of each single video sequence reduced potential biases.

However, there are some methodological limitations in this study. The raters could only be partially blinded to the NPI group due to the clear visibility of the FT procedure, which could cause possible bias. Nevertheless, raters did not know if the infant was in the FT only group or in the combination group. A further limitation was the exclusion of a no intervention control group due to ethical considerations. Long-term consequences of repeated doses of sucrose were investigated in a small number of studies, but there are no conclusive findings regarding the risk for poor neurological outcomes. In the present study we did not follow up infants for neurological outcomes. Further research regarding consequences of prolonged use of sucrose is

needed. Another limitation of this study was that although the original protocol was for infants in the FT only group to receive FT for all painful procedures during the time they were enrolled in the study, staff shortages made it impossible for nurses to implement this procedure consistently for non-study painful procedures [47]. Consequently, these infants were treated with oral sucrose 20% (the standard of care in the units) for non-study procedures. The impact that this had on the efficacy of FT alone is unknown but it is possible that consistent use of this procedure may have altered its efficacy.

3.5 CONCLUSIONS

The results of this randomized controlled trial provide evidence that oral sucrose alone or combined with FT remains effective in reducing heel-stick related pain over time in preterm infants (24-32 weeks), during the critical phase of the first 14 days of NICU stay. These findings have important clinical implications for the management of pain in preterms of low GA, who are at risk for a high frequency of painful procedures during their NICU stay. During the recovery phase of the heel-stick, the combination of FT and oral sucrose was slightly more effective in reducing pain than sucrose alone. This difference was not however; clinically meaningful particularly given the additional resources needed to implement the combined intervention.

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CHAPTER IV

FACILITATED TUCKING AS NON-PHARMACOLOGICAL INTERVENTION FOR NEONATAL PAIN RELIEF: IS IT CLINICALLY FEASIBLE?

Cignacco EL, Axelin A, Stoffel L, Sellam G, Anand KJ, Engberg S

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ABSTRACT

There is an impressive body of knowledge on pain management in infants hospitalized in neonatal intensive care units. However, deficits in the clinical management of pain in these infants remain. One reason is the gap between research evidence and translation of this knowledge into the clinical setting. This is particularly true for non-pharmacological pain relieving methods. Effective performance of some of these methods requires additional staffing and time. This viewpoint articles describes the clinical challenges associated with implementing facilitated tucking. Although facilitated tucking is described as an efficient method for acute pain relief, the clinical facilitators required to successfully implement such a resource consuming intervention remain unclear.

Conclusion: Translational research on the feasibility of using facilitated tucking in the management of neonatal pain is warranted, including the economic impact of this intervention. Increased manpower costs need to be weighed against the possible long-term economic consequences of pain exposure in infants.

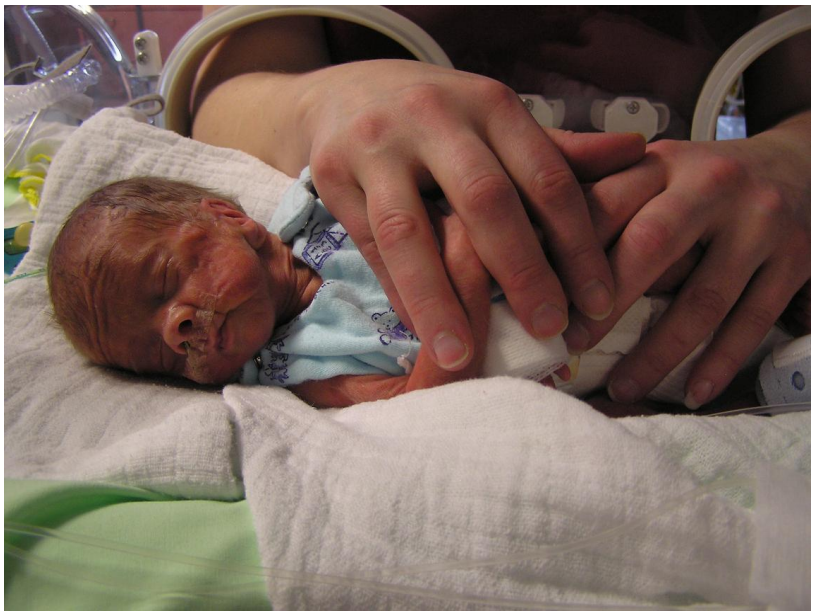
An increasing number of research studies are being performed to examine the effectiveness of non-pharmacological pain relieving interventions (NPIs) [1-6] for the treatment of infants exposed to acute painful events in the neonatal intensive care unit (NICU). As evidenced by several studies, preterm infants are experiencing a high number of routine skin breaking procedures associated with pain [7, 8], which may have a long-term impact on cognitive and behavioural outcomes [9].

Pharmacological analgesia has well-known side effects and questionable impact on neonatal outcomes [10, 11]. The drive to find suitable non-pharmacological alternatives is, therefore, highly commendable in the context of acute pain related to diagnostic and therapeutic procedures. This is particularly true for resource challenged countries.

Recent reports on the impact of NPIs are usually limited to their effectiveness to reduce pain. There is no report describing their feasibility defined as the time, costs and ease of implementing these interventions in the clinical setting. In addition to such economical and organizational factors, the performance of non-pharmacological methods requires a paradigm of care which is highly patient and parent oriented, driven by empathy and focused attention. This focus may require a change in existing attitudes and values within a NICU-team, as this kind of interaction in patient care does not seem to be taken for granted [12].

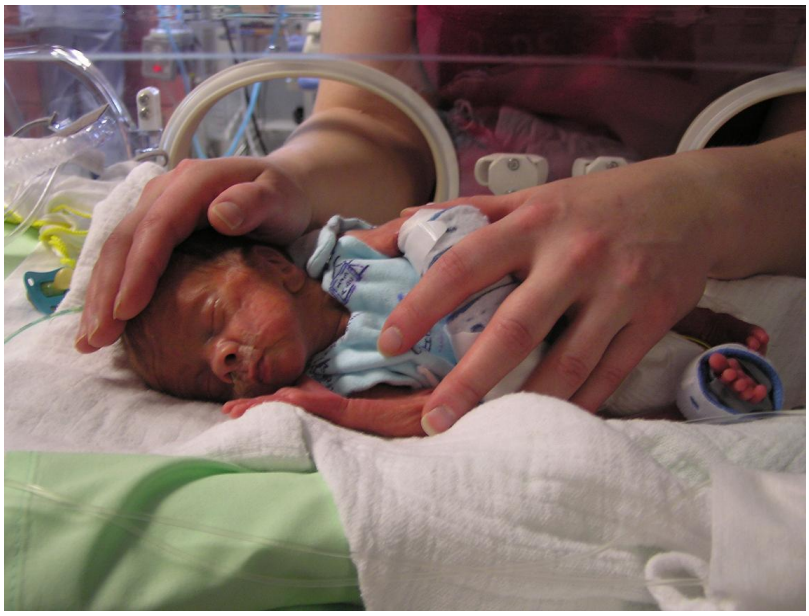
As an example, facilitated tucking (FT) was shown to be effective in the relief of acute neonatal pain [13-16]. It is defined as containment of the infant's arms and legs in a flexed, midline position close to the trunk. Depending on the painful intervention needed, different holding techniques are warranted. For suctioning procedures e.g. the close holding of infant's arms and legs is recommended (see figure 1).

Figure1: Facilitated tucking position for suctioning procedure



For the heel-stick one hand holds the infant's trunk, while the other holds softly the head (see figure 2). The effective utilization of this intervention requires about 10 minutes of interaction with the infant in order to provide the infant with the sense of being held and attended to through the unpleasant experience of pain. FT should be started about 3 minutes prior to the painful procedure to help the infant adapt to the tactile stimuli of two adult hands holding him. Relaxation of the infant is generally observed after about 3 minutes of FT, so the painful procedure itself should not start until after the infant is relaxed. The same holds true for the post-procedural period: the FT needs to continue for at least 3 minutes to give the infant the opportunity to recover and return to baseline-status. FT requires an increase in manpower as two nurses are needed during the painful procedure: one to tuck the child and the other to perform the painful intervention. Given current financial restrictions and staff shortages in health care settings, the feasibility of implementing such a resource consuming intervention must therefore seriously be questioned [17].

Figure 2: Facilitated tucking position for heel-stick procedure.



This fact was shown in a current multi-site RCT testing the effectiveness of FT across repeated pain exposures of infants < 32 weeks of gestation [18]. Within this RCT, the intervention was designed to be performed by nurses during their regular shift. This meant that two nurses involved in daily routine care were needed for the study intervention, one to perform the heel-stick for blood sampling and the second to comfort the infant. These are the same personal resources that would be needed in a "real life" clinical situation of an NICU offering FT as a pain relieving method. Our experience within the mentioned RCT showed that the allocation of a second staff nurse to do the FT was difficult due to the work load during the shift. Nurses involved in the study questioned why an intervention was being tested for its effectiveness, if the personnel needed to use this method within routine clinical care is questionable. Another concern was that such research could provide nurses with new knowledge about an effective pain relieving intervention, but also burden them with the awareness that they are not to be able to perform this "good clinical practice" due to limitations in resources. These concerns expressed by dedicated nurses need to be taken seriously by the research community. Other NPIs like kangaroo care, swaddling, use of non-nutritive sucking and sucrose have evidence of efficacy [1-6] and are less resource consuming and may, therefore, be more feasible and practical

for care providers to utilize in clinical practice. However, in light of the increasing interest towards non-pharmacological pain relief in a NICU, the question of facilitators for successful implementation of these methods remains to be investigated in the future.

Scientists should avoid accumulating evidence without exploring the clinical feasibility of implementing FT as a non-pharmacological intervention through translational research. The goal of translational research is to accelerate the movement of scientific findings into clinical practice and, thereby, improve health care outcomes for patients [19,20]. Related to the pain relieving intervention of FT, translational research has the potential to close the gap between what we know from existing evidence related to its effectiveness [13-16] and what nurses really practice or are able to practice in the clinical setting under given economic circumstances. In the future FT must be tested in real practice NICU settings, which are characterized very often by uncontrolled and often uncontrollable conditions. Through translational research, scientist and clinicians can identify problems of utilization of this specific pain relieving intervention and collaboratively work on practical and setting-tailored solutions. One possible solution might be to enhance the parent's involvement in the care of their infant by utilizing them do perform the FT as was already explored by our Finnish colleagues [15] or by engaging support personnel or individuals working as volunteers in the hospital. Translational research has the potential to provide a "win-win situation" not only for the infants and their families, but for research scientists and clinical personnel as well. Therefore, future translational research on the clinical feasibility of FT is warranted, including the economic impact of this intervention. In such a study the increased manpower costs need to be weighed against the possible long-term economic consequences of repeated and harmful pain exposure of preterm infants.

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CHAPTER V

CONTEXTUAL FACTORS INFLUENCING PAIN RESPONSE TO HEEL-STICK PROCEDURES IN PRETERM INFANTS. WHAT DO WE KNOW? A SYSTEMATIC REVIEW

Sellam G, Cignacco EL, Craig KD, Engberg S

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ABSTRACT

Background: Major efforts to develop objective measurement tools for neonatal pain assessment have been made. However, the challenge of measuring pain in neonates remains suggesting that contextual factors (CFs) might alter their responses to pain. Although the role of CFs is increasingly discussed as crucial for pain assessment, they are not well described in the literature and are rarely considered in the clinical setting despite their importance.

Aim: To systematically examine studies investigating the impact of CFs on pain response in preterm infants.

Method: A literature search was undertaken for the period from 1990-2009. Studies reporting the relation between one or more CFs and pain response in preterm infants during a heel-stick procedure were considered for inclusion.

Results: Twenty-three studies satisfied inclusion criteria. The studies varied relative to their design, sample, analysis procedures, and variables examined. Six categories of CFs emerged: age, pain exposure, health status, therapeutic interventions, behavioral status, and demographic factors. The examined CFs varied in the strength of their association with pain response, although none were invariably related, as evidenced by contradictory findings. In some cases the inconsistencies appeared attributable to methodological limitations in studies. Behavioral and physiological pain responses were not always in agreement as would be expected.

Conclusion: This review supports the influence of some CFs on pain response. However, the results remain inconclusive which may be, in part, related to the heterogeneity of the studies. Contextual factors need further investigation for a better understanding of the magnitude of their effect on pain response.

5.1 INTRODUCTION

Due to new reproductive technologies and advancing maternal age, preterm birth rates continue to rise in many industrialized countries [1,2]. To ensure survival preterm infants need neonatal intensive care (NICU), which is associated with high procedural pain exposure [3,4]. The developing nervous system undergoes extensive changes postnatal [5] and repeated exposure to pain early in life may lead to significant changes in pain processing while the infant remain in NICU [6-9] and to altered pain response later in infancy [6,10,11] and later in life [12,13].

The challenges of assessing pain in neonates are recognized, and major efforts have been devoted to the development of measurement tools for neonatal pain [14]. Despite these major commitments, it is also recognized that the neonatal response to pain is influenced by more than the invasive procedure itself, and contextual influences must be considered in the evaluation of pain.

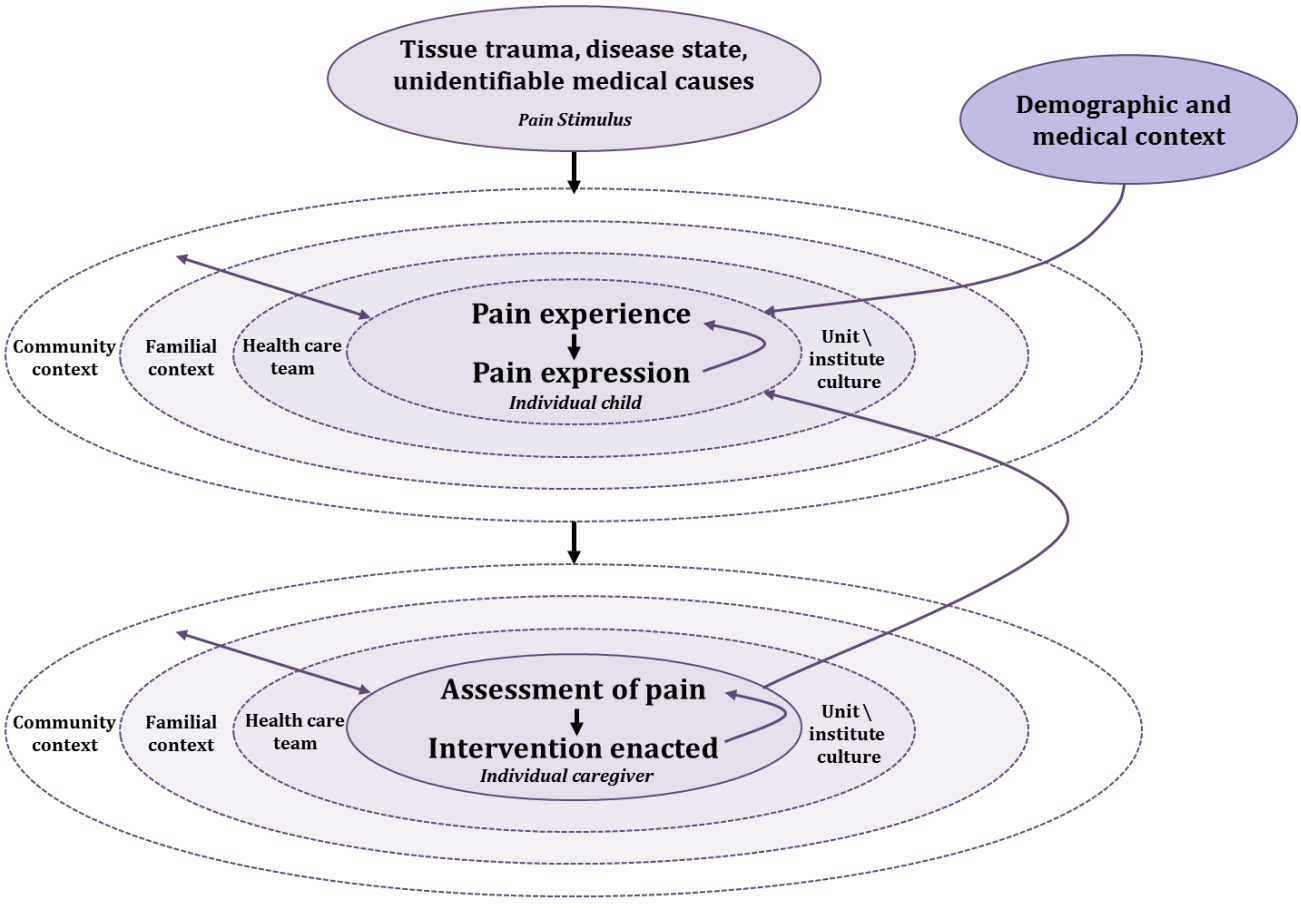
The impact of immediate contextual factors (CFs) as determinants of the high variability in pain responses of preterms also contributes to complicating their pain assessment. Cignacco et al. [15] described higher within than between subject variability in pain responses among preterms across 5 heel-sticks during a 14-day period. The inter-rater agreement in pain assessment was high for the 3 first heel-sticks and lower for later heel-sticks, suggesting that there may be alterations in preterms' pain response across time. This variability could be due to a combination of low gestational age (GA) and high pain exposure, which provokes changes in response over time [16] with an impaired level of response sensitivity as post-natal age increases [17,18].

Craig and Pillai Riddell [19] provide a basis for conceptualizing the importance of CFs. In their model pain should be viewed as a dynamic and interactive process between infant's pain

response and the caregiver. The model examines three central features: painful event, child, and caregiver. The influence of familial, community and cultural environments is recognized as well. When focusing on preterm infants in the NICU, immediate medical and demographic factors appear to have a more direct influence on pain experience and expression than cultural and community factors. In our formulation of the model (Figure 1), we provide a focus on medical and demographic CFs influencing pain by putting the health care team next to the preterm infant within those circles of environments that influence pain.

Although the role of CFs is increasingly discussed as crucial, they are not well described in the literature and rarely considered in pain assessment. There are no published systematic reviews of the impact of CFs on pain response in preterm infants. The aim of this study was to systematically review existing literature examining the relationship between CFs and pain response in preterm infants.

Figure 1: The Socio Communication Model of Infant Pain – Modified Model



Craig & Pillai Riddell (2003)- modified model

5.2 METHODS

This systematic review is reported according to the present PRISMA guidelines (Preferred Reporting Items for Systematic reviews and Meta-Analyses) [20]. Searches were undertaken in the following databases: Medline, Cochrane library, CINAHL, Web of Science, and PsycInfo covering the period from 1990 to November 2009. Our search was restricted to studies published in English and with human subjects. In PubMed and Cochrane, the MeSH headings "Infant, Premature" and "Pain" were entered in combination with the following searching terms: "response", "response AND heel-stick", "response AND contextual factors", "response AND determinants", "response AND confounders", "response AND contextual indicators", "response AND risk factors", "response AND predictors", "response AND gestational age", "response AND severity of illness", "response AND co-morbidities", "response AND neurological impairment", "response AND analgesics", "response AND medications", "response AND number of painful procedures", "response AND time from last painful procedure". The same search was performed with the search terms "expression" and "reaction" instead of "response". In databases without MeSH possibilities the search terms "preterm infants" and "pain" were combined consistently with the search terms "response", "expression", "reaction" and the other combined search terms.

The databased systematic search for studies including CFs was challenging, as few studies referred exclusively to them. Contextual factors are rather "lurking" variables in the measurement of pain in neonates. We, therefore, used the 'snowball method' also looking for references in the most recent publications relating to CFs. Two of the authors (SE and GS) evaluated the papers independently for eligibility according to the inclusion criteria. In the case of disagreement between the two reviewers, a third author (EC) participated in the evaluation process. After discussion a decision was made about inclusion of the study.

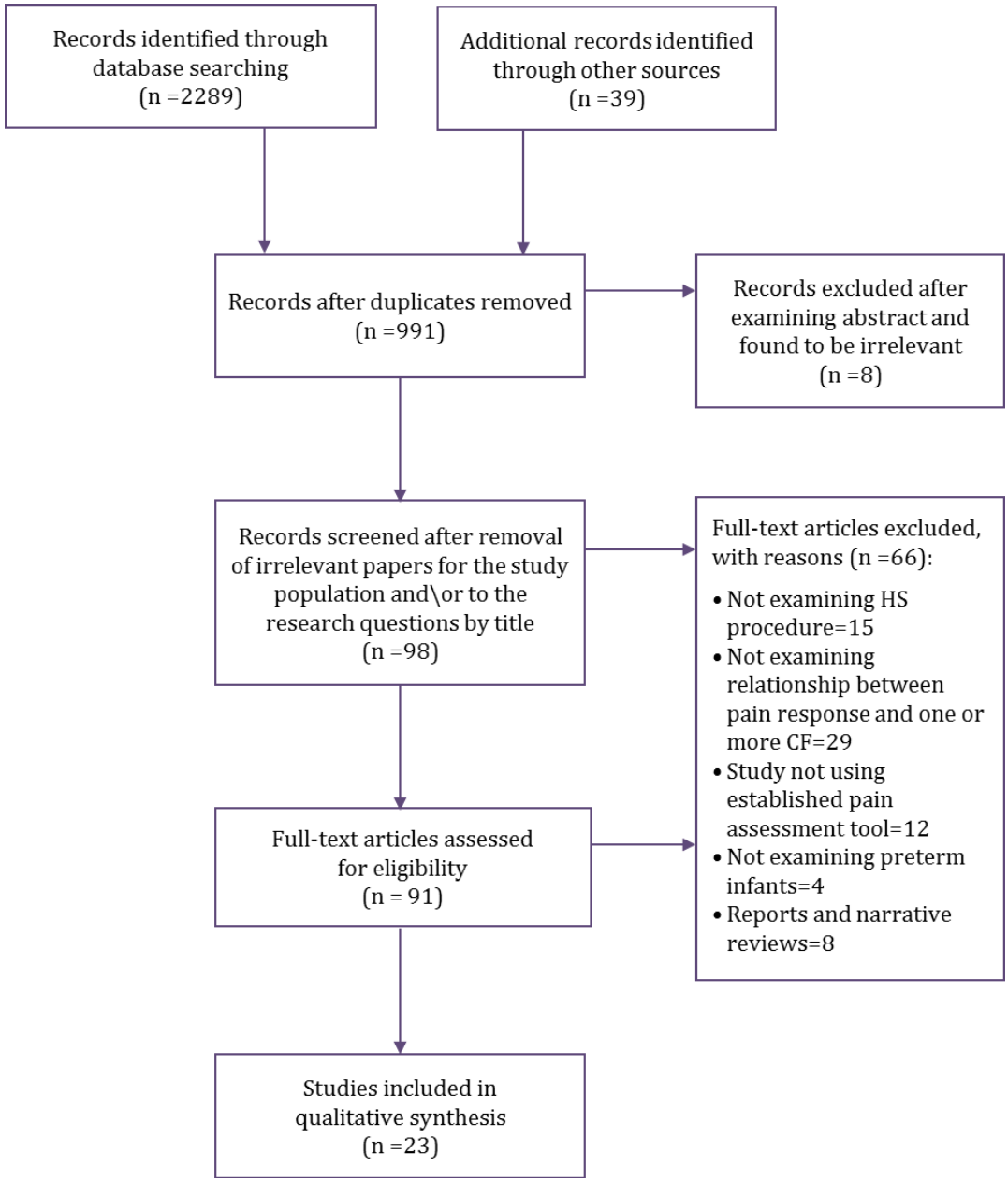
5.2.1 Selection process

The systematic search of the 5 databases returned 2,289 citations while an additional 39 studies were identified when the references of recent publications were hand searched. After deleting duplicate articles, 991 were screened by title or abstract for relevance. Based on this review, 91 articles were retrieved for detailed review based on the inclusion criteria (see Table 1 and Figure 2). A total of 23 studies met the selection criteria and were included in this review. Studies examining the association between CFs and pain responses in preterm infants (up to 37 weeks of gestation) hospitalized in a NICU were included regardless of the study design. Heel-stick procedure was chosen as a representative painful procedure as it was a focus of many of the studies, it is one of the most common painful procedures performed in the NICU setting and it is performed internationally using similar methods.

Table 1: Inclusion criteria

Inclusion Criteria
1. Published as peer-reviewed article in English.
2. Reported neonatal subjects ventilated or not ventilated infants.
3. Examined heel-stick procedure as painful intervention.
4. Examined the relationship between one or more CFs and pain responses.
5. Relied on a validated pain assessment tool.
6. All possible research study designs were considered.

Figure 2: Flow diagram of study selection process (PRISMA)



5.2.2 Quality assessment process of the included studies

We were unable to identify a standardized instrument designed to assess the quality of studies examining factors associated with a particular outcome. Consequently, we adapted a quality criteria checklist used in a prior systematic review examining fall risk factors [21]. The checklist contains 12 dichotomous questions (Table 2). The 23 selected studies were evaluated independently by three of the authors (GS, ES and EC). In case of disagreement, a discussion took place until consensus was reached. No studies were excluded on the basis of the quality assessment.

Table 2: Quality Assessment

	REFERENCE	1A	1B	2	3A	3B	3C	3D	4A	4B	4C	5A	5B
1	CRAIG ET AL_1993	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
2	EVANS ET AL_2005	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
3	GIBBINS ET AL_2003	Y	Y	Y	Y	Y	Y	Y	N	N	Y	Y	Y
4	GIBBINS ET AL_2008_I	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N
5	GIBBINS ET AL_2008_II	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N
6	GRUNAU ET AL_2001_I	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y
7	GRUNAU ET AL_2004	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N
8	GRUNAU ET AL_2005	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
9	HOLSTI ET AL_2006	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
10	JOHNSTON & STEVENS_1996	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
11	JOHNSTON ET AL_1995	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
12	JOHNSTON ET AL_1996	Y	Y	Y	Y	N	N	Y	Y	Y	Y	Y	Y
13	JOHNSTON ET AL_1999	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
14	MORISON ET AL_2003	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	N
15	OBERLANDER ET AL_2002	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
16	PORTER ET AL_1998	N	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y
17	SLATER ET AL_2009	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y
18	STEVENS ET AL_1994	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
19	STEVENS ET AL_1999	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
20	WALDEN ET AL_2001	Y	Y	Y	Y	N	N	Y	Y	Y	Y	Y	Y
21	WARNER_2001	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y
22	WILLIAMS ET AL_2009	Y	N	Y	Y	N	Y	Y	Y	Y	Y	Y	Y
23	XIA ET AL_2002	Y	Y	Y	Y	Y	?	N	N	N	Y	Y	N

Legend: Criteria for quality assessment of the included papers

1A, Definition of preterm infants; **1B**, Definition of CF; **2**, Description of study period-how many HS; **3a**, Definition of criteria for inclusion and exclusion; **3B**, Sample size based on power analysis or ≥ 20 per predictor; **3C**, Drop out less than 20%; **3d**, Specification of sample characteristic; **4A**, Adequate description of primary data collection or procedure of secondary data analysis; **4B**, Data collection by standardized procedures; **4C**, Used a validated pain assessment tool; **5a**, Reported relationship between CF and pain statistically; **5b**, Using methods of multivariate analysis (Multivariate analysis was defined as: Analysis that controlled for the effects of one or more confounding variables or covariates).

Y=yes (in agreement); **N=**no (in agreement); **?**= not specified.

5.3 RESULTS

5.3.1 Study characteristics

The systematic search revealed studies that examined the association between the following CFs and pain response: (1) age related CFs [GA, post-natal age(PNA), post-conceptual age]; (2) pain exposure related CFs (time since last painful procedure, total number of previous painful procedures or the number within the 24 hours prior to the heel-stick procedure); (3) health status related CFs (severity of illness, neurological impairment and other co-morbidities, Apgar scores,

length of stay in NICU); (4) therapeutic intervention-related CFs (administration of medications such as opioids, mechanical ventilation, handling before the heel-stick, and positioning); (5) behavioral CFs (sleep state prior to the heel-stick procedure, temperament of the infant); and (6) demographic factors (race, gender, and weight). The included studies were of various designs. However they could be allocated to either the category of observational studies (78%, n=18) or interventional studies (22%, n=5) (Table 3). All included preterm infants in their study population, with 78% (n=18) examining preterm infants exclusively and 22% including term infants as a control group (n=5). Most studies excluded infants at risk for neurological impairment (91%, n=21). All of them assessed pain during a heel-stick procedure, either for single (74%, n=17) or repeated (26%, n=6) heel-sticks. Pain response was assessed with different behavioral and physiological assessment instruments, which were described as reliable and valid. The studies using physiological measures examined heart rate and oxygen saturation as pain indicators. The majority of studies used one of two behavioral instruments used for pain measurement: the Premature Infant Pain Profile (PIPP) and the Neonatal Facial Coding System (NFCS). Thirty percent of the studies (n=7) used the PIPP, and 65% used the NFCS (n=15). One study only used the Neonatal Infant Pain Scale (NIPS). Most of the studies examined behavioral outcomes separately from physiological outcomes. Ninety-one percent of the studies (n=21) examined both physiological and behavioral outcome measures, while 7% (n=2) only reported findings related to behavioral outcomes. Of the 21 studies that examined both physiological and behavioral outcomes, 74% (n=17) examined the outcomes separately while 17% (n=4) examined them in combination. Most of the studies reported a total score on the outcome measures (61%, n=14), while the remaining studies reported findings on the specific behavior/physiologic components assessed by the measurement tool. In the quality assessment, ten studies met all the quality criteria [16,17,22-29]. All the studies met the following criteria: description of study period, reporting how many heel-sticks were performed (2); use of a validated pain assessment tool (4c); and reporting the relationship between CFs and pain statistically (5a) (Table 2). Six studies did not report that their sample size was based on a power analysis or did not include $\geq$ 20 subjects per CF examined (criteria 3b) [18,30-

[35], while five did not examine the relationship between the CF and pain responses multivariately (5b) [31,36-39] and four had a drop-out rate of ≥ 20% (3c) [18,32,33,39]. With the exception of one study [39] which failed to meet 5 criteria, the rest of the studies met all but one or two criteria.

5.3.2.1 Age-related contextual factors

While infant age was the most commonly examined CF, it was measured in a variety of ways. In addition, the terminology utilized to describe age varied. Information presented in the articles indicated age was classified as (1) GA, the age of the infant at birth; (2) PNA, the age of the child at the time of the heel-stick, i.e. days since birth; and (3) post-conceptual age, the sum of the age at birth + age since birth.

Gestational age was the most commonly examined CF. Eleven studies (48%) examined GA as a CF influencing behavioral pain responses [16,17,24,26,30,31,34,36,37,39,40] with eight of these examining physiological response as well [16,17,24,26,31,36,37,39]. Five studies reported a statistically significant GA effect on behavioral response to pain with greater behavioral response as GA increased [17,24,30,37,39]. Four studies reported that GA was unrelated to behavioral responses to pain [16,26,34,36]. Three of the eight studies examining the impact of GA on physiologic responses reported a significant effect of GA on oxygen saturation and/or heart rate [17,30,37]. Gibbins et al. [37] reported that GA had a significant effect on oxygen saturation but not on heart rate while Craig and colleagues [17] reported that there was a significant GA effect on heart rate but not on oxygen saturation. While there was a negative correlation between GA and heart rate variability in the study of Morison et al. [31] (r = -0.64), the correlation between GA and post-lance heart rate was weak (r = 0.16). Most of the studies examining the effect of GA on physiological response did not find any significant effect on heart rate or oxygen saturation [16,24,26,31,36,39]. Four studies measured pain with the PIPP, which included behavioral and physiological parameters. Three reported a significant GA effect on the total PIPP score [22,25,41], while one described a non-significant effect [29].

Post-natal age (PNA) was examined by 5 studies[16,18,25,29,34]. All three studies that examined the impact of PNA on behavioral outcome measures reported a significant relationship with vigor increasing with PNA [18,25,34]. Two studies examined the impact of PNA on total PIPP scores and neither found a significant effect during multivariate analysis [25,29]. Two studies examined the impact of post conceptual age on pain responses [33,40]. One of these studies reported that post conceptual age was significantly positively related to 'time to facial response' during heel-stick and that infants with no responses had significantly lower post conceptual ages than those with facial responses [40]. The other study [33] reported that post conceptual age was not significantly related to facial responses (eye squeeze or brow bulge), total PIPP scores, heart rate or oxygen saturation. In this study, the only behavioral response that showed a significant post conceptual age effect was finger splay with significantly fewer younger infants showing a response compared to older infants.

5.3.2.2 Previous pain exposure- related contextual factors

Investigators have examined the relationship between (1) previous number of painful procedures, (2) the time since the last painful procedure and (3) the number of painful procedures during the 24 hours prior to the heel-stick. The number of prior painful procedures was examined in eight studies [16,18,22,23,25,29-31]. Three studies [16,30,31] reported that the total number of previous painful procedures was significantly inversely related to behavioral pain responses and two described a significant positive association with total PIPP scores [22,29]. In contrast, two studies reported that there was no significant relationship between the number of previous painful procedures and behavioral responses to pain [18,23] and one did not find a significant relationship with PIPP scores [25]. Five studies examined the relationship between the number of prior painful procedures and heart rate [16,18,23,30,31]. Three reported that the relationship was not significant [16,18,23] while one study found that the number of previous painful procedures was significantly related to heart rate variability [30] and another reported a moderate but non-significant correlation with heart rate (r = 0.40) [31]. Two studies examined the relationship between the number of previous painful procedures and oxygen saturation and the relationship

was not statistically significant in either study [16,18]. One study concentrated on the time since the last painful procedure and found a non-significant negative relationship to PIPP scores [25]. Two studies examined the relationship between painful procedures during the previous 24 hours and pain response [31,34] but found no significant relationship.

5.3.2.3 Health status-related contextual factors

A number of studies examined the association between health status variables (severity of illness, neurologic impairment, sepsis, Apgar scores and length of stay in the NICU) and preterm infants' pain responses. Seven studies examined severity of illness [16,18,22,28-31]. Four studies found no significant associations between severity of illness and behavioral and/or physiologic pain responses [16,18,29-31]. In one study [28], findings were mixed with a significant effect on cry variables but no significant relationship to NFCS scores was found. Only one study reported that severity of illness was significantly related to pain scores. Evans et al. [22] reported that there was small but significant negative association between severity of illness and PIPP scores.

The influence of neurological impairment on pain response was examined in three studies [27,34,40]. While neurologic impairment was not related to most of the measures of pain response in these studies, it was significantly related to tongue protrusion in one study [27]. One study examined the association between sepsis and pain responses [34] and found no significant relationship. Apgar scores at 5 min. were examined in 5 studies [16,25,30,31,34]. In three of the studies, Apgar scores were not significantly related to pain responses [25,30,34]. In contrast, Johnson and Stevens [16] reported that 5-minute Apgar scores were significantly inversely related to brow bulge and nasolabial furrow scores but not to eye squeeze, heart rate or oxygen saturation, while Morison et al. [31] described a significant positive correlation between 1-minute Apgar scores and total facial responses during the heel lance (r = 0.80) and non-significant correlations with eye squeeze scores during heel lance and post-lance heart rate as well as a non-significant correlation with stress cues. In the study of Morison et al. [31] stress cues were measured by the Neonatal Individualized Developmental Care Assessment (NIDCAP). The purpose of NIDCAP is

to assess - according to a comprehensive set of body movements - the stability or the state of stress the premature infant expresses. Care based on NIDCAP aims at enhancing infant's stability throughout a highly individualized care. The observed stress cues were tremor, startle, twitch, extended arms/legs, diffused squirm, arch, fisting, finger splay, airplane, salute, sitting on air, stretch/drown, and flaccid arms/legs.

Length of stay in NICU was examined by one study and was not significantly related to pain response [34].

5.3.2.4 Therapeutic interventions- related contextual factors

Relationship between selected interventions and pain responses were examined. These interventions were medications, mechanical ventilation, handling, and positioning. Preterm infants in the NICU receive a wide range of medication. Medications reported in the literature were mainly opioids, steroids, and caffeine. Three studies examined the relationship between infants receiving analgesics during the time of the study and pain responses [23,30,40]. In one study, receiving morphine prior to the heel-stick was significantly positively related to latency of facial expression [40]. In the second study, total morphine administration was negatively related to heart rate variability but was not significantly related to pain behaviors [30]. The latter study reported that total days of dexamethasone was negatively related to the pain behaviors and positively related to heart rate variability. One study examined caffeine and found no significant relationship to pain response [25].

Other therapeutic interventions were investigated in a smaller number of studies. Mechanical ventilation was examined in three studies [18,30,34]. None of the studies found a significant relationship between ventilator status and pain response. Two studies examined handling of the infant prior to the heel-sticks procedures and found significant negative effects on behavioral responses to pain [24,32]. One of the studies [32] also reported a significant effect on heart rate, while other [24] did not. Another one compared pain responses in infants in prone and supine position

prior to their heel-stick and found no significant interaction between positioning and the phase of the heel-stick for total facial activity, heart rate or sleep/wake state [38].

5.3.2.5 Behavioral contextual factors

The relationships between behavioral factors (sleep-wake cycle and temperament) and pain responses were examined in three studies [25,35,40]. Two studies described sleep/wake state as a predictor for pain response [25,40]. In one of the studies [40] sleep/wake state was positively significantly related to NFCS scores (meaning the more alert the infant the less the response), but not to changes in facial expression measured by the PIPP or to cry variables. In the other study, sleep/wake state was not significantly related to PIPP scores [25].

Temperament was examined by Warner [35]. While there was no significant relationship between mother's perceptions of their infant's pre or post-natal activity levels, there were significant relationships between temperament and pain response as measured by some items of the NIDCAP and PIPP scores. In this study, temperament was operationalized by high or low motor reactivity, high or low motor intensity, and high or low threshold. Infants who were higher reactivity responders based on the NIDCAP had significantly higher PIPP scores than those who were lower reactivity responders. The relationships to heart rate and oxygen saturation were non-significant.

5.3.2.6 Demographic factors

Demographic factors, specifically weight [16,30,31,34], race [25,34], and gender [18,24,25,34] were examined in several studies. Two of the studies describing the relationship between infant weight and pain responses reported that the relationships were not significant [30,34]. In contrast, Johnson and Stevens [16] reported that there was a significant negative relationship between birth weight and oxygen saturation response to the heel-stick. Birth weight was not significantly related to any of the other pain response variables measured in this study (brow bulge, eye squeeze, nasolabial furrow or heart rate). Morison and colleagues [31] reported that birth weight was significantly negatively correlated with total facial responses (r = -0.61) and with stress

cues (*r* = -0.73). In addition, there were non-significant correlations between birth weight, eye squeeze scores, and heart rate variability. Investigators found that there was no significant relationship between race [25,34] or gender [18,24,25,34] and pain responses.

5.4 DISCUSSION

The systematic review provided insight into the influence of CFs on preterm infant's pain response during heel-stick procedures. Certain CFs exerted substantial effects. Other findings suggested subtle, inconsistent and probably not clinically important impact. Many of the findings were inconclusive and deserving of studies with more methodological rigor.

Age - The most commonly examined CFs group, age, was the CF that most consistently affected pain responses in these studies. The results showed that facial expression response to pain tended to increase with age. This effect can be explained by the development of the nervous system. The cephalocaudal development of facial musculature may influence the magnitude of facial activity as evidenced by the fact that preterm infants have less muscular strength, posture, tone and body movement compared to term infants [31,36,41]. Moreover, preterm infants are significantly more hypotonic than term infants with smooth movements improving as PNA increases [18]. Most of the studies (57%) that examined the relationship between GA and facial responses or PIPP scores (which include assessment of facial responses) during heel-sticks reported statistically significant relationships, while 42% of the studies did not find a significant relationship between GA and pain expression. These findings could be explained by limitations of studies. Gibbins et al. [36] explored physiological and behavioral pain response; however, they included only infants with extremely low GA (23-25 6/7; 26-28 weeks). They found no differences in pain response between the two groups. Both groups showed more total facial expression at the lance phase compared to baseline. It is noteworthy that facial expression is a behavioral response to pain seen with even the earliest viable newborn infant, attesting to its inherent biological nature. Stevens et al. [29] also reported a non-significant relationship between GA and pain responses. Similar to Gibbins et al. [36] they examined only extremely low GA infants.

The studies finding a significant effect of GA included infants born at term or a group of infants greater than 28 weeks GA. Three of the studies with non-significant results [16,29,34] did a multivariate analysis and examined PNA as well as GA. Two of them found significant relation to PNA. By incorporating post-natal age into the analysis, the effect of GA may be reduced or not valid any longer.

Previous pain exposure - Most of the studies examining the impact of number of prior painful procedures reported a positive significant relationship with pain response, meaning that higher numbers of painful procedures were related to a lower behavioral pain response. Reduced pain expression based on the number of painful procedures has a variety of explanations. Infants with a very low GA (24-28 weeks) were found to have a higher procedural exposure due to their general immaturity and need for a higher degree of intensive care and their length of stay in the NICU [15]. However, the only study examining length of stay in the NICU did not find that it was significantly related to pain responses. The blunted pain responses, mainly reported in relation to facial activity, in very preterm neonates may also be a sign of exhaustion as a result of all of the procedures and handling they experienced during hospitalization in the NICU [25,32]. Logically, these explanations could be related to the GA of the infant. The proximity to the last painful procedure might explain variation in pain response as well [25]. A further possible explanation for low responsiveness is the endogenous production of endorphins in response to the last procedure, which might have protected the infant from the pain resulting from the subsequent procedure [25]. However, the validity of this explanation may be limited depending on the time from the last painful procedure. Only one study examined the relationship between the time since the last painful procedure and pain responses and the relationship was not significant.

Health status - Most of the studies that examined the relationship between health status and pain responses did not find significant relationships. In the three studies examining the relationship between neurological impairment and pain responses, there was no significant

relationship. It seems possible that the biological substrates for pain are not that readily affected by the health status of the infant. An explanation for these findings is that during this period of immaturity, the brain injury has not yet expressed and that identifiable differences in pain response and functional impairment might only be observed at a later age [27].

Therapeutic interventions - Although preterm infants in the NICU usually receive medications, their impact was examined in only a very small number of studies. Scott et al. [42] reported that morphine reduced facial activity associated with heel-stick. However, the present review found contradictory findings regarding the influence of morphine on pain expression [23,30,40]. Preterm infants who are critically ill and undergoing mechanical ventilation typically receive continuous fentanyl infusion or other sedatives. Some pharmacological sedation interventions may also dampened pain experience and/or pain expression [43]. None of the studies examining mechanical ventilation found a relationship with pain response. In one of these studies, dexamethasone administration was negatively related to NFCS scores and positively related to heart rate [30]. These results were interesting and should be examined in future studies. As preterm infants in a NICU setting are being expose constantly to various medications, further research on this topic is required.

Behavioral state - Temperament, measured by the NIDCAP, was significantly positively related to the PIPP scores in one study [35]. Although the findings of Warner are interesting, they need to be interpreted cautiously, as temperament was only examined with the NIDCAP, which is a developmental assessment and not a valid tool for the assessment of temperament.

Sleep/wake state was positively significantly related to NFCS scores [40] but not to changes in facial expression measured by the PIPP [25,40]. The positive relation between the sleep/wake state and NFCS scores is supported by two additional studies which were not included in the present systematic review because they did not meet our inclusion criteria. Slater et al. [44] reported typical cortical pain response (measured by near infrared spectroscopy) to heel-stick in infants between 25-45 weeks of gestation. The responses were significantly greater in awake

compared to sleeping infants. Grunau and Craig [45] reported, that awake infants responded with significantly more facial movement compared to infants who were asleep. These studies suggest that sleep/wake state is an important factor in pain response and support the need for more research to confirm these relationships.

5.4.1 Limitations

There are some methodological shortcomings that need to be taken into account when considering the findings of this review. The CFs were not always defined or measured in the same manner. The age of the infants included in the studies varied as did the time point of first data collection after birth. The number of studies examining some of the CFs was quite small. Due to all of these limitations, a meta-analysis was not possible.

Furthermore, most of the studies measured facial activity as the pain indices. In the majority of studies, the NFCS and the PIPP were used as pain assessment tools. Both of the tools focus mainly on facial activity without including body movements. Yet, body movements are a major indicator of pain, which can be observed at the earliest GA and could also be sensitive to the influence of CFs.

5.5 CONCLUSIONS

The results suggest that contextual factors play an important role in preterm infants' responses to pain and should be considered when pain is measured. However, there was variable impact, with none of the CFs examined consistently related to pain response, and there was an inconsistency between behavioral and physiological responses to pain. One of the issues in interpreting these findings across studies was the inconsistencies in the characteristics of the samples and designs. Our understanding of the role of CFs will be advanced by well-designed prospective studies that use consistent definitions of CFs such as GA and the neonatal groups' ages being compared and an adequate sample of extremely low and low GA infants. We acknowledge that one of the challenges of examining the effects of

various CFs on pain response is that there is a high degree of correlation between factors. Consequently, it is challenging to disentangle the effect of any single factor on pain response. Due to this interrelationship, the ideal study would need to be adequately powered to allow a multivariate study of all the CFs identified in this review as potentially influencing pain responses. One of the strengths of the studies that have been done is that they used reliable and validated pain measures. Since there is evidence, however, to suggest that CFs may have a differential effect on behavioral and physiologic pain responses, future studies should examine them separately. Studies should report the magnitude of the effect of these factors on pain responses in this population. Once we better understand the factors that influence neonatal pain responses, neonatal pain assessment instruments have to be revised in order to determine whether a preterm neonate is in pain or not.

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Table 3: Studies examining Contextual Factors and pain response in preterm infants

Nr	Study	Design/Settings/Sample	Variables	Measurement of primary outcomes	Findings
1	Craig et al 1993 (Canada)	**Design:** Cross sectional. **Settings:** Special care nursery. **Sample:** n=56, 5 groups of GA wks: (1) 25-27, n=8; (2) 28-30, n=15; (3) 31-33, n=16; (4) 34-36, n=10; (5) 37-41, n=7. **Mean Postnatal age (PNA)**-total sample: ~4days. **Ventilated at observation:** n=3. No infants with neurological impairment (NI) were included.	**CFs:** Gestational age (GA) **Outcome:** Responses across phases of the heel-stick (HS) procedure (baseline, swab, lance and recovery).	Neonatal Facial Coding System (NFCS)	Significant main effects for GA (F[4,51]=7.22, p<0.0001) and phase (F [3,153]=58.44, p<0.00001) as well as significant interaction between GA and heel-stick phase (F[12,153]=3.91, p<0.00001). No significant differences across phases for group 1; there were significant differences for the other four groups (p<0.0002 for group 2 and <0.00001 for groups 3, 4 and 5).
				Infant Body Coding System (IBCS)	Significant main effects for gestational age group (F[4, 51]= 4.34, p<0.004) and for the procedure phase (F[3, 153]=47.71, p<0.00001). No significant interaction between gestational age group and procedure phase (p=0.65). Infants at all gestational ages reacted to the swab and appeared to react more to the heel-stick.
				Heart rate (HR)	A significant phase effect (p<0.00001) and a significant GA group by procedure phase effect (F[16, 204]=3.14, p=<0.0001). No significant different in HR across phases in group 1; significant differences across the phases in the other four GA groups (p<0.001 - <0.00001).
				Oxygen saturation (SaO_2)	SaO_2 levels were not significantly different among the GA groups. Significant phase effect (F [4, 180]=6.80, p<0.00001) but no significant GA by phase effect.
2	Evans et al, 2005 (USA)	**Design:** Comparative longitudinal cross sectional. **Settings:** Regional level III NICU. **Sample:** n=81, 4 groups of GA: (1) <28; (2) 28-30; (3) 31-33; and (4) 34-36 wks. **PNA:** < 72 hours since birth. Proportion of ventilated infants was not reported. No infants with NI were included.	**CFs**: GA, severity of illness, number of painful procedures. **Outcome:** Pain response during 1-12 HSs per infant	Premature Infant Pain Profile (PIPP)	Infants from group 3 had significantly higher PIPP scores (M=10.5; SD=3.7) than all other groups (group 1: M=9.8, SD=3.1; group 2: M=7.7, SD=2.3; group 4: M=9, SD=9.0) (p<0.02). Both severity of illness and the number of prior painful procedures were significantly related to PIPP scores (F[2, 305]=3.83, p=0.023). When considered together, there was a small but significant negative association between PIPP scores and NTISS scores and total prior painful procedures (p<0.047) [the more severe the infants condition &\or more painful procedures experienced prior to the HS, the lower the PIPP scores].

Nr	Study	Design/Settings/Sample	Variables	Measurement of primary	Findings
3	Gibbins & Stevens, 2003 (Canada)	**Design:** Secondary data analysis of a RCT. **Settings:** NICU. **Sample:** n=190, 3 groups of GA: (1) 27-31 6/7 wks, n=63; (2) 32-30 wks; (3) 32-35 6/7 wks, n=63; (4) 36-43 wks, n=64. **PNA:** <7 days since birth. Proportion of ventilated infants was not reported. Infants with NI were excluded.	**CF**: GA. **Outcome:** Pain response during one heel-stick.	PIPP pain indicators HR & SaO_2	Significant GA differences were found in percentage of change from baseline in: • Brow bulge during the heel lance (F[187,2]=25.83, p<0.001) and heel squeeze (F[187,2]=18.47, p<0.001), • Eye squeeze during the heel lance (F[187, 2]=32.13, p<0.001) and squeeze (F[187,2]=29.84, p=0.01), and • Nasolabial furrow during the heel lance (F[187,2]=12.31, p=0.01) and squeeze (F[187,2]=12.84, p=0.01). The most mature neonates showed the most facial activity while the least mature showed little facial activity. Significant GA differences in percentage of change from baseline in SaO_2 during the heel lance (F[187, 2]=5.55, p=0.005) and squeeze (F[187, 2]=35.8, p=0.001) and in HR during the lance (F[187, 2]=70.79, p<0.001)and squeeze phases (F[187, 2]=35.8, p<0.001) with the least mature neonates having the least amount of change from baseline.
4	Gibbins et al 2008_I (Canada)	**Design:** Secondary data analysis of data collected in two studies. **Settings:** NICU. **Sample:** n=161infants at varying risk for neurological impairment. GA in 4 strata: (1) 23-27 6/7 wks (n=41); (2) 28-31 6/7wks (n=50); (3) 32-35 6/7wks (n=21); (4) >36wks (n=49). **PNA:** Not reported. Proportion of ventilated infants was not reported.	**CF:** GA **Outcome:** Pain response during one HS.	NFCS SaO_2 HR	Differences in total facial activities from baseline to lance phase indicated significant differences (F[3, 145]=6.5, p<0.001) based on GA with the least mature infants having significantly less change from baseline to lance compared to the 3rd and 4th strata. Significant differences in mean SaO_2 between baseline and heel lance based on GA (F[3, 145]=3.1, p<0.03) with the greatest change seen in strata 2 and the least in strata 3. No significant differences in mean heart rate change from baseline to heel lance based on GA.
5	Gibbins et al, 2008_II (Canada)	**Design**: Prospective descriptive crossover design. **Settings:** Major regional level III NICU. **Sample:** n=53 in two GA strata: (1) 23-25 6/7wks (n=23 and (2) 26-28wks (n=30). **PNA**: <7 days Proportion of ventilated infants was not reported. No infants with NI were included.	**CF:** GA. **Outcome:** Pain response during one HS and a diaper change.	NFCS Body movement HR SaO_2	No significant differences between the 2 GA in total facial activity. No significant differences in total body movements were between the 2 GA groups. No significant GA effect. No significant GA effect.

Nr	Study	Design/Settings/Sample	Variables	Measurement of primary outcomes	Findings
6	Grunau et al 2001 (Canada)	**Design:** Prospective cohort study. **Settings:** Level III NICU. **Sample:** n=136. GA 23.6-32.7 wks, mean = 28.1wks. **PCA(GA+PNA):** 32 wks. Proportion of ventilated infants was not reported (mean number of days was reported). No infants with NI were included.	**CFs:** GA, birth weight, 5 minute Apgar, mechanical ventilation, number of surgeries, severity of illness, number of painful procedures, medications exposure (morphine, dexa-methasone, indomethacin, fentanyl and pancuronium). **Outcome:** Pain behavior and HR variability (HRV) based on the lance and squeeze phases during one HS.	Pain Behavior =(0.73*$NFCS_{lance}$) +(0.87*$NFCS_{squeeze}$) +(0.79*state) +(-0.19*ΔLF power) HRV =(0.31*$NFCS_{lance}$) +(0.07*$NFCS_{squeeze}$) +(-0.13*state) +(-0.95*ΔLF power)	In a regression analysis- GA (β=-0.60, p<0.0001), number of pain procedures (β=-0.51, p<0.0001)) and number of days on dexamethasone (β=-0.28, p<0.05) were predictors of pain behaviors. GA (β=0.56, p<0.0001), number of painful procedures (β=0.75, p<0.0001)), number of days on dexamethasone (β=0.43, p<0.01) and total morphine exposure (β=-0.20, p<0.05) were predictors of heart rate variability.
7	Grunau et al 2004 (Canada)	**Design:** Comparison study. **Settings:** Level III NICU. **Sample:**: n=38. GA-25-32 wks. **PCA:** 32 wks Proportion of ventilated infants was not reported (mean number of days was reported). No infants with NI were included.	**CF:** Prone position. **Outcome:** Pain response during one HS.	NFCS HR Sleep/wake state	Significant increase in total facial activity from baseline to heel lance in both the supine and prone group (F[1, 36]= 68.32, p=0.0001) but no statistically significant main effect for position (F[1, 36]= 0.88, p=0.35) and no significant interaction between the phase of the heel-stick and position F[1, 36]= 0.03, p=0.88). Significant increase in HR in both groups (F[1, 36]= 68.96, p=0.0001), but no significant effect for position (F[1, 36]= 1.89, p=0.18) and no position by phase effect (F[1, 36]= 0.002, p=0.96). The groups did not differ statistically significantly during the HS (Mann Whitney U = 158.0; p = 0.56).

Nr	Study	Design/Settings/Sample	Variables	Measurement of primary outcomes	Findings
8	Grunau et al. 2005 (Canada)	**Design:** Prospective cohort study. **Settings:** Level III NICU. **Sample:** n=87. GA: 22-32 wks, 2 groups: (1) 22-28, n=30; (2) 29-32, n=57. **PNA:** 32 wks ± 7 days. Proportion of ventilated infants was not reported (mean days=17.52+14.67 days in group 1 and 1.40+3.31 days in group 2. No infants with NI were included.	**CFs:** Number of prior pain exposure, severity of illness, morphine exposure. **Outcome:** Pain response during one HS.	NFCS	No significant association between prior pain exposure (skin breaking procedures since birth) and facial response to the heel-stick in group 2. After controlling for severity of illness and cumulative morphine exposure, the association was not statistically significant in group 1.
				HR	No statistically significant association between prior pain exposure and heart rate variability in either group.
				Cortisol levels	Group 1: controlling for early illness severity and morphine exposure, higher neonatal procedural pain predicted lower plasma cortisol responses to stress (adjusted R^2=0.23; standardized β=-0.59, t=-2.22, p=0.039). / Group 2: no significant relationships.
9	Holsti et al. 2006 (Canada)	**Design:** Randomized-within subjects, cross over design. **Settings:** Level III NICU. **Sample:** n=42. GA-25-32 wks (mean 30 wks) **PCA:** 32 wks ± 7 days Proportion of ventilated infants was not reported (median=5.5+9 days). No infants with NI were included.	**CFs:** GA, rest period (RP) versus series of routine nursing interventions (clustered care -CC) prior to heel-stick, gender. **Outcome:** Pain response during two HS.	NFCS	Significant increase in facial activity in both the RP (F=10.1, p<0.0001) and CC groups (F=21.6, p<0.0001) with significantly more facial activity in the CC group than the RP group during the heel lance (t=2.1, p<0.05). GA was significantly related to facial activity (F=4.3, p<0.04) with increased facial response seen only in infants > 30 weeks of age. No gender effects.
				HR	There was no significant difference in the change in HR from baseline to lance in the two care groups and no significant GA or gender effects.
				Sleep/wake state	No differences in the sleep/wake state in the RP and CC groups and no gender or GA effects.

Nr	Study	Design/Settings/Sample	Variables	Measurement of primary outcomes	Findings
10	Johnston & Stevens. 1996 (Canada)	**Design:** Cross sectional comparative. **Settings:** Two level III NICU. **Sample:** n=89. 2 groups based on PNA at the time of the heel-stick: (1) PNA=4 weeks (mean GA=27.3 wks, n=36); (2) PNA= 4 days (mean GA=32.3wks, n=53). **PCA:** 32 wks ± 7 days. Proportion of ventilated infants was not reported. No infants with NI were included.	**CFs:** PNA, GA, 5 minute Apgar score, birth weight, weight at data collection, severity of illness, number of painful procedures. **Outcome:** Pain response during one HS.	NFCS Brow bulge	Group 2 had significantly higher scores (F[1,82]=5.06, p<0.027), there was an increase over time (heel-stick phase) (F[3,80]=50.99; p<0.0001) and there was a significant group by time effect (F[3,80]=4.80; p<0.004). Total number of painful procedures (β=-0.42474; p<0.0003) accounted for 18% of the variance. APGAR scores (β=-0.279926, p<0.015) accounted for 7.8% of the variance. Not significant: GA, birth weight, weight at data collection, severity of illness.
				Eye squeeze	Group 2 had higher scores (F[1, 82]=9.85; p<0.001); there was a significant increase over time (F[3,80]=36.94; p<0.0001) and there was a significant group by time effect (F[3,80]=4.60; p<0.005). Total number of painful procedures (β=-0.35131; p<0.001) accounted for 10.9% of the variance. Not significant: GA, birth weight, weight at data collection, severity of illness, 5 minute APGAR.
				Nasolabial furrow	No significant group effects (F[1, 85]=3.23; p=0.076), significant time effect (F[3, 83]=134.69; p<0.0001); significant group by interaction effect (F[3, 83]=4.40; p<0.039). Total number of painful procedures (β=-0.40339; p<0.0007) accounted for 16.3% of the variance. APGAR scores (β=-0.23259, p<0.05) accounted for 5.4% of the variance. Not significant: GA, birth weight at data collection, severity of illness, total number of painful procedures.
				HR	HR was significantly higher (F[1, 86]=25.23; p<0.0001) in group 1 & was different across HS procedures phases (F[3, 84]=38.77; p<0.0001). The interaction between group and phase was not significant (F[3, 83]=1.96; p=0.126) GA at birth (β=-0.34658; p<0.0009) accounted for 12% of the variance. Not significant: birth weight, weight at data collection, severity of illness, total number of painful procedures, 5-minute APGAR.
				SaO_2	SaO_2 was significantly lower for group 1 (F[1, 85]=20.41; p<0.0001) and was different across phases of the procedure (F[3, 83]=19.41; p<0.0001) but there was no significant group by time interaction (F[3,83]=0.73; p=0.538). Birth weight (β=-0.34159; p<0.0018) accounted for 11.6% of the variance. Not significant: GA, weight at data collection, severity of illness, total number of painful procedures, 5 min APGAR.

Nr	Study	Design/Settings/Sample	Variables	Measurement of primary outcomes	Findings
11	Johnston et al. 1995 (Canada)	**Design:** Crossover. **Settings:** Two level III NICU. **Sample:** n=48. Mean GA at birth: 27.5 wks **PCA:** 29wks, ~11 days. **Ventilated at observation**: 49.5%. No infants with NI were included.	**CF:** GA **Outcome:** Pain response during one real and shame HS.	NFCS Brow buldge HR HR standard deviation Minimum SaO_2	No significant (1) multivariate GA effect (regardless of condition – real or sham and phase). (2) 2-way (age by condition) or (3) 3-way (age by condition by phase) effect. No significant multivariate, 2-way or 3-way effects. Significant multivariate GA effect (F[5, 35]=3.094; p<0.020) but not significant 2-way or 3-way effects. No significant multivariate, 2-way or 3-way effects.
12	Johnston et al. 1996 (Canada)	**Design:** Crossover. **Settings:** Two level II-III NICU. **Sample:** n=28. Mean GA at birth: 28.3 wks. **PNA:** 12-59 days. Proportion of ventilated infants was not reported. No infants with NI were included.	**CF:** PNA, ventilatory status, gender, severity of illness, number of painful procedures. **Outcome:** Pain response during repeated real and shame HS (4 real and 4 shame) over 8 weeks.	NFCS: Brow bulge Eye squeeze Nasolabial furrow HR SaO_2	Significant difference across time points (greater responses as PNA increased) (F[3, 25]=3.99; p<0.026) and no significant condition (real vs. sham HS) by time effect (F[3, 25]=0.999; p=0.409). No significant effect for frequency of invasive procedures, severity of illness, ventilatory status or gender. Significant time (PNA) effect (F[3, 25]=26.225; p<0.0001) and significant condition by time effect (F[3, 25]=8.183; p<0.001). No significant effect for frequency of invasive procedures, severity of illness, ventilatory status or gender. Significant time effect (F[3, 25]=6.72; p<0.002) and a significant condition by time effect (F[3, 25]=3.38; p<.036). No significant effect for frequency 0f invasive procedures, severity of illness or gender. Ventilatory status was significantly related to nasolabial furrow at time 2 only (t=13.25, p=0.05). No significant time effect (F[3, 25]=1.428; p=0.258) or condition by time effect (F[3, 25]=0.4162; p=0.743). No significant effect for frequency of invasive procedures, severity of illness, ventilatory status or gender. No significant time effect (F[3, 25]=0.77; p=0.516) or condition by time effect (F[3, 25]= 1.413; p=0.262). No significant effect for frequency of invasive procedures, severity of illness, ventilatory status or gender.

Nr	Study	Design/Settings/Sample	Variables	Measurement of primary outcomes	Findings
13	Johnston et al. 1999 (Canada)	**Design:** Cross-sectional design based on secondary analysis of the control session of a randomized crossover design (Stevens et al., 1999) **Settings:** Four level III NICU. **Sample:** n=120. Mean GA at birth: 28 wks (27-31) **PNA:** 10 days Proportion of ventilated infants was not reported. No infants with NI were included.	**CFs:** GA, PNA, gender, race, medications (caffeine or other stimulant drugs), number of invasive procedures, Apgar (5 minute), time since last painful procedure, wake\ sleep state. **Outcome:** Pain response during one HS.	PIPP	The following variables were retained in the final regression model: PNA at the time of the study (*OR*= 0.9975, 95% *CI*= 0.9944-1.0005); GA at time of birth (*OR*= 0.9351, 95% *CI*= 0.8883-0.9844); time since last painful procedure more recent (*OR*= 0.9988, 95% *CI*= 0.9974-1.0002); and sleep/wake state (*OR*= 0.1787, 95% *CI*= 0.218-1.4664). Younger infants who had a lower GA at the time of birth, had a more recent painful procedure or\and sleeping were less likely to demonstrate behavioral and physiologic indicators.
14	Morison et al. 2003 (Canada)	**Design:** Cross-sectional. **Settings:** One level III NICU. **Sample:** n=10. Mean GA at birth: 30.84 wks (23-32). **PNA:** M=30.8, SD=1.58. **Ventilated at observation:** 1 infant. Infants with major congenital anomalies were not included.	**CFs:** GA, Apgar (1 minute), birth weight, number of painful procedure from birth and in the past 24 hours, severity of illness at day 1. **Outcome:** Pain response during one HS.	20s NFCS	Total facial responses during the lance were positively related to GA (*r*=0.49, *ns*), birth weight (*r*=0.61, *p*<0.10), APGAR at 1 minute (*r*=0.80, *p*<0.001), number of invasive procedures during the previous 24 hours (r=0.08, *ns*) and severity of illness (*r*=0.21, *ns*) were negatively related to the number of invasive procedures since birth (*r*=-0.60, *p*<.10).
				Eye squeeze	Eye squeeze during the lance were positively related to GA (*r*=0.53, *ns*), birth weight (*r*=0.49, *ns*), APGAR at 1 minute (*r*=0.44, *ns*). Number of invasive procedures during the previous 24 hours (*r*=0.03, *ns*) and severity of illness (*r*=-0.43, *ns*) were negatively related to the number of invasive procedures since birth (*r*=-0.56, *p*<0.10).
				HR	No significant relationship between post lance HR and GA (*r*=0.16, *ns*), birth weight (*r*=0.09, *ns*), APGAR at 1 minute (*r*=0.33, *ns*), number of invasive procedures during the previous 24 hours (*r*=0.40, *ns*) and since birth (*r*=-0.08, *ns*). Greater Severity of illness in day 1was negatively associated with a smaller increase in mean HR (*r*=-0.77, *p*<0.05)
				HR variability	HR variability was negatively correlated with GA (*r*=-0.64, *p*<.10), birth weight (*r*=-0.37, *ns*), APGAR at 1 minute (*r*=-0.23, *ns*) and severity of illness (*r*=-0.23, *ns*) and positively correlated with the total number of painful procedures (*r*=0.37, *ns*) and the number in the prior 24 hours (*r*=0.23, *ns*)

				NIDCAP (stress and stability cues during the 10 min post lance compared to 10 min at baseline)	Post-heel lance, stress cues were negatively correlated with GA (r=-0.75, p<0.05), birth weight (r=-0.73, p<0.05) and APGAR at 1 minute (r=-0.75, p<0.05). There were positively correlated with the number of painful procedures since birth (r=0.79, p<0.01) and recent painful procedures (r=0.32, ns). Stability cues were negatively correlated with severity of illness (r=-0.51, *ns*) and the number of painful procedures since birth (r=0.27, *ns*) although neither correlation was significant (i.e., associated with a *p-value*<0.10).
15	Oberlander et al. 2002 (Canada)	**Design:** Cross sectional comparative. **Settings:** One level III NICU. **Sample:** n=12 with parenchymal brain injury, and 12 healthy controls with similar characteristics. Mean GA at birth: 26.3wks (24-28). **PCA:** 32 wks ±6 days. Proportion of ventilated infants was not reported.	**CF:** Parenchimal brain injury. **Outcome:** pain response during one HS.	NFCS- total score facial activity NFCS-Tongue protrusion NFCS-finger splay HR Sleep/wake state	Repeated measures ANOVA showed a statistically significant main effect across events (F=16.6; *df* 1,50; p <0.01), no significant group differences or interaction were found (p<0.05). Infants with PBI had significantly higher tongue protrusion at lance (x^2 *df* [1]=4.8; p=0.03). Proportion of infants displaying finger splay increased in both groups during heel squeezing but not significantly (x^2 *df* [1]=2.40; p=0.17), with no difference between the 2 groups. Mean HR increased significantly from baseline to lance (F[1,2]= 57.0; p<0.01). No significant group (PBI vs. controls) differences in mean HR. No significant differences in the pattern in the two groups.
16	Porter et al. 1998 (USA)	**Design:** RCT **Settings:** One level III NICU. **Sample:** n=48. Mean GA at birth: (1) Handled (n=21) M=35.3w, (2) Non-handled (n=27) M=35.6. **PNA:** Handled: M=3.2 ± 1.4, Non-handled: M= 3.3 ± 1.2. Proportion of ventilated infants was not reported.	**CF:** Handling. **Outcome:** Pain response during one HS, one group with previous handling (n=21), a second-without (n=27).	NFCS Behavioral state HR	During the HS phase the handled group showed an average increases in facial activity (2.5% *vs.* 1.5%, p=0.001). In response to the HS, handled infants exhibited significantly greater increases in behavioral responses (2.91 vs. 1.82, p=0.0001). Compared to baseline, during the HS the handled group had a mean HR increase of 30.5 ± 14.9 bpm and the non-handled group had a mean increase of 22.6 ± 15.8 bpm (p= 0.03).

Nr	Study	Design/Settings/Sample	Variables	Measurement of primary outcomes	Findings
17	Slater et al. 2009 (UK)	**Design:** Observational correlational design. **Settings:** Two NICUs. **Sample:** n=95. 25-44 wks GA (mean=31.8 wks). Proportion of ventilated infants was not reported	**CFs:** PCA, Intra ventricular hemorrhage (IVH), sleep (behavioral) state, administration of morphine at the time of the HS. **Outcome:** The latency to the change in facial expression during 1-8 HSs.	PIPP Any facial Response Time to facial response	Mean PCA of infants with response was significantly higher than the mean PCA of infants with no response (t_{170}=2.50, p=0.013; unpaired *t-test*). Responders were on average 1.6 wks older. On multivariate analysis, PCA (standardized β=0.29, p<0.001) and morphine administration (standardized β=0.30, p<0.001) were significantly related to time to facial expression change while presence of IVH (standardized β=0.12, p=0.19) and sleep state (standardized β=0.02, p=0.80) were not significantly related.
18	Stevens et al. 1994 (Canada)	**Design:** Cross-sectional (Secondary analysis for Stevens & Johnston, 1994) **Settings:** One level III NICU. **Sample:** n=124. Mean GA at birth: 32.9w (230.6 days, SD=6.32). **PNA:** M=3.3 days, SD=1.5. No infants included in the study were ventilated. Proportion of ventilated infants was not reported No infants with NI were included.	**CFs:** Severity of illness (Physiologic Stability Index-PSI), behavioral state, gender. **Outcome:** Pain response during one HS.	NFCS Cry	When the effect of weight and GA was controlled there was no multivariate main of PSI group (F[21, 366]=1.0843, p<0.363) or gender (F[7,114]=1.0322, p=0.395) on facial responses; there was a significant for behavioral state (F[21,336]=3.20, p<0.0001). Controlling for weight and GA at the time of data collection, there was a significant multivariate main effect of PSI on cry variables (F[12, 153]=2.00, p<0.027); no significant effect for behavioral state (F[12,153]=0.56, p<.873) or gender (F[5, 109]=1.17, p=0.660)

Nr	Study	Design/Settings/Sample	Variables	Measurement of primary outcomes	Findings
19	Stevens et al. 1999 (Canada)	**Design:** Prospective randomized crossover trail. **Settings:** Three tertiary care NICUs. **Sample:** n=122. Mean GA at birth: 28.6+15 wks. **PNA:** ≤28 days. Proportion of ventilated infants was not reported. No infants with NI were included.	**CFs:** GA, PNA, severity of illness, number of painful procedures, frequency and intensity of therapeutic procedures (measured by the Neonatal Therapeutic Intervention Scoring System). **Outcome:** Pain response during one HS in infants randomized to one of 4 groups: (1) control, (2) prone position, (3) pacifier with water or (4) pacifier with 24% sucrose.	PIPP scores	GA: Main effect (F[1,122]=20.95, p<0.0001); no significant interaction (multivariate)effect (F[3, 351]=0.42, p=0.738). PNA: Main effect (F[1, 118]=19.70, p<.0001); no significant interaction effect (F[3, 347]=1.58, p=0.195). Severity of illness: Main effect (F[1, 267]=0.85, p=0.36); no significant interaction effect (F[3, 355]= 1.97, p=0.118). Frequency and intensity of therapeutic procedures: Main effect (F[1, 267]=5.37, p=0.021); no significant multivariate effect (F[3, 371]=1.44, p=0.231). Number of painful procedures: Main effect (F[1, 133]=15.78, p<0.0001); significant interaction effect (F[3.350]=3.59, p=0.014). PIPP scores increased with higher numbers of painful procedures.
20	Walden et al. 2001 (USA)	**Design:** Quasi-experimental repeated measures design. **Settings**: Two tertiary care NICUs. **Sample:** n=11. GA at birth: 24-26wks (M=24.63+0.67 weeks). **PCA:** 27-32 wks **Ventilated at observation:** 91% (n=10). No infants with NI were included.	**CF:** PCA. **Outcome:** Pain response during 6 HSs per infant at PCA (wks): 27, 28, 29, 30, 31, and 32.	HR & SaO2 NIDCAP (behavioral response to HS) PIPP	There were no significant differences in heart rate responses between 27 and 32 weeks PCA (p=0.29). The only variable that showed significant PCA effects was finger splay. At 27 weeks, 45.5% of infants showed finger splay during the heel-stick/squeeze phase compared to 100% of the infants at 32 weeks (p=0.03). No significant differences in infant response patterns as a result of PCA were noted for, brow bulge, eye squeeze, or PIPP scores (p= 0.29).

Nr	Study	Design/Settings/Sample	Variables	Measurement of primary outcomes	Findings
21	Warner et al. 2001 (USA)	**Design:** Descriptive correlational design (Secondary analysis). **Settings:** One level III NICU. **Sample:** n=20. GA at birth: 30-36wks (mean-32.8wks). **PNA:** <3 days. **Ventilated at observation**: 63%. No infants with NI were included.	**CF:** Temperament (measured by the NIDCAP for 2 minutes prior to and following the HS). **Outcome:** Pain response during two HS procedures. The first between days 1 and 4 (mean GA =33 wks 1 day) and the second between days 2 and 15 (Mean GA=33 weeks 4 days) measured by the PIPP and NFCS.	Total motor activity PIPP Physiological responses	No relationship between mothers' perception of in-utero or post-birth activity levels and total motor activity at the time of HS 1 (r=-0.245) or 2 (r=-0.002). There was a significant temperament effect on PIPP scores at HS-1 and HS-2. Mean PIPP scores for low reactivity responders = 8.90$\pm$4.90 and 7.20$\pm$9.82 at HS 1 and 2 respectively compared to mean scores of 9.80$\pm$4.47 (HS-1) and 11.70$\pm$2.36 (HS-2) for high reactivity responders (P<.05 for both HSs). There were no significant within group differences between HS-1 and HS-2 for either group. Temperament type was not significantly related to physiologic responses (HR and SaO_2).
22	Williams et al. 2009 (USA)	**Design:** Longitudinal. Settings: Two tertiary care NICUs. **Sample:** n=35. GA at birth: <30wks. **PCA:** 26.3 wks + 1.8 days. Ventilated at observation: 63%. 29% of the included infants had grade III IVH.	**CFs**: PNA , GA, ventilation, IVH, recent painful procedure (within previous 24 hours), APGAR score at 1 or 5 min, race, gender, sepsis, severe IVH, birth weight, length of NICU stay. **Outcome:** Pain response during multiple HS procedures.	NIPS	For each passing week the recorded pain scores increased 0.23 (95% $C.I$= 0.08, 0.37p=0.002). No effect on the pain scores by demographic data, IVH, recent painful procedure and APGAR score at 1 or 5 min. Although GA, birth weight, mechanical ventilation, and length of stay in the NICU were significantly related to pain scores when examined in individual separate mixed models that included PNA and HS phase, when they were included in the same mixed model, only PNA and HS phase were significantly associated with pain scores.

Nr	Study	Design/Settings/Sample	Variables	Measurement of primary outcomes	Findings
23	Xia et al. 2002 (China)	**Design:** Cohort longitudinal study. **Settings:** Two tertiary care NICUs. **Sample:** n=100. Stratified into 3 groups of: (1) 29-32wks (n=30); (2) 33-36wks (n=30); (3) full terms (n=40). **PNA:** 5-10 days. No ventilated infants were included. No infants with NI were included.	**CF:** GA. **Outcome:** Pain response during one HS.	NFCS HR	The NFCS scores of groups 2 were significantly higher than group 1 during the HS (M=4.8 ± 0.7 vs. 3.7 ±2.4, p< 0.05) and squeeze (M=5.7±0.6 *vs.* 4.7±2.5, p< 0.05). The mean scores of group 3 were also significantly higher than those of group 1 during HS (5.1±1.8 *vs.* 3.7± 2.4, p< 0.01) and squeeze (6.2±0.9 vs. 4.7±2.5, p< 0.001). Significantly more infants in group 3 showed horizontal (n=21, 53% vs. 6, 20%, p<0.01) taut tongue (n=34, 85% *vs.* 19, 63%, p<0.05) and hand to mouth (n=14, 35% *vs.* 3, 10%, p<0.05) behaviors than group I. While mean heart rate increased significantly compare to baseline in all three GA groups, there were no significant differences in the mean heart rates during the heel lance (group 1=152.5±17.9, group 2=155.6±15.2, group 3=154.7±17.2). There were significant GA group differences in maximal heart rate variability (group 1=16.8±12.2, group 2=16.2±11.3, group 3=23.3±12.2; p<0.05) in HR variability.

CHAPTER VI

CONTEXTUAL FACTORS ASSOCIATED WITH PAIN RESPONSE OF PRETERM INFANTS TO HEEL-STICK PROCEDURES

Sellam G, Engberg S, Denhaerynck K, Craig KD, Cignacco EL

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ABSTRACT

Background: Evidence indicates that medical and demographic contextual factors (CFs) impact pain responses in preterm neonates, but the existing evidence is very heterogeneous.

Aim: To explore the effect of CFs on pain responses to heel-stick procedures of preterms infants.

Methods: This study was a secondary analysis of data collected during a randomized controlled trial examining pain response to non-pharmacological interventions across repeated heel-sticks. Five heel-sticks across the first 14 days of life were videotaped. Pain response was rated with the Bernese Pain Scale for Neonates (BPSN) by four raters blinded to the heel-stick phases (baseline, heel-stick, and recovery). Demographic and medical CFs were extracted from medical charts. Mixed single and multiple regression analyses were performed controlling for the intervention group, site and heel-stick phase.

Results: Apgar scores at 1 minute were negatively associated with behavioral (p=0.002) BPSN scores, while Apgar scores at 5 minutes after birth were positively associated with behavioral (p=0.006) scores**.** Accumulated number of painful procedures (p=0.002) and gender (p = 0.02) were positively associated with physiological scores while CPAP (p=0.009) and mechanical ventilation (p=0.005) were negatively associated.

Conclusion: Higher exposure to painful procedures, male infants and having CPAP or mechanical ventilation were CFs associated with physiological response. The only variables significantly associated with behavioral BPSN scores were Apgar scores but these relationships were inconsistent.

6.1 BACKGROUND

Pain management in neonates remains an important area for research and discussion, despite significant progress over the past 25 years. In particular, comprehensive pain assessment that considers preterm infants' differing neurologic and developmental status is urgently needed [1]. Physiologic and behavioral pain responses in preterm infants show high variability [2], are less vigorous than in term infants, and influenced by more than the invasive procedure itself [3]. Medical and demographic contextual factors (CFs) (e.g. gestational age, previous pain exposure, and severity of illness) appear to impact pain response and failure to understand their impact can lead to erroneous pain assessment [4-9]. A recent systematic review revealed associations between CFs (e.g., GA, postnatal age, previous pain exposure) and pain response [10], but findings were inconsistent. Thus, the impact of CFs needs to be further explored to determine which CFs need to be taken into consideration when evaluating the preterm infant's pain response, particularly behavioral components of their response [2,11-13]. Furthermore, based on the repeated recommendations to assess pain multidimensionally, it is imperative that the impact of CFs on both behavioral and physiological pain responses should be examined.

Multidimensional pain assessment is important due to the complexity of pain in preterm infants. Johnston and Stevens [7] reported that preterms demonstrated physiological changes, including decreases in oxygen saturation (SaO_2) and increases in heart rate (HR), in response to painful events across time, but behavioral responsiveness was blunted. Contextual factors may explain both the variability in pain response between and within preterm infants and the low correlation between behavioral and physiologic responses [2,7,14]. While there is evidence that non-pharmacological interventions are effective in reducing acute pain in minor and moderate procedures in preterms [15,16], their impact is variable, and may be influenced by CFs. Slater and

colleagues [17] compared behavioral pain responses [measured using Premature Infant Pain Profile (PIPP) scores] and pain–specific brain and spinal cord activity to a noxious stimulus in newborns receiving sucrose compared to sterile water. PIPP scores were significantly lower in infants receiving sucrose, but there were no significant differences in the latency of the nociceptive brain activity between the two groups. Although the findings of this study were heavily criticized in terms of methodology [18-22], these findings were not examined in relation to CFs, which might have had an influence on the results reported.

In this sub study we explored the association between medical and demographic CFs and behavioural and physiologic pain responses to heel-stick procedures in preterm infants (<32 weeks of gestation).

6.2 METHODS

6.2.1 Design, Sample and Setting

This secondary data analysis utilized data collected during a multicenter randomized controlled trial using a repeated measures design to examine pain responses of three groups of preterm infants receiving non-pharmacological pain relief interventions across repeated routine heel-sticks [14]. Seventy-one premature infants between 24 and 32 weeks of gestation needing neonatal intensive care were recruited during the first 2 days of life in three University Hospital neonatal intensive care units (NICUs) in Switzerland (Basel, Zurich and Bern). Infants were randomly assigned to one of three intervention groups receiving (1) oral sucrose, (2) facilitated tucking, or (3) combined oral sucrose and facilitated tucking. Infants were stratified by GA prior to randomization: (1) 24-28 weeks, and (2) 29-32 weeks. Five heel-sticks during the first 12-14 days of life were videotaped for each infant and pain was scored by four independent raters, who were blinded to the phase (baseline, heel-stick, and recovery) of data collection. The videotaping procedure was designed to ensure that the raters could not see if the heel-stick procedure was being performed.

Infants admitted to the NICU during this time period were assessed for eligibility according to predefined inclusion and exclusion criteria. Inclusion criteria were: preterm infants born between 24 0/7 and 32 0/7 weeks of gestation, anticipation of at least five routine heel capillary blood samples over the first 14 days of life and parental consent. Infants were excluded if they had a high grade intra-ventricular hemorrhage (III and IV grade), severe life-threatening malformations or a congenital malformation affecting brain circulation or the cardiovascular system, had undergone a surgical procedure, had blood pH of less than 7.00, had any condition involving partial or total loss of sensitivity or had any reason that could cause inability to express pain. Infants treated with mechanical ventilator or CPAP (continuous positive airway pressure) were included if they met other eligibility criteria (for detailed information regarding the study procedure, please see Cignacco et al., 2012).

6.2.2 Variables and Measures

6.2.2.1 Pain predictor variables (CFs)

The CFs were chosen based on the findings of our recently published systematic review [10]. The following CFs were significantly associated with pain response in several studies. Demographic CFs included: **birth weight**; **gender; post-natal age (PNA)** measured as weeks and days since birth documented for each heel-stick being videotaped; **gestational age (GA)** measured as weeks and days at birth and **post-menstrual age (PMA)**. PMA was calculated by combining GA at birth with PNA (GA at birth + PNA = PMA) [23]. All demographic data except post-natal and post-menstrual ages were documented at the beginning of data collection. Medical CFs included: **Apgar scores** at 1 and 5 minute after birth; **medications** - daily doses of: sedatives (Propofol lipoid, Phenobarbital, Valium, Midazolam, and Chloralhydrate); narcotics (Morphine, Fentanyl, Pethidin, and Remifentanyl) and non-narcotics (Paracetamol, Tylenol, and Becetamol) pain relief medications, steroids and caffeine given during the 14 days of data collection; **the number of previous procedures** - painful and uncomfortable diagnostic and therapeutic procedures; **the duration of the heel-stick procedure; CPAP or mechanical ventilation** at

the time of the heel-stick procedures; the seven most common **co-morbidities** seen in preterm infants during a NICU stay (broncho-pulmonary dysplasia, necrotizing entero-colitis, respiratory distress syndrome, patent ductus arteriosus, septic events, cardiac events, and respiratory arrest).

6.2.2.2 Measurement of Pain Response

Pain response was the dependent variable in this study and was measured by the BPSN, a nine-item instrument, which includes three physiologic (HR, respiratory rate, and SaO_2) and six behavioral (grimacing, body movements, crying, skin color, sleeping patterns, consolation) items. As the infants were continuously monitored, HR and SaO_2 were downloaded from the clinical database for the same time frame as the three phases of the data collection. Respiratory rate was counted by the raters during the rating of the video sequences. Each item was scored on a 3-point scale (range 0-3); for the behavioral items higher scores indicated greater distress while for physiological items it was a greater change from baseline. The BPSN is a valid and reliable pain assessment instrument for preterm and term infants currently widely used in Swiss and some German NICUs. Cignacco et al. [24] performed a validation study of the BPSN. Construct validity was good ($f = 41.3$, $p < 0.0001$) and concurrent and convergent validity of the BPSN compared to VAS (Visual Analogue Scale) and PIPP were $r = 0.86$, and $r = 0.91$, $p < 0.0001$, respectively. Initial psychometric testing (Brunner et al., 2011, unpublished) of the BPSN instrument supported good construct validity with differentiation between painful and non-painful procedures ($f = 41.27$, $p \leq 0.0001$), inter-rater reliability coefficients of $r = 0.86 – 0.97$ and intra-rater reliability coefficients of $r = 0.98$ to 0.99. For the purpose of this study, inter-rater reliability testing was performed and the Cronbach's alpha ranged from 0.90 and 0.95 for the different phases. Based on this high inter-rater reliability, mean BPSN scores were calculated across the raters and used in the analysis. For this instrument, a score of 11 or less is considered non-painful. Based on previous studies reporting low correlations between the behavioral and physiological dimension of pain assessment [2,7,14], two BPSN scores were calculated: a behavioral BPSN score (B-BPSN); and a physiological BPSN score (P-BPSN).

6.2.3 Ethical Considerations

The randomized controlled trail (RCT) was approved by the ethical boards of the Canton Basel, Bern and Zurich. Potential infant participants were screened by a trained study nurse. Written informed consent was obtained from a parent according to the protocol approved by the ethical boards. The RCT was registered, number NCT00758511 in http://www.clinicaltrials.gov.

6.2.4 Data Collection

Data for the CFs were collected using an investigator-developed data collection sheet. Data on pain responses were collected during five routine heel-sticks (T1-T5) performed between postnatal day 2 and 16 as part of the neonate's routine clinical care. The first data collection (T1) was performed no later than the fourth day of life. All heel-sticks procedures were videotaped in three phases (baseline, heel-stick, and recovery). In total, 1065 sequences were produced; ten sequences were discarded due to quality problems (in terms of ability to rate the sequence). DVDs were prepared for the rater with the 1055 sequences presented in random order in relation to the number of the heel-sticks being recorded (T1 – T5) and the phase (baseline, heel-stick, recovery phase) of the procedure. The DVDs were provided to four trained nurses for assessment of pain responses during each sequence. Raters scored the behavioral items and breathing; HR and SaO_2 data were added and the total pain scores were calculated by the first author (GS) and a research assistant. The raters rated the video sequences independently at their own pace within a time period of two months. Relationships between these data and the interventions are reported elsewhere [14].

6.2.5 Data Management and Analysis

Descriptive statistics (means, standard deviations (SD), frequencies, and/or inter quartile ranges depending on level of measurement and distributional properties) were used to describe the demographic and medical characteristics of the infants. Mean number of painful procedures was compared per GA group utilizing a t-test. The correlation between physiological and behavioral items of the BPSN was calculated with Pearson correlation coefficient. We

logarithmically transformed the BPSN scores to obtain normally distributed residuals. The CFs were tested for possible association with mean BPSN scores (behavioral, and physiologic) across all 5 heel-stick procedures during the heel-stick and recovery phases using random intercept regression analysis, with infants as the random variable and CFs as fixed effects. The analysis controlled for the baseline phase BPSN score of each heel-stick, non-pharmacological intervention group, and site. We screened each CF separately in a simple mixed regression model and included only those which reached a probability level of $p < 0.20$ in the multiple regression models. During the analysis we observed co-linearity between GA and PNA. Consequently, we used only the post-menstrual age in the multivariate analysis. Apgar scores at 5 minutes, PMA and accumulated number of painful procedures were included in the multivariate models as well (even if not reaching a probability level of $p < 0.20$), as we hypothesized they might be associated with pain response based on findings from previous studies [10]. The final alpha of the mixed regression models was considered to be significant if $p < 0.05$. Data were analyzed using IBM SPSS statistics software® version 19 (IBM, Inc., Armonk, NY, USA) and SAS version 9.1 (SAS Institute, Inc., Cary, NC, USA).

6.3 RESULTS

6.3.1 Sample characteristics

Between January 2009 and December 2010, a total of 201 infants were assessed for eligibility; 130 infants were excluded due to various reasons (e.g. not meeting inclusion criteria, parents declined participation, enrolled in another study etc.) [14] and 71 infants were enrolled in the study. All but one completed the data collection period of five heel-sticks. The fifth heel-stick was missed for one infant. The mean GA of the total sample at birth was 29.24 (±1.83) weeks, with a mean PMA at heel-stick 1 of 29.58 weeks (±1.81), and 30.64 (±1.82) at heel-stick 5. The mean birth weight was 1174.44 grams (±337.00). Mean number of co-morbidities per infant was 1.59 (±1.39). While 25.4% (n = 18) of the infants had no co-morbidities, 2.8% (n = 2) had a total of five co-morbidities. None of the infants had necrotizing enterocolitis. The mean (M) number of painful procedures during the

first 14 days of life was 201 (±104.1) procedures per infant [14.38 (+ 7.43) per day]. There were significant differences in the mean number of painful procedures between the two GA groups (p < 0.0001) with the younger group having twice as many procedures (M = 21 ±9.0/day) than the older group (M = 11 ±7.0/day). There were also significant differences in the number of painful procedures between recruitment site I (M = 18.5 ±10.2/ day, p < 0.001) and site II (M = 7.8 ±4.0/day) and between site II and site III (M = 16.9 ±6.2/day, p < 0.001) but not between site III and site I. There were no significant differences in the demographic characteristics of the non-pharmacological interventions groups. The sample characteristics are summarized in table 1.

Table 1: Demographic characteristics of the sample

Demographic characteristic		n (%)
Sample		71 (100)
Gender	Female	32 (45.00)
	Male	39 (55.00)
Ventilation	HS 1	4 (5.60)
	HS 2	3 (4.20)
	HS 3	2 (2.80)
	HS 4	0 (0)
	HS 5	0 (0)
CPAP	HS 1	28 (39.40)
	HS 2	22 (31.00)
	HS 3	22 (31.00)
	HS 4	18 (25.40)
	HS 5	17 (23.90)
Co-morbidities	Broncho-pulmonary disease	6 (8.50)
	Respiratory distress syndrome	44 (62.00)
	Patent ductus arteriosus	14 (19.70)
	Septic events	8 (11.30)
	Cardiac events	22 (31.00)
	Respiratory arrest	19 (26.80)
GA at birth (weeks)		29.24 (1.83)
PMA at time of HS (weeks)	HS 1	29.58 (1.81)
	HS 5	30.64 (1.82)
Birth weight (gr)		1174.44 (337)
Apgar scores	1 min	5.97 (2.19)
	5 min	7.58 (1.61)
Number of painful procedures per day		14.38 (7.43)
Number of HS per infants per day		1.07 (1.21)
Duration of HS (minutes)		4.86 (4.35)

GA= gestational age; **HS**=heel-stick; **PNA=** post-natal age

6.3.2 Pain response

The correlations between the behavioral and physiological pain scores were low for all five heel-sticks (r = 0.179 - 0.396).

6.3.3 Contextual factors associated with BPSN scores

Although data about medication administration was collected, very few of the infants received any of the target medications. Consequently, medication was not included in the regression model (behavioral or physiological). Table 2 presents the simple regression models results. The multivariate models of the B-BPSN and P-BPSN scores are presented in table 3.

Based on the univariate results, when examining the mixed model with B-BPSN scores as the outcome, we included cardiac events (p = 0.189), Apgar scores at 1 minute (p = 0.080), and weight (p = 0.135) as well as Apgar scores at 5 minutes (p = 0.424), PMA (p = 0.395) and accumulated number of painful procedures (p = 0.331). Most of the examined CFs were not significantly associated with the behavioral scores, 1 minute Apgar was negatively associated (p = 0.002) and 5 minute Apgar was positively associated (p = 0.006) with the B-BPSN scores.

For the mixed regression model with P-BPSN scores as the outcome, we included gender (p = 0.012), CPAP (p = 0.162), mechanical ventilation (p = 0.009), respiratory distress syndrome (p = 0.113), PMA (p = 0.009), weight (p = 0.103), duration of heel-stick procedure (p = 0.068) and accumulated number of painful procedures (p = 0.011). Apgar scores at 1 minute (p = 0.428) and Apgar scores at 5 minutes (p = 0.526) were included in the model as well. In the multivariate model PMA was no longer significant (p = 0.878). Weight (p = 0.330), Apgar 1 and 5 minutes (p = 0.771 and 0.551 respectively) remained non-significant. Male infants showed higher P-BPSN scores (p = 0.020). Mechanical ventilation (p = 0.005) and CPAP (p = 0.009) at the time of heel-stick were negatively associated with P-BPSN. Infants exposed to a higher number of painful procedure showed higher physiological scores (p = 0.002). Although not statistically significant, there was trend for P-BPSN scores to increase as the duration of the heel-stick increased (p = 0.055).

Table 2: Contextual factors influencing pain response – simple mixed regression model

	Behavioral BPSN scores					Physiological BPSN scores				
Contextual factor	**B**	**SE**	**df**	**t-value**	***p*-value**	**β**	**SE**	**df**	**t-value**	***p*-value**
GA-groups	-0.194	0.308	63.188	-0.630	0.531	0.123	0.153	65.472	0.801	0.426
Gender	0.118	0.296	63.111	0.400	0.690	0.367	0.141	65.502	2.597	0.012*
CPAP	0.180	0.275	304.213	0.655	0.513	-0.215	0.153	219.292	-1.404	0.162
Mechanical ventilation	-0.046	0.701	461.708	-0.067	0.947	-1.053	0.399	329.658	-2.639	0.009**
Broncho-pulmonary dysplasia	0.204	0.524	62.752	0.390	0.698	-0.041	0.262	64.463	-0.158	0.875
Respiratory distress syndrome	0.142	0.334	62.639	0.426	0.671	0.262	0.163	64.252	1.606	0.113
Patent ductus arteriosus	-0.383	0.364	63.724	-1.052	0.297	-0.174	0.182	65.896	-0.954	0.344
Septic events	0.046	0.457	62.527	0.102	0.919	-0.160	0.228	64.973	-0.704	0.484
Cardiac events	0.628	0.472	63.029	1.328	0.189	0.106	0.238	64.463	0.448	0.655
Respiratory arrest	0.291	0.407	63.569	0.717	0.476	0.223	0.202	65.370	1.104	0.274
PMA	-0.187	0.220	692.968	-'0.852	0.395	0.344	0.131	676.836	2.627	0.009**
Apgar (1 min)	-0.121	0.068	62.706	-1.779	0.080	-0.027	0.034	64.541	-0.798	0.428
Apgar (5 min)	0.077	0.096	63.885	0.806	0.424	-0.030	0.048	67.493	-0.638	0.526
Weight	-0.0006	0.0004	64.364	-1.512	0.135	0.0003	0.0002	65.742	1.655	0.103
Duration of HS procedure	0.014	0.036	673.645	0.382	0.702	0.040	0.022	686.621	1.825	0.068
Number of painful procedures	-0.001	0.001	468.821	-0.974	0.331	0.002	0.0009	347.094	2.551	0.011*

*$p<0.05$; **$p<0.01$ **SE**= standard error **df**= degrees of freedom **HS**= heel-stick **GA**= gestational age **PMA**= postmenstrual age

Table 3: Contextual factors influencing pain response – multivariate mixed regression model

Outcome variable	Contextual factor	β	SE	df	t-value	*p*-value
B-BPSN	**Cardiac events**	0.571	0.456	62.366	1.253	0.215
	PMA	0.288	0.371	390.406	0.775	0.439
	Apgar (1 min)	-0.289	0.087	60.765	-3.289	0.002**
	Apgar (5 min)	0.347	0.122	61.293	2.831	0.006**
	Weight	-0.0007	0.0004	68.224	-1.793	0.077
	Number of accumulated painful procedures	-0.002	0.001	459.340	-1.346	0.179
P-BPSN	**Gender**	0.324	0.136	65.102	2.381	0.020*
	CPAP	-0.400	0.152	252.950	-2.618	0.009**
	Mechanical ventilation	-1.106	0.393	263.964	-2.809	0.005**
	Respiratory distress syndrome	0.286	0.156	61.842	1.833	0.072
	PMA	0.051	0.221	351.397	0.234	0.815
	Apgar (1 min)	-0.004	0.044	58.539	-0.101	0.920
	Apgar (5 min)	-0.036	0.063	63.025	-0.571	0.570
	Weight	0.0004	0.0002	71.562	1.959	0.054
	Duration of HS procedure	0.043	0.022	685.427	1.952	0.051
	Number of accumulated painful procedures	0.003	0.0009	458.362	3.088	0.002**

*$p<0.05$; **$p<0.01$

B-BPSN= behavioral BPSN scores **P-BPSN**= physiological BPSN scores

PMA= post-menstrual age **HS**= heel-stick **SE**= standard error **df**= degrees of freedom

6.4 DISCUSSION

Mechanical ventilation or CPAP at time of heel-stick, gender and accumulated number of painful procedures were the CFs associated with changes in physiological pain scores. Only Apgar scores at 1 and 5 minutes after birth was associated with behavioral pain scores. Generally, the CFs under investigation influenced P-BPSN rather than B-BPSN scores.

In the present study, there was a low correlation between the behavioral and physiological BPSN scores across the 5 heel-sticks (r = 0.179 - 0.396). This finding is consistent with previous

studies [1,2,25,26]. In addition, studies showed that behavioral response varied across infants. Johnston et al. [27] reported that some of the infants had no cry response during skin-breaking procedures. This phenomenon was explained in part, by examining the CFs associated with different pain responses [10]. One explanation for the low correlation may be that behavioral and physiological factors represent different components of the complex response mounted to combat painful insult.

Apgar scores at 1 and 5 minutes were found to be associated with B-BPSN scores but not with the P-BPSN. Some studies have examined Apgar scores as CFs influencing pain response. All but one examined only one of the Apgar scores. Grunau et al. [5] and Johnston et al. [28] examined Apgar scores at 5 minutes and did not find any association with either behavioral and physiologic pain responses. The only study examining both Apgar (at 1 and 5 minutes) scores did not find them to be significantly associated with behavioral pain responses, measured by the NIPS (Neonatal Infant Pain Scale) [29]. Johnston and Stevens [7] examined the relationships between pain responses and 5 minute Apgar scores, using the Neonatal Facial Coding System (NFCS), HR and SaO_2. Apgar scores were found to be associated with NFCS but not with the physiologic measures. Our findings that higher Apgar scores at 5 minutes were associated with higher B-BPSN scores were consistent with Johnston and Stevens' results. Morison et al. [30] examined the association between Apgar scores at 1 minute and NFCS and physiological parameters (HR and HR variability) in 10 preterm infants. The Apgar score (1 min) was significantly related to NFCS scores but not to physiologic scores. Apgar scores at 1 minute were positively associated with behavioral pain scores, i.e. infants with lower Apgar scores at 1minutes had lower behavioral scores. In contrast, we found a negative association between Apgar scores at one minute and B-BPSN scores. One explanation for this inconsistency may be the limited reliability of Apgar scores in preterm infants [31]. Further research is needed in order to have a better understanding of these clinical parameters and how they relate to preterms' pain responses.

We found a significant positive relationship between the number of previous painful procedures and physiological pain responses measured by the P-BSPN but no significant association with B-BPSN scores. In previous studies, the number of previous painful procedures was one of CFs most often associated with pain responses. Most of studies finding this association examined pain response with the PIPP which includes HR and SaO_2 in calculation of the total score, but behavioral and physiologic scores were not examined separately [4,28,32]. Johnston and Stevens [7] however, examined both physiologic (HR and SaO_2) and behavioral responses measured by the NFCS. They compared two groups of infants; one born at 28 weeks of gestation and assessed at 32 weeks the second born at 32 weeks and assessed within 4 days. The number of previous invasive procedures was the variable that explained most of the variance in facial pain response. They did not, however, find the same effect on the physiological scores. Exposure to repeated pain can alter pain processing and can cause neurological damage due to the immaturity of the preterm nervous system [33,34]. Our findings indicate that infants who were exposed to a higher number of painful procedures had greater physiologic response.

Infants treated with mechanical ventilation and CPAP during the heel-stick showed lower P-BPSN pain responses. However, no association was found with B-BPSN. Our results are not in line with the existing literature. Johnston et al. [35], Grunau et al. [5], and Williams et al. [29] found no association between ventilatory status and either behavioral or physiologic pain scores. Williams et al. [29] reported that mechanical ventilation was significantly related to behavioral pain scores when examined bivariately. However, when examined in a multiple mixed model with other CFs that were significantly related to pain responses bivariately, mechanical ventilation was no longer significant. While the reason that our findings were not consistent with previous research is unclear, none of the previous studies examined the association between mechanical ventilation and pain responses in infants being treated with non-pharmacological interventions during painful procedures

In our study, gender was associated with physiologic scores. Boys had a higher physiologic pain response than girls. We did not find significant associations between gender and behavioral pain response. Our findings were not consistent with two previous studies examining the relationship between gender and physiologic responses and pain [35,36], which both found no significant relationship. The reason our findings were not consistent with Johnston and Holsti's findings is unclear as in the exploratory analysis of the parent study (RCT), no further significant differences in the characteristics of boys and girls were found. Valerie and colleagues [37] examined pain reactivity in male and female preterms as measured by the NFCS, sleep-wake state and HR. They found no significant behavioral differences between boy and girls but did find significant differences in the magnitude of HR response. Boys demonstrated higher HR changes from baseline to heel-stick. Due to the inconsistent results in the existing literature, further research is needed to determine if there are gender differences in preterm infants' pain responses, and to explore reasons for these differences.

Infant age has been the most commonly examined CF in relation to pain responses [10]. To take into consideration both GA and age since birth (PNA) we combined GA and PNA in to one variable, post-menstrual age (PMA). PMA was not significantly associated with any kind of pain response (behavioral or physiologic) in our study. Mixed findings regarding the association between preterm age variables and both behavioral and physiologic pain response were described recently [10]. These conflicting findings support the need for additional research examining these relationships and possible explanations for the variability in findings. One possible explanation is the other CFs (in addition to the age variable) that studies examined.

6.4.1 Limitations and strengths

There are a number of limitations that need to be considered when interpreting the findings of this study. This study is a secondary data analysis and the parent study was not designed to specifically examine CFs. Consequently, some potentially informative CFs such as an overall measure of severity of illness was not examined. Furthermore, the number of infants with some

CFs was small (e.g. medications, co-morbidities) which may have limited our ability to detect significant effects during the bivariate analysis. While we were able to include a variety of CFs in the multivariate analysis, a larger sample would have allowed us to include more CFs in the multivariate models. The small sample size could also be one explanation for the limited results of the present study.

This study also has a number of strengths. We examined a variety of CFs that previous studies have suggested may influence preterm infants' pain responses. The use of multivariate analysis while controlling for potential confounders as site, non-pharmacological intervention group and phase 1 allowed us to examine the independent contribution of each examined CF in explaining pain responses. A further strength of this study was the utilization of a pain assessment instrument that allowed us to examine the impact of the CFs on both behavioral and physiologic pain responses.

6.5 CONCLUSIONS

The findings of this study reinforce the importance of CFs in pain response and the need for their consideration in pain assessment. We also confirmed the low correlation between behavioral and physiologic pain scores in preterm infants. These findings support the need for pain assessment instruments that incorporate both behavioral and physiologic measures as these measures represent different constructs. Our findings also add to the growing body of research that suggests that CFs need to be considered when assessing pain in this population. However, given that findings about the impact of CFs are mixed and not definitive across studies and within the results of the present study, additional research is needed to determine the CFs that need to be incorporated into pain assessment instruments. Multi-center studies are recommended to ensure that sample sizes are large enough to permit the examination of multiple CFs analyzed in multivariate models. Studies should examine the impact of CFs on both aspects of pain response (behavioral and physiologic).

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CHAPTER VII

SYNTHESIS OF FINDINGS,

DISCUSSION, AND CONCLUSIONS

SYNTHESIS, DISCUSSION, AND PERSPECTIVES

This dissertation addressed a gap in the existing literature regarding the comparative effectiveness of two non-pharmacological interventions (NPI) over time, and the association of contextual factors (CFs) with pain response of preterm infants receiving these interventions.

The unique physiology of preterm infants continues to provide major challenges to both practical and academic arenas. Due to reproductive technologies and higher maternal age, preterm birth rates have been increasing worldwide [1]. Moreover, significant advances in medical technology have contributed tremendously to the increased survival of critically ill preterms [2]. Preterm infants, as an extremely vulnerable population due to their rapidly developing central nervous system, are exposed to enormous amount of procedural pain which can cause short and long term adverse effects [3]. Therefore comprehensive pain assessment followed by pain treatment is an essential key to safer development and improved outcomes for these infants. An impressive increase in the body of evidence about neonatal pain assessment and treatment has appeared since the recognition that the fetus actually can feel pain [4-6]. Despite the evidence existing on pain management methods, the number of uncomfortable and painful procedures being performed in the NICU setting on preterms is still enormous, and pain treatment is still not optimal and not universally administrated despite existing recommendations of the American Academy of Pediatrics and the Canadian Pediatric Society [7-13]. Non-pharmacological pain relief interventions are recognized as one of the important keys for coping with the challenge of high pain exposure in preterms. There is evidence to support their effectiveness for pain treatment during minor painful procedures [14,15]. Despite the extensive existing knowledge regarding the benefits of NPIs, information about their efficacy and safety over time is limited, along with

research about the comparative effectiveness of these interventions. Therefore, there is a need for well-designed research studies to address this gap in knowledge.

While there is compelling evidence that pain is a major problem in preterm neonates and that it may have a sustained negative impact on their development, the challenges of assessing pain are also widely recognized. Nowadays term and preterm pain is recognized as a complex experience involving not only the functional dimension, but also cognitive and emotional processing [16]. Accurate pain assessment is an essential pre-requisite to safe and efficacious pain treatment. Great advances have been made in pain assessment in this population over the past quarter century. However, assessing pain in preterms remains one of the most difficult challenges facing clinicians, researchers, and parents due to infants' incapacity for verbal report. Substantial evidence confirms that preterm and term infants have the autonomic and functional capacity for mounting a response to noxious stimulation [4,17,18]. Currently, pain assessment instruments rely on the different dimensions of pain responses such behavioral (e.g. facial, body movement, crying) and physiological measures (e.g. increase heart rate, decreased oxygen saturation, increased breathing rate) to diagnose pain in infants. Another focus of pain assessment taking place in the research arena is the role of medical and demographic CFs in pain responses [19]. High variability in pain responses of preterm infants receiving the same noxious stimulation was found by Cignacco et al. [20], which could not be explained by the painful insult alone, suggesting that CFs can explain this high variability. Most of the studies examining CFs influencing pain response have found age variables [e.g. gestational age (GA), post-natal age (PNA), and post-conceptual age] to be the most influencing factors [21-30]; however, other studies have not supported these relationships [31-36]. Other important CFs identified in studies is number of previous exposures to pain [24,25,34] and severity of illness [7,21], but similar to the age variables, the results are inconsistent. These inconsistencies demand a deeper exploration in to this complex phenomenon of CFs, their influence on pain response with NPIs and their place in pain assessment.

The findings of the studies of this thesis added to the knowledge base on preterm neonatal pain by (1) comparing the effectiveness of two NPIs, sucrose and facilitated tucking (FT), over repeated minor painful procedures; (2) systematically examining published evidence on the impact of CFs on neonatal pain responses; and (3) providing an insight in to the association between numerous CFs and pain response of preterm infants under the therapeutic effect of sucrose and FT. These three contributions provide a basis for further discussion around the challenges in assessing and managing pain in preterms.

The parent study (chapter 3) was an RCT, which compared the effectiveness of sucrose, FT, and the combination of the two interventions across five heel-sticks in preterm infants. While sucrose was significantly more effective than FT, the combination of the two procedures was significantly more effective than sucrose only in the recovery phase. In this context of the effectiveness of sucrose and FT, our research team and others have found variability in pain response, which prompted us to examine the influence of CFs on preterm infants' pain responses. During the data collection phase of the parent project, a systematic review was carried out in order to examine the existing literature about CFs influencing pain responses. While the results of this systematic review did not reveal any CF that was consistently related to pain responses in preterm neonates, the three factors that were most consistently related were GA, previous pain exposure, and severity of illness (chapter 5). Subsequently, an exploratory sub-study of the parent study examined eleven CFs for their association with pain response under the therapeutic effect of sucrose and FT. The results of this study showed an association of Apgar scores at 1 minutes and 5 minutes after birth for the behavioral BPSN scores, while the accumulated number of painful procedures, having CPAP or mechanical ventilation, and gender were associated with the physiological BPSN scores. Our finding reinforce the importance of CFs in pain response and the need for their consideration in pain assessment. We also confirmed the low correlation between behavioral and physiologic pain scores in preterm infants. Our findings also add to the growing body of research that suggests that CFs need to be considered when assessing pain in this population. However, given that findings about the impact of CFs are

mixed across studies, additional research is needed to determine the CFs that need to be incorporated into pain assessment instruments. Furthermore, this study contributes some insights in to the complexity of analyzing the association between pain responses and CFs, and their integration into the pain assessment process (chapter 6).

In the following pages we discuss the limitations and findings of this dissertation taking a broad perspective that goes beyond the discussion of the individual manuscripts (Chapters 3 to 6). Implications for further research and suggestions for clinical practice will also be presented.

7.1 METHODOLOGICAL ISSUES RELATED TO PAIN ASSESSMENT

7.1.1 Pain assessment measures, how should clinicians choose?

The gate control theory [37] shaped the conceptualization of pain mechanism in the early 1960s. This theory changed and shaped the way we think about pain today. Subsequently, the principles of this basic theory were substantiated which reinforced its conceptual value and utility over time [38,39]. The most important contribution of this theory according to Melzack [40] is that pain is a multidimensional experience produced by a wide pattern of nerve impulses generated by the brain. More specifically, the transmission of pain from the peripheral nerve through the spinal cord is subject to modulation by both intrinsic neurons and controls being driven from the brain[39]. In infants, the brain's ability to modulate pain is limited due to the immature nervous system and limited cognitive capabilities [41]. Therefore, a fundamental starting point for conceptualizing infants' pain assessment is the recognition of infants' reliance on the caregiver [42]. Furthermore, the socio-communication model of infant's pain [31] asserts that infants' pain should not be interpreted outside the context of the caregiver.

In the past 2 decades, about 30 pain assessment instruments for infants were developed [43-45]. The early pain assessments instruments for neonates were uni-dimensional including only behavioral dimensions such as facial activities [46-48], or body movements[49]. Uni-dimensional physiological indicators and biomarkers were proposed as well [45]. Anand and Craig [50] assert that

during this past 2 decades of pain assessment development, the scientific community also came to realize that physiological response to pain are evidence of pain and not just a 'surrogate measure' of pain. The most common bio-marker used is cortisol. Cortisol levels as a stress response can be measured during pain exposure through saliva. A number of studies have shown that salivary cortisol levels of hospitalized preterm infants are higher than in healthy infants [51-54]. However, the cortisol literature is inconsistent with some studies reporting higher levels [55] during pain response of preterm infants and others reporting lower levels [52,56] or no changes [20]. The use of this method has been limited to research, as results cannot be provided rapidly in the clinical setting. An additional physiological measure is the Near-infrared spectroscopy (NIRS). Studies in adults and preterm infants have shown that painful stimuli lead to activation of specific cortical and subcortical areas, including circulatory and metabolic mechanisms [16,63]. NIRS is a non-invasive technique that can detect specific hemodynamic responses in the somatosensory cortex produced by painful and tactile stimuli [16,57,58]. This technique was assessed as a measure of pain in preterm infants in only three published studies [16,57,59]. Another sub-analysis of the parent study compared pain responses measured by NIRS and other physiological measures, i.e. heart rate and oxygen saturation (Gerull et al., manuscript in preparation). The main limitations of NIRS are primarily related to its lack of feasibility in the clinical setting as a pain assessment method and its' limited ability to filtrate artifacts. Other physiological measurements that have been used alone to measure pain are heart rate [60], heart rate variability [61], vagal tone [62] (measured through heart rate), oxygen saturation [63,64], respiratory rate [60,65], and blood pressure [65]. While many physiological measures are available for use in studies of neonatal pain responses, they are index measures of reactivity and not direct measures of pain [45]. No single marker is able to capture all aspects of neonatal pain. In addition to the need to measure multiple behavioral and physiological parameters when assessing pain in infants, there is evidence to suggest that environmental and medical factors influence pain responses in preterm infants [24,26,66].

Based on gate theory and the complex nature of neonatal pain, a multidimensional approach is recommended when assessing pain [45]. This recommendation is justified given the consistently reported low correlations between behavioral and physiological pain indicators [25,60,67]. A multidimensional measurement approach can be achieved by a simultaneous employment of objective (physiological) and subjective (behavioral) components [68,69], and by using a multidimensional approach within a particular measurement domain (e.g. multidimensional behavioral measure that include grimacing, cry, and body movements [70-72]). However, these measures vary widely in relation to the extent to which their psychometric properties have been examined, as well as their clinical feasibility and utility [73]. Moreover, a large proportion of these pain measures have been developed for research purposes, which makes them less feasible for use in the clinical setting [74]. The most widely used, valid and reliable (Cronbach's alphas range: α = 0.59–0.76 for the different items) composite pain assessment instrument in the clinical setting and research is the Premature Infants Pain Profile (PIPP) [68,73]. The PIPP is a multidimensional pain assessment instrument that includes 3 behavioral facial indicators, 2 physiological indicators (heart rate and oxygen saturation), and 2 CFs (GA and sleep-awake state). Stevens et al. [73] recently reported, in a review, that the PIPP was used in 59 studies from 1996 to 2009 and conclude that it remains reliable and valid as an outcome measure in pain interventions study. Nevertheless PIPP is not recognized as "gold standard" for pain assessment. In Switzerland, clinical setting uses the Bernese Pain Scale for Neonates (BPSN). The BPSN is a validated pain assessment instrument for term and preterm neonates, which was developed by nurses of a NICU in the capital city of Switzerland, Bern [69]. The uniqueness of the BPSN is that its development was a clinical initiative, based on recognition for the need for a more comprehensive pain assessment instrument. One of the limitations of PIPP is that facial activity is the only behavioral indicator measured. The use of only facial indicators can hamper pain assessment when the infant is mechanically ventilated or treated with CPAP. The BPSN was developed to address this limitation by including additional behavioral indicators (in addition to crying and facial expression). The additional behavioral indicators are consolation

(time needed to comfort/calm the infant), sleep quality, and posture. In our study, pain was assessed with the BPSN. In a feasibility study performed prior to the comparative effectiveness study, the BPSN was compared with the PIPP [20]. Interrater reliability for the BPSN was similar to the PIPP (Cronbach's alphas range α = 0.69–0.99 and 0.60–0.99 respectively). While there is evidence to support the reliability and validity of both the PIPP and BPSN, there are still concerns about their sensitivity in very preterm infants. One of the reasons for limited sensitivity is likely to be influence of CFs on preterm infants' pain responses. While the PIPP does incorporate two CFs (gestational age behavioral status in terms of sleep/awake status), the BPSN does not include one (sleep/awake status). However, research findings in relation to which CFs need to be incorporated into pain assessment instruments are mixed, including findings in relation to the two CFs incorporated into the PIPP [19].

The non-specificity of various pain indicators is a challenge for pain assessment in infants. One of the issues is that the individual behavioral and physiological indicators might also be present/abnormal for reasons other than pain. Investigators have examined the relationship between behavioral pain indicators and cortical hemodynamic activity in response to noxious stimuli using NIRS. Slater et al. [59] compared NIRS and the PIPP in preterm infants. They reported an overall significant correlation between somatosensory cortical hemodynamic activity and PIPP scores (correlation coefficient = 0.72, p = 0.001). However, at 13 out of 33 measurement points, infants with cortical responses did not display facial changes resulting in low PIPP scores that could be interpreted as infants having no pain or pain of low severity. These findings are consistent with the findings of Johnston et al. [26] who reported that approximately 20% of infants did not respond behaviorally or physiologically to a painful stimulus. Cortical activation is a promising indicator of acute pain in infants but still needs to be validated adequately across infant populations and procedures [73]. Future research is needed to compare findings of composite measures such as the BPSN and PIPP with NIRS in order to affirm these results.

There is evidence suggesting that CFs can impact some pain responses [75]. Based on previous studies and the results of our study (chapter 6), CFs may not impact behavioral and physiologic responses in the same way. Grunau and collogues [24] found that GA was negatively associated with behavioral pain response measured by the Neonatal Facial Coding System (NFCS), while it was positively associated with physiological response measured by heart-rate variability. They found the same direction of associations with the number of painful procedures. Findings in relation to the impact of CFs on both behavioral and physiologic responses are inconsistent. While there is more support in the literature for selected CFs such as GA and the previous painful exposure, findings in relation to their direction of association are not consistent. Methodological issues such as the age ranges of infants in the studies, other CFs that were also examined and the method of data analysis may be one explanation for the inconsistencies reported across studies. These issues support the need for additional research examining the impact of CFs on preterm infants' pain responses using different methods in order to find consistent effect. Future studies should examine the relationship between severity of illness in multivariate models that include other variables such as PMA and prior pain exposure to determine if it's independently related to pain responses in preterm infants. To conclude, CFs needs to be examined in a broad standardized way, in order to draw clear implications for clinical pain assessment.

As there are many pain assessment instruments for neonates and no consensus exists about which should be used [45], we are not recommending a specific pain assessment tool. When assessing pain, health care provider also needs to be aware of several dimension such as the time point pain when the infant's pain is being assessed (e.g., was the infant disturbed prior to the painful insult), and the other medical and demographic factors that may impact his/her pain responses. We believe that current evidence supports use of multidimensional pain assessment instruments. While additional research is needed to identify the specific factors that impact behavioral and/or physiologic pain responses, there is evidence that CFs does influence infants' responses to pain and should be incorporated in pain assessment instruments. Including CFs as

integral part of pain assessment process is likely to provide a more sensitive assessment of the responses of the preterm infant. Pain scores should be adjusted for the effects of CFs showed to be important predictors of pain responses in this population. Using the number of previous painful procedures as an example, if the infant was exposed to X number of painful procedures he would get XX points added to his pain score.

7.1.2 CFs study statistical analysis strategy-which is the right way to go?

In the analysis of the CFs sub-study (chapter 6) we encountered a major challenge while deciding how to conduct the statistical analysis. A decision had to be made about which phase(s) of the heel-stick (baseline, heel-stick, and recovery) to include in the analysis. There were a number of questions that we considered in planning the analysis. Should we include the BPSN scores for all phases, only phase 2 (heel-stick), or phases 2 (heel-stick phase) and 3 (recovery phase) as the outcome in the regression model? Should we calculate changes scores between phases? It was challenging to decide since the strategy used in previous studies varied. While some studies focused on only the heel-stick phase, other studies used two (baseline and heel-stick) phases, and some three (baseline, heel-stick, and recovery) to six phases (baseline, contact, swab, heel-stick, squeeze, and recovery).

In the first analysis we performed, we included all phases by computing an average score across the 3 phases in the mixed regression model. None of the CFs examined were significantly related to total BPSN scores. GA at birth and PNA were not significantly related to either behavioral or physiological scores. When GA and PNA were combined to form a single variable, PMA, it was negatively associated (p = 0.005) with behavioral scores, and positively associated (p = 0.01) with physiological scores. The number of painful procedures were negatively related to behavioral scores (p = 0.02), but positively associated with physiological scores (p = 0.004). Gender (p = 0.001) was positively and mechanical ventilation (p = 0.01) was negatively related to physiological scores. When the results were discussed in our research team, we wondered if including all phases was the correct approach given that our aim was to examine the

associations between CFs and pain responses. Since all infants were not disturbed for 1/2 hour prior to the heel-stick and were expected to be free of any pain experience at baseline, we questioned the validity of including baseline scores in the calculation of the mean score representing the infants' pain responses. As a result of this discussion we decided to change strategy and performed the analysis with phase 2 (heels-stick) only. The results of this analysis were challenging to explain. Total and behavioral BPSN scores were negatively associated with patent ductus arteriosus, while physiological BPSN score was negatively associated with mechanical ventilation and positively associated with gender and respiratory distress syndrome. Therefore, another research group discussion took place, where the question of the strategy was discussed again. During these discussions, one of the issues raised was the time needed to recover following a painful procedure and the likelihood that recovery scores are influenced by the second phase (heel-stick) scores. Including scores across all heel-stick phases was also reconsidered. The basis for that discussion was a personal discussion with a neonatal expert who recommended including phase 1 (baseline) on the basis that it is also influenced by CFs (Dr. Steen Hertel, personal communication, October 14, 2011, Nova Scotia, Canada). The final analytic approach selected for examining the impact of CFs on pain scores was to use the average of phase 2 and 3 scores as the outcome (dependent) variable in the final analysis while controlling for phase 1 scores.

In addition to the method utilized to measure pain, other methodological issues need to be considered. These issues may account for some of the variability seen in findings across studies examining the impact of CFs on pain response in preterm infants. They include how CFs were measured, which pain assessment instrument was used to measure pain, study sample sizes and how the relationships between CFs and pain responses were analyzed (bivariately or multivariately). As supported by our systematic review (chapter 5), the existing literature presents a wide variation in the CFs measured, pain assessment instruments, and statistical analysis strategies. In order to move science forward and provide clear and robust results regarding the CFs influencing pain response, we recognize a need for development of a strong

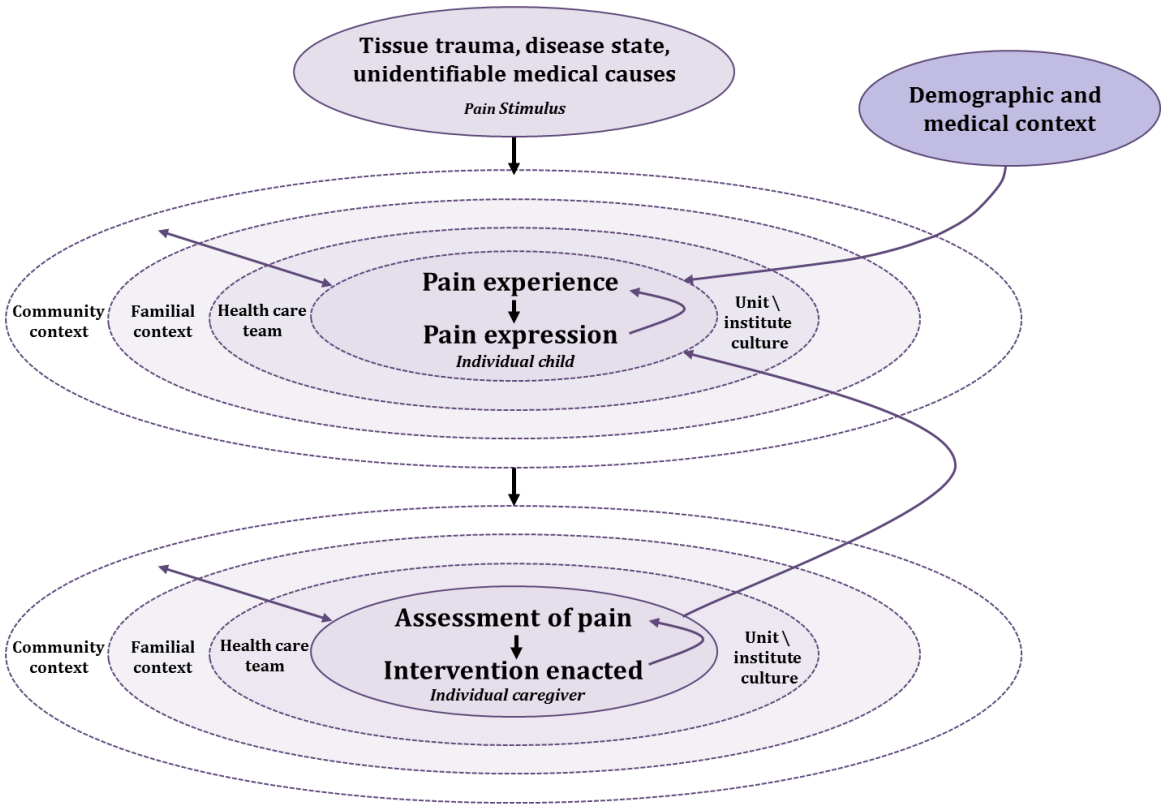
theoretical framework to explain how CFs may influence pain responses in preterm infants. Such a framework will provide guidance in CF selection and help to advance science in this area. There is also a need for standardized methods of data collection, large sample sizes, and consistent use of multivariate approaches when examining the relationships between CFs and pain responses.

The lack of clear guidelines on how to treat pain scores when examining the impact of CFs and our findings that the CFs significantly related to pain scores varied with the methodological approach we utilized, suggest that some of the variability that we see in the literature in relation to the impact of CFs on pain responses in preterm infants may be related to variations in how the pain score outcome is calculated. There is a need to get consensus and clear guidelines for how to calculate pain scores, including which phases of the procedure (baseline, painful procedure, and recovery) should be included in the analysis.

7.2 PAIN MANAGEMENT OF PRETERM INFANTS, THE SOCIO-COMMUNICATION CONTEXT

Infants' pain is complex and involves more than the painful intervention itself. In addition to the medical and demographic CFs, the familial and social contexts are crucial factors in understanding pain as a broad concept. The dynamics between the infant and the caregiver are important to take under consideration while assessing and managing neonatal pain [76]. To explain the complexity of pain in a broad concept of infant, caregiver, family, and community we used the Scio-communication Model of Infants' pain developed by Craig and Pillai Riddell [31]. For the purpose of this dissertation, where the focus is preterm infants hospitalized in a NICU, we modified the model by adding the medical and demographic CFs, and placed the health care team and unit environment in the first circle next to the infant (Figure 1).

Figure 1: The Socio Communication Model of Infants' Pain – modified model



Craig & Pillai Riddell (2003)- modified model, Sellam et al. (2011)

The implementation of evidence based practice regarding pain assessment and management is widely recognized and had been addressed in several studies [77-80]. Yet, our understanding of how the health care provider and organizational factors influence pain management in preterms remains a challenge. Stevens et al. [81] have recently carried out a qualitative study, which explored 147 health care professionals' perception of the influence of factors at an organizational level on pain practice in the NICU. Three themes emerged from the data which captured influences on optimal pain practices: 1) culture of collaboration and support for evidence-based practice, 2) threat to autonomous decision-making, and 3) complexities in care delivery. The results of this study support the modification we have performed to the socio-communication model of infants' pain, placing the institute/unit culture, and caregiver in the first circle influencing pain assessment and management in neonates. However, further research is still needed to determine how these characteristics impact pain management and to develop and examine the efficacy of interventions designed to address these issues.

7.3 NON-PHARMACOLOGICAL PAIN RELIEF INTERVENTIONS: RECOMMENDATIONS FOR CLINICAL PRACTICE

As reported in chapter 3 and other studies, preterm infant are exposed to a large numbers of painful procedures each day [8,9]. Studies have supported the efficacy of NPIs such as sucrose, pacifier, breast feeding, skin-to-skin contact, FT etc. for minor painful procedures [14,15]. However, little is known about the comparative effectiveness of these existing NPIs, moreover, even less is known about their effect over time. In the study reported in chapter 3 we found that both sucrose and FT were effective in preventing pain over time and that there was little additive value of using both interventions together. From a clinical perspective, both sucrose and FT were effective for pain management (achieving BPSN pain scores below 10 which results to "no pain") although sucrose was statistically more effective than FT. Given that both interventions are effective, other issues need to be considered in selecting which will be utilized in the clinical setting. These include the safety of the interventions and the resources needed to implement them. Additional research is needed to determine the long-term safety of repeated administration of sucrose in this population. The other issue that must be considered in clinical recommendations is the staff resources required to implement the interventions. While FT was effective in preventing pain (as evidenced by BPSN scores) and had a small additive effect when combined with sucrose during the recovery phase of the heel-stick, it did require more staff resources than sucrose alone that was more effective in preventing pain. As discussed in chapter 4, this is an important issue for many clinical settings where current staffing levels may be a barrier to implementation of this intervention. Rather than rejecting FT as a pain relieving intervention, we recommend this intervention to be performed by the parents. In addition to enhancing pain relief, parental involvement in the pain management of their infant may enhance bonding. This suggestion is supported by Axelin et al. [82] in a recent study examining 23 mothers of 29 preterms regarding their different styles of involvement in their child's pain care. They showed that mothers are willing to actively take part of their child's pain care. Thus, parental participation in FT may be a good option for some parents.

7.4 CONCLUSIONS

While great strides have been made in pain assessment and treatment in preterm infants, pain management remains a major challenge in this vulnerable population of preterm infants. The findings of this dissertation support the efficacy of sucrose over time and recommend it over FT. While our findings support the importance of considering CFs as influencing pain responses in this vulnerable population, the specific CFs that need to be incorporated into pain assessment scales remains unclear. Our findings raise important methodological issues that need to be considered as future studies are designed to examine the impact of CFs on the pain responses of preterm infants.

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APPENDIX

BERNESE PAIN SCALE FOR NEONATES

Bernese Pain Scale for Neonates					
Indicator	**0**	**1**	**2**	**3**	**Score**
Sleeping	Quiet sleep or physiologically alert	Light sleep with eye movement	Spontaneous waking	Cannot get sleep	
Crying	Not crying	Short period of crying (less than 2 minutes)	Increased crying (more than 2 minutes)	Increased, shrill crying (more than 2 minutes	
Consolation	No consolation necessary	Consolable in less than 1 minute	More than 1 minute needed for consolation	Not consolable for more than 2 minutes	
Skin color	Pink	Plethoric	Slightly pale, possibly Mottled	Pale, mottled, bluish	
Facial expression	Face relaxed	Brief grimace	Increase grimace and trembling of chin	Permanent grimace of face and trembling of chin	
Posture	Body relaxed	Mainly relaxed, short bouts of tension	Frequent bouts of tension but relaxation possible	Permanently tense	
Breathing	Normal and regular (baseline)	Superficial; increase in rate by 10-14 within 2 minutes and/or retractions	Superficial; increase in rate by 15-19 within 2 minutes; more frequent retractions	Superficial and irregular; marked increase in rate by 20 or more within 2 minutes and/or marked retractions	
No pain: 0 - 8 points **Pain: ≥ 9 points**				**TOTAL FOR SUBJECTIVE INDICATORS →**	
Heart-rate	Normal (Baseline)	Increase of 20 bpm or more over the baseline with return to baseline within 2 minutes	Increase of 20 bpm or more over baseline without return to baseline within 2 minutes	Increase of 30 bpm or more over baseline or more frequent episodes of bradycardia within 2 minutes	
Oxygen saturation	Decrease of 0% to 1,9%	Decrease of 2% to 2,9%	Decrease of 3% to 4,9%	Decrease of 5% or more.	
No pain: 0 – 10 points **Pain: ≥ 11 points**				**OVERALL TOTAL →**	

Total points for subjective indicators: 21; total overall score: 27

CURRICULUM VITAE

PERSONAL DATA

NAME:	Gila Sellam, MScN, RN
DATE OF BIRTH:	September 24th, 1979
NATIONALITIES:	French, Israeli
ADDRESS:	Rufacherstrasse 6, 4055 Basel, Switzerland
MOBILE PHONE:	+41 (0)76 335 42 55.
E-MAIL ADDRESS:	gilsellam@gmail.com

ACADEMIC EDUCATION

GRADUATE

2009 - 2012 **PHD STUDY PROGRAM**
Institute of Nursing Science, University of Basel

2006 - 2009 **MASTER OF SCIENCE IN ONCOLOGY NURSING**
Hadassah School of Nursing, faculty of medicine, Hebrew University, Jerusalem, Israel

2004 - 2005 **ADVANCED COURSE OF CLINICAL INSTRUCTION**
Hadassah School of Nursing, faculty of medicine, Hebrew University, Jerusalem, Israel

UNDERGRADUATE

1999 - 2003 **BACHELOR OF SCIENCE IN NURSING & REGISTERED NURSE IN GENERAL NURSING**
Hadassah School of Nursing, faculty of medicine, Hebrew University, Jerusalem, Israel

1994 – 1997 **ABITUR – MATRICULATION CERTIFICATE**
Rabbai Baharn Religious High School for Girls, Kfar Eliyahu, Gedera, Israel.

APPOINTMENTS AND POSITIONS

2009 – to present Research assistant and doctoral student, Institute of Nursing Science, University of Basel, Switzerland.

05-09/2007 Long term follow up clinic nurse in the pediatric hematology- oncology unit (in addition to other duties in the unit).

2005 – 2008 Clinical supervisor at the Hadassah Ein Kerem pediatric hematology-oncology unit.

2003 – 2008 Staff nurse at the Hadassah Ein Kerem pediatric hematology- oncology unit.

PROFESSIONAL EXPERIENCE

2009 – to present Research and teaching assistant, and organization of a pediatric conference at the Institute of Nursing Science, University of Basel.

2006 – 2008 Member of the unit's palliative team, and member of the unit's leadership group.

2003 – 2008 Certified full time nurse at the pediatric hematology-oncology unit. Shift manager since September 2004. Responsible for developing pain management protocol in the unit, as well as writing protocols for other relevant topics for the unit. Responsible for clinical instruction and introducing new nurses to the procedures of the unit.

2001 – 2003 Nurse assistant during nursing studies at the Hadassah Ein Kerem hospital department of pediatrics.

PARTICIPATION IN PROFESSIONAL CONFERENCES

2011 8th International Forum on Pediatric Pain, New Concepts in Complex and Recurrent Pain (IFPP) which took place in Nova Scotia, Canada (October 2011, **Poster presentation**)

2011 2nd Symposium of the Swiss Clinical Trial Organization, Clinical Research in Pediatrics (SCTO) which took place in Basel, Switzerland (June 2011, **Poster presentation**)

2011	`Assembling Health`: Challenges in Research and Treatment of Complex Health Conditions which took place in Haifa, Israel (March 2011, **Poster presentation**)
2010	The 3rd congress of the European Academy of Pediatric Societies (EAPS) which took place in Copenhagen, Denmark (October 2010, **Poster presentation**)

AWARDS AND SCHOLARSHIPS

2010	Scholarship for participation at the International Multi-Professional Pediatric Palliative Care Course in Haltern, Germany
2009 – 2012	Grant for PhD studies at the University of Basel funded by the BOTNAR foundation.
2009	Grant for PhD studies from Hadassah University Hospital, Jerusalem, Israel.
2008	Excellence scholarship from the Eve Rosen Lieberson Nursing Scholarship for MScN studies.
2006	Nominated for the oncology unit's Itay Ramon Foundation exceptional nurse award at Hadassah Ein Kerem

RESEARCH

2009 – 2012	PAMINA (PAin Management In neoNAtes) project. Testing the efficacy of pain relieving non-pharmacological interventions in preterm infants undergoing repeated heel-sticks in neonatal intensive care units. A pilot randomized controlled trial
2009 – 2012	Contextual factors associated with pain response of preterm infants receiving non-pharmacological pain relief interventions for heel-stick procedures.
2008	Study nurse of a randomized cross-over study of curcumin for prevention of oral mucositis in children receiving Doxorubicin based chemotherapy.
2006 – 2009	Master thesis on body image and sexual self-perception in young adults who have completed treatment for a malignant illness

PUBLICATIONS

PEER REVIEWED JOURNALS

2013 **Sellam G,** Engberg S, Denhaerynck K, Craig DK, Engberg S. Contextual factors associated with pain response of preterm infants receiving non-pharmacological pain relief interventions for heel-stick procedures. *The European Journal of Pain,* 2013;17:255-263.

2013 Gerull R, Cignacco EL, Stoffel L, **Sellam G,** Nelle M. The influence of non-pharmacologic analgesic interventions in preterm infants on physiologic parameters: a randomized trial. *Resubmitted on February 2013 to the Neonatology.*

2012 Cignacco EL, **Sellam G,** Stoffel L, Gerull R, Nelle M, Anand KJS, Engberg S. Oral sucrose and 'facilitated tucking' for repeated pain relief in preterms. A randomized controlled trial. *Pediatrics* 2012;129:299-308.

2011 Sellam G, Cignacco EL, Craig KD, Engberg S. Contextual factors influencing pain response to heel-stick procedures in preterm infants: What do we know? A systematic review. *European Journal of Pain,* 2011;15:661 e1- e15.

2011 Elad S, Meidan I, Zeevi I, **Sellam G**, Saman S, Waldman E, Wientraub M, Revel-Vilk S. Randomized cross-over study of curcumin for prevention of oral mucositis in children receiving Doxorubicin based chemotherapy. *Submitted on December 2011 to the Journal of Alternative Therapies in Health and Medcine*.

2010 Cignacco E, Axelin A, Stoffel L, **Sellam G**, Anand KJ and Engberg S. Facilitated tucking as a non-pharmacological intervention for neonatal pain relief: Is it clinically feasible? *Acta Paediatr* 2010; 99, 1763–1765.

OTHER JOURNALS

2011 Sellam G, Schulz C, Hammerli NS, Cignacco E. [Neonatal intensive care: optimizing pain management in newborn infants]. Krankenpfl Soins Infirm 2011;104:32-3.

LEADERSHIP PROJECT

2010 – 2012	Conceptualization and co-organization of conference: *Challenges in Pediatric Care: Innovations through Advanced Nursing Practice*, taking place on January 13th, 2012 in Basel, Switzerland.

TEACHING EXPERIENCE

2009 – 2012	Quantitative Research course. Lectures for bachelor students of the Institute of Nursing Science, University of Basel, Switzerland
2009-2011	Quantitative Proposal Writing Seminar. Lectures for master students of the Institute of Nursing Science, University of Basel, Switzerland
2009-2011	Quantitative Master Seminar. Lectures for master students of the Institute of Nursing Science, University of Basel, Switzerland