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The efficacy of kangaroo mother care, sucrose and pacifier to reduce responses of preterm infants to procedural pain

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The efficacy of kangaroo mother care, sucrose and pacifier to reduce responses of preterm infants to procedural pain

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Figura da capa e separadores |*Maternidade I,* Humberto Seabra Santos, 1994
Grafismo e paginação |José Joaquim M. Costa

À minha Mãe,
às minhas filhas, Aline e Cristina

(...)
Two roads diverged in a wood, and I—
I took the one less traveled by,
And that has made all the difference.

Robert Frost, 1920

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Aos meus companheiros do PICH.

Aos meus amigos.

À minha família.

ABSTRACT

Preterm neonates in intensive care units endure frequent procedures that may cause pain, warranting the study of interventions that will decrease this pain. The primary aim of this study was to compare the efficacy of the combination of sucrose, pacifier and kangaroo mother care (S+KMC), with that of sucrose and pacifier (S), in reducing the pain responses of preterm infants undergoing venepuncture. Secondary objectives addressed to babies in S+KMC were to examine the relationship between maternal anxiety and the pain responses of the babies; and to explore mothers' perceptions of KMC during venepuncture.

A randomized-controlled trial was conducted in two neonatal intensive care units in Portugal. One-hundred and ten preterm infants without severe illness, stratified by gestational age, were randomly assigned to receive S+KMC or S for venepuncture. Measures of pain responses were the Premature Infant Pain Profile, heart rate, oxygen saturation, facial actions, behavioral state, heart rate variability and recovery time, which were analysed with repeated-measures ANOVA. Mothers' anxiety was measured with the State-Trait Anxiety Inventory. Their perceptions were obtained through content analysis of semi-structured interviews.

Compared to infants in S, infants in S+KMC displayed significantly less facial action; were more likely to have recovered heart rate baseline values at 60 and 90 seconds after the procedure, if they were 32 weeks gestational age and above; and changed from sleep to wake states significantly less. Maternal anxiety was low to moderate and was not correlated to specific pain responses. Mothers emphasized their feelings of well-being in comforting and protecting the babies.

In conclusion, combining sucrose, pacifier and kangaroo mother care is effective and safe in preterm infants undergoing venepuncture for blood-draw; low to moderate levels of anxiety of mothers do not interfere with the pain responses; mothers appreciate holding the baby skin-to-skin when the infants are enduring pain.

Keywords: Pain, preterm neonate, sucrose, kangaroo mother care, venepuncture

RESUMO

Os recém-nascidos pretermo que necessitam de cuidados intensivos são frequentemente submetidos a procedimentos diagnósticos e terapêuticos que podem causar dor. Contrariamente ao que se pensava há duas décadas, a evolução ontogenética da dor inicia-se cedo e, a partir das 24 semanas de gestação, o feto dispõe do equipamento neurosensorial necessário à experiência de dor. Todavia, as vias de controlo descendente não se encontram ainda suficientemente desenvolvidas, resultando em hipersensibilidade dolorosa. As consequências da exposição repetida à dor no período neonatal têm vindo a ser estudadas, sendo hoje conhecidos os efeitos a curto prazo da dor não tratada, como a hiperalgesia e a alodinia nos recém-nascidos, e alguns efeitos a médio e longo prazo como as alterações da sensibilidade e da reactividade ao stress em crianças de idade escolar. O alívio da dor nesta população vulnerável é, pois, uma tarefa imperiosa. Dado o reduzido leque de fármacos disponíveis para estas idades e o seu potencial para efeitos adversos, torna-se necessária a investigação de intervenções não-farmacológicas. Entre estas, a sacarose oral com chupeta tem sido exaustivamente demonstrada como eficaz, sendo utilizada por norma em muitas unidades neonatais antes da realização de procedimentos como a punção do calcanhar e a punção venosa. Durante estes procedimentos, também o contacto pele-a-pele entre mãe e bebé, conhecido como canguru materno, pode ser utilizado como forma de reduzir as respostas de dor dos recém-nascidos.

Desconhecia-se, todavia, se ao adicionar o canguru materno ao uso da sacarose com chupeta seria possível reduzir ainda mais as respostas de dor dos recém-nascidos pretermo. Por outro lado, dada a co-regulação fisiológica mãe-bebé, colocava-se a questão de saber se a ansiedade materna poderia comprometer o efeito analgésico do canguru materno. Finalmente, as percepções das mães sobre a realização de canguru materno durante a venopunção não haviam sido exploradas.

Assim, os objectivos definidos para este estudo foram: 1) comparar as respostas de dor dos recém-nascidos pretermo aos quais é proporcionado canguru materno, sacarose oral

e chupeta durante a punção venosa para colheita de sangue, com as respostas dos recém-nascidos aos quais é proporcionada apenas sacarose oral com chupeta; 2) analisar a relação entre a ansiedade materna e as respostas de dor dos recém-nascidos que efectuaram canguru materno; e 3) explorar as percepções maternas sobre a realização de canguru materno durante a venopunção.

Para dar resposta ao primeiro objectivo, foi realizado um estudo randomizado, controlado, cego, em duas unidades de cuidados intensivos neonatais portuguesas. Cento e dez recém-nascidos sem doença grave, estratificados por idade gestacional (28 a 31 semanas e seis dias, e 32 a 36 semanas e seis dias) foram aleatoriamente alocados a dois grupos: um recebeu sacarose oral com chupeta (grupo Sacarose); o outro recebeu sacarose oral com chupeta e canguru materno (grupo S+CM) antes, durante e após venopunção. As respostas de dor foram medidas através da escala Premature Infant Pain Profile (PIPP) e foram analisadas a frequência cardíaca, a saturação de oxigénio da hemoglobina, as acções faciais (percentagem de tempo em saliência interciliar, olhos apertados e prega nasolabial), o estado comportamental, a variabilidade da frequência cardíaca (baixa frequência, alta frequência e ratio entre ambas) e o tempo de recuperação da frequência cardíaca inicial após o final do procedimento. As acções faciais foram gravadas em vídeo e as variáveis fisiológicas foram registadas através do Somté® Compumedics, ao longo de cinco fases: antes do procedimento, preparação da pele, punção, compressão e repouso. Para a determinação do score PIPP, a análise das gravações foi efectuada por codificadores cegos aos propósitos do estudo.

Para dar resposta ao segundo objectivo, foi realizado um estudo descritivo-correlacional analisando a relação entre a ansiedade materna medida pela escala de Estado de Ansiedade do State-Trait Anxiety Inventory (STAI) e as respostas de dor dos recém-nascidos (N= 60).

As percepções maternas foram estudadas através da análise de conteúdo das entrevistas semi-estruturadas realizadas às mães que tinham efectuado canguru materno (N= 52).

A comparação dos dois grupos de intervenção quanto a variáveis socio-demográficas e clínicas não revelou diferenças significativas.

Em todos os testes foi utilizado como nível de significância $\alpha < .05$. A ANOVA de medidas repetidas (fases do procedimento) a dois factores (intervenção e idade gestacional) revelou o efeito principal da intervenção sobre a percentagem de tempo em saliência interciliar, $F(1, 98) = 5.12$, $p = .026$, e olhos apertados, $F(1, 98) = 6.02$, $p = .015$. A análise post-hoc mostrou que no momento da punção, a saliência interciliar ocorria durante menos tempo nos recém-nascidos do grupo S+CM ($M = 15.89$, $EP = 4.58$) do que no grupo Sacarose ($M = 29.22$, $EP = 4.75$). O mesmo se verificou para o tempo em olhos apertados ($M = 13.85$, $EP = 4.36$ no grupo S+CM e $M = 29.13$, $EP = 4.52$, no grupo Sacarose). O efeito principal

da idade gestacional verificou-se na frequência cardíaca mínima e média e no índice baixa frequência da variabilidade da frequência cardíaca.

A reactividade dos recém-nascidos durante o procedimento foi semelhante nos dois grupos de intervenção, observando-se o efeito principal da fase do procedimento sobre a PIPP, a frequência cardíaca, a saturação máxima de oxigénio, as expressões faciais e o índice baixa frequência da variabilidade da frequência cardíaca. Tal indica uma variação significativa destes sinais de dor ao longo das fases do procedimento, com aumento dos sinais de dor desde o momento antes do procedimento até à punção, seguido de uma diminuição desses sinais até ao repouso.

O teste de Qui-Quadrado para cada fase do procedimento mostrou uma associação significativa entre intervenção e estado comportamental: em todas as fases, a proporção de bebés em estado de sono (versus estado de alerta) era significativamente mais elevada no grupo S+CM.

Apesar não ter havido uma diferença significativa no tempo médio de recuperação da frequência cardíaca de base após o procedimento, a probabilidade (odds-ratio) de ter recuperado aos 60 e 90 segundos após o procedimento foi cerca de 3 vezes mais elevada nos recém-nascidos do grupo S+CM com 32 ou mais semanas de gestação, do que nos do grupo Sacarose.

Durante o procedimento não se verificaram efeitos adversos em qualquer dos grupos de intervenção.

A ansiedade materna foi baixa, sendo significativamente mais baixa nas mães do grupo S+CM (M= 37.78, SD= 9.13) do que nas mães do grupo Sacarose (M= 43.48, SD= 9.82), t(87)= 2.65, p= .009.

Nas entrevistas, as mães salientaram a sensação de bem-estar em ter o bebé em contacto pele-a-pele, o contentamento em poder protegê-lo da dor e a importância que esse acontecimento havia tido para a realização do seu papel parental.

Estes resultados demonstram que a combinação sacarose, chupeta e canguru materno é eficaz e segura em recém-nascidos pretermo, permitindo reduzir a expressão facial e o tempo de recuperação quando comparada com a utilização de sacarose com chupeta; níveis baixos e moderados de ansiedade materna não interferem na redução das respostas de dor dos bebés; as mães apreciam o contacto pele-a-pele durante o procedimento doloroso e sentem o seu papel parental reforçado por poderem participar no alívio da dor do seu bebé.

Em conclusão, o canguru materno pode ser adicionado ao uso da sacarose com chupeta para reduzir as respostas de dor de recém-nascidos pretermo acima das 28 semanas de gestação durante a colheita de sangue por venopunção.

Palavras-chave: dor, recém-nascido pretermo, sacarose, canguru materno, punção venosa.

Acronyms

ADHD - Attention deficit and hyperactivity disorder
CNS - Central nervous system
CPAP - Continuous positive airway pressure
DAN - Douleur-Aigue du Nouveau-Né
EDIN - Échelle Douleur et Inconfort du Nouveau-Né
ELGA - Extremely low gestational age
FBW - Full birth weight
HF - High frequency
HPA - Hypothalamic-pituitary-adrenocortical
HRV - Heart rate variability
KC - Kangaroo Care
KMC - Kangaroo Mother Care
LBW - Low birth weight
LF - Low frequency
MD - Mean difference
NEC - Necrotizing enterocolitis
NFCS - Neonatal Facial Coding System
NIDCAP - Newborn Individualized Care and Assessment Program
NIPS - Neonatal Infant Pain Scale
NNS - Non-nutritive sucking
PIPP - Premature Infant Pain Profile
RSA - Respiratory sinus arrhythmia
STAI - State-Trait Anxiety Inventory
VAS - Visual analogue scale
VLBW - Very low birth weight
WMD - Weighted mean difference

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INTRODUCTION

INTRODUCTION

It is currently well documented that infants who are born prematurely feel pain. Their pain was ignored for many years because of common beliefs that the immaturity of the nervous system protected them from feeling pain and that there was no pain memory in infancy, and therefore no long-term consequences of suffering in early life. Difficulties in interpreting the infant's expressions as being related to pain or to other stress conditions and lack of mastery over medications for pain relief for this age group have also contributed to poor pain-management in preterm infants (Schechter, Berde & Yaster, 1993).

In the past twenty-five years, however, research on pain in neonates has produced four major contributions to knowledge: the demonstration that even the smallest preterm neonates are equipped to and indeed experience pain; that neonates are able to respond to tissue-damaging stimuli through physiological and behavioral indicators that can be consistently measured; that repetitive and prolonged pain in the neonatal period has long-term consequences; and that a number of interventions, both pharmacological and non-pharmacological, can be effective and should be used to manage pain.

Studies of the neurobiology of pain development have put into evidence since the late eighties that innervation of the peripheral tissue and the basic connections between primary sensory neurons and the cells in the dorsal horn of the spinal cord occur early in fetal development and that maturation of the afferents and chemical changes needed for pain processing at the spinal level are in place well before the third trimester of gestation (Fitzgerald & Walker, 2009). However, the lack of inhibitory control at the spinal cord level, as well as the ineffectiveness of inhibitory pathways descending from the brain stem, results in hypersensitivity to painful stimuli in preterm neonates. Knowledge about the supraspinal processing of pain is more recent and it has been shown that pain perception may occur in the absence of full cortical activity (Hall & Anand, 2005a). Yet, cortical activity is present in response to painful stimuli and has

now been measured through real-time near-infrared spectroscopy (Bartocci, Bergqvist, Lagercrantz, & Anand, 2006; Slater *et al.*, 2006).

Infants born preterm usually need to be admitted to neonatal intensive care units (NICU). The environment of these units, as well the clinical condition of the infants, supplies multiple sources of stress and pain. These infants undergo a very high number of diagnostic and therapeutic procedures in order to improve their survival and most of these procedures, such as heel lance, venepuncture and suctioning, are invasive and cause acute pain (Carbajal *et al.*, 2008; Cignacco *et al.*, 2008). Neonatal diseases and surgery, as well as prolonged ventilation, are sources of established and prolonged pain. Infants born at early gestational ages, with very low birth weight and sick infants are, by virtue of their clinical condition, more exposed to pain (Hall & Anand, 2005b).

Preterm infants respond to stress and painful events with physiological and behavioral changes. The intensity of these responses is related to their gestational age, severity of illness and previous exposure to pain, younger and sicker infants' responses being less robust than the responses of healthy term babies (Gibbins *et al.*, 2008a; Lucas-Thompson *et al.*, 2008; Johnston & Stevens, 1996; Johnston, Stevens, Craig, & Grunau, 1993). Increase in heart rate, decrease in hemoglobin oxygen saturation levels, and cortisol release are observed in the presence of painful stimulation. Facial grimacing, cry and body movement can be found in response to a painful procedure, facial grimacing being a more specific response than others (Stevens *et al.*, 2007).These indicators of pain have been analyzed to build consistent assessment tools that facilitate the measurement of pain intensity and are valuable for clinical practice and research.

Early exposure to repetitive pain associated with maternal separation is not without consequence. Permanent changes in pain processing at the peripheral, spinal and supraspinal levels, in neuroendocrine function and in neurologic development may be manifested later by alteration in pain thresholds, in the response to stressful events, in cognitive functions, and by an array of long-term disabilities (Grunau & Tu, 2007; Anand & Scalzo, 2000; Gunnar & Barr, 1998).

The pain endured by neonates during their stay in the hospital is a major concern of parents (Gale, Franck, Kools, & Lynch, 2004). Being unable to protect their infant from pain and feeling dispossessed of their role as primary carers are referred by parents as important sources of distress (Franck, Cox, Allen, & Winter, 2004). Measures to enhance maternal-infant interaction and empower parents in the care of their infants in neonatal intensive care units must therefore be considered part of a global approach of developmental, family-centered care.

The use of potent pharmacological agents like morphine and fentanyl for neonatal surgery was demonstrated to successfully reduce mortality and morbidity more than two decades ago (Anand, Sippell, & Aynsley-Green, 1987). However, the use of these pharmacological agents for procedural pain such as related to heel lance, intra-venous cannulation and endotracheal suctioning, is not an option given the high frequency of these procedures and the potential of those agents for adverse effects. Morphine has not consistently been reported to be effective for acute procedural pain (Carbajal *et al.*, 2005) and neither has Paracetamol (Shah, Taddio, & Ohlsson, 1998). Lidocaine-prilocaine cream, known as EMLA™ (Eutectic Mixture of Local Anesthetics), although safe in proper dosing, is not effective in preterm infants to reduce pain from heel lance (Stevens *et al.*, 1999; Larsson, Jylli, Lagercrantz, & Olsson, 1995).

This obviously limited choice of pharmacological agents for common procedures has warranted research on Non-pharmacological interventions. Many studies have highlighted the positive effects of interventions like oral sweet solutions such as sucrose or glucose, non-nutritive sucking elicited through a pacifier, facilitated tucking, breastfeeding and skin-to-skin contact between mother and infant, also known as kangaroo mother care, among others, in reducing the pain response of preterm infants during routine painful procedures. The mechanisms of action of some of these Non-pharmacological interventions are well known while others are still unclear and remain under research.

More important, when compared to placebo, the efficacy of these interventions in reducing the pain responses have been shown but more research is needed to devise combinations of interventions that will further decrease procedural pain levels.

The above considerations comprise the problem statement and can be represented in Figure 1.

Pain as a human response is a focus of nursing practice, pain control is an expected outcome and Non-pharmacological interventions for common procedural pain are within the scope of nursing practice (International Council of Nurses, 2010).

For this reason, with the aim of contributing to improve pain management practices in neonatal care, we have considered the need to study interventions that, if effective, will have a good potential to be integrated into clinical practice. Oral sucrose, with or without non-nutritive sucking, is currently considered standard care to manage procedural pain in many neonatal units, and pacifiers are used frequently as a soothing intervention. Kangaroo Mother Care, on the other hand, is used more or less systematically in these units to reduce parental stress and improve parent-child bonding but, as far

we know from published and unpublished reports, it is not currently used for pain management (American Academy of Pediatrics, Committee on Fetus and Newborn and Section on Surgery, Canadian Paediatric Society, & Fetus and Newborn Committee, 2006). Adding kangaroo mother care to the standard use of sucrose and pacifier would therefore be feasible, since these interventions are known to neonatal staff, and might further reduce the pain responses of preterm infants during painful procedures. The question, however, was that the effect of this combination in reducing the pain responses of preterm infants during a painful procedure had not been studied before and was therefore unknown.

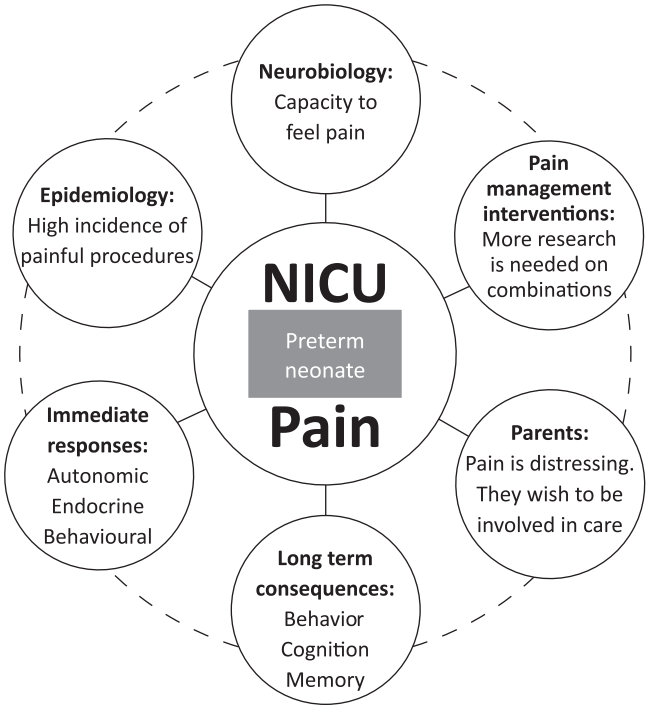

Figure 1. The preterm infant in the NICU and pain: problem statement. Preterm infants have the capacity to feel pain. During their stay in neonatal intensive care units, they endure a high number of painful procedures and events. Their physiological and behavioral immediate responses to pain can be measured consistently. The long-term consequences of repetitive exposure to pain are reflected on alterations of pain pathways, behavior and cognition. Pain is a major concern for parents, who wish to be involved in the care of their infants. There are efficacious and safe available interventions to reduce pain responses during procedures but more research is needed on the combination of these interventions to improve pain management.

Oral sucrose has been exhaustively studied for procedures such as heel lance and venepuncture and its efficacy has been demonstrated in very low doses, without more adverse events than with placebo (Stevens, Yamada, & Ohlsson, 2010). Infants given sucrose cry less and present lower pain scores. The stimulation of taste buds in the tongue by sweet-taste activates the release of endogenous opioids, responsible for cessation of cry in infants (Blass & Ciaramitaro, 1994). Pacifiers also reduce pain responses, either

alone or in combination with oral solutions (Cignacco *et al.*, 2007). There are two hypotheses for the calming effect of sucking, none of them involving opioid mechanisms: it promotes self-regulation and it is a strong sensory stimulation mobilizing the limited attentional resources of the neonate (Carbajal, Chauvet, Couderc, & Olivier-Martin, 1999). Kangaroo Mother Care is another well studied intervention, especially for heel lance. It promotes sleep states, reduces cry and pain scores and facilitates recovery of altered physiological parameters after procedural pain (Johnston, Campbell-Yeo, & Fernandes, 2009; Warnock *et al.*, 2009). Opioid and non-opioid mechanisms may be at stake, since during skin-to-skin contact between mother and infant, sensorimotor, thermal, olfactive and tactile interactions take place, which are hidden regulators of infant physiology and behavior (Hofer, 1994).

The main question of this study was therefore: are the pain responses of preterm infants to a painful procedure reduced when kangaroo mother care is added to the standard care use of sucrose and pacifier? Given the known co-regulation of mother-infant physiology and behavior (Morelius, Theodorsson, & Nelson, 2005; Matthiesen, Ransjo-Arvidson, Nissen, & Uvnas-Moberg, 2001), a secondary question was raised: does maternal anxiety interfere with the potentially beneficial effects of kangaroo care? And finally, because mothers are an essential part of this intervention and their presence during painful procedures is often an issue for health professionals, a last question was raised: how do mothers perceive doing kangaroo care during a painful procedure, i.e., holding the baby skin-to-skin while the baby is enduring pain?

The painful procedure chosen to be examined was venepuncture because it is the most frequently performed needle-related procedure in our neonatal intensive care units.

In order to respond to the questions above, one main and two secondary objectives were defined: 1) to compare the efficacy of the combination of kangaroo mother care, sucrose and pacifier, with that of sucrose and pacifier, in reducing the pain responses of preterm infants undergoing venepuncture in the Neonatal Intensive Care Unit; 2) to examine the relation between maternal anxiety and the pain responses of preterm babies in KMC; and 3) to explore mothers' perceptions of doing kangaroo care during venepuncture.

To attain the main objective of this study a single-blind randomized-controlled trial was conducted and, to respond to the secondary questions, mothers who performed kangaroo care were interviewed. The study took place in two level II/III Neonatal Intensive Care Units in Coimbra, Portugal. After authorization and consent

procedures, one-hundred and ten preterm babies (N= 110) with gestational ages between 28 and 37 weeks, stratified into two gestational age groups (from 28 weeks to 31 weeks and six days and from 32 weeks to 36 weeks and 6 days) participated in this study. Infants were randomly assigned to receive one of two interventions to reduce pain during venepuncture for blood draw: 1) Oral sucrose with pacifier; or 2) Oral sucrose, pacifier and kangaroo mother care. Physiological indicators, namely heart rate and oxygen saturation, as well as facial behaviors were digitally recorded before, during and after the venepuncture. The pain responses were examined using a composite pain scale, the Premature Infant Pain Profile (Stevens, Johnston, Petryshen, & Taddio, 1996) and analyzing its components separately: heart rate, oxygen saturation, facial actions and state. Heart rate variability and recovery time were also analyzed. The two intervention groups were compared across the procedure using repeated-measures ANOVA.

The second objective was attained undertaking a cross-sectional, correlational study. Mothers' anxiety level was measured before the painful procedure using the State scale of the State-Trait Anxiety Inventory (Spielberger *et al.*, 1983) and the relation with infants' pain responses was examined.

Finally, the third objective was reached through content analysis of the semi-structured interviews to mothers who performed kangaroo care.

The design of this study is represented in Figure 2.

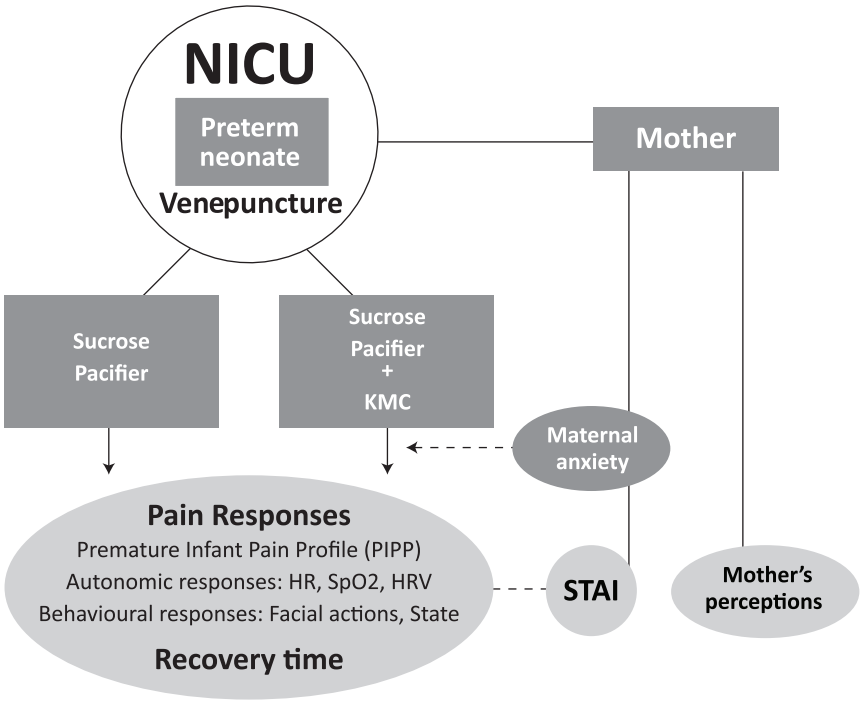

Figure 2. Study design. Note: KMC - Kangaroo mother care; STAI - Stait-Trait Anxiety Inventory; NICU - Neonatal Intensive Care Unit.

This research report is organized in two parts — the background and the empirical study — and follows the recommendations contained in the Publication manual of the American Psychological Association, 5th edition (American Psychological Association, 2001).

In the first part, divided in five chapters, the theoretical and empirical background for studying non-pharmacological pain interventions in the context of neonatal care is presented. In the first chapter, the vision of the preterm neonate within the Model of the Synactive Organization of Behavioral Development (Als, Butler, Kosta & Anulty, 2005) offers a framework for integrating pain relief as an important part of the developmental care approach. Pain as a multidimensional experience and evidence from neurobiology of the capacity of preterm neonates to feel pain are examined next. Short term responses to pain and tools to assess pain intensity as well as long-term consequences of early exposure to pain are also addressed in the second chapter. The third chapter focuses the epidemiology of pain and pain management in neonatal intensive care units, confirming that pain occurs frequently and is often undertreated. The fourth chapter offers a comprehensive review of studies of non-pharmacological pain interventions for procedural pain in newborns. Parental concerns and the importance of a family-centered approach for pain management in the NICU are analyzed in the last chapter of the first part.

The second part presents the empirical study. The methods are described, namely the study design, the research settings, the sampling method, the outcome measures, the research procedure including the experimental protocol, the procedures for data extraction and statistical analysis, and the way in which ethical concerns were dealt with. The results are displayed in chapter seven, beginning with a presentation of participants' characteristics, followed by the pain responses of infants to the interventions studied. In the third and fourth sections of the results chapter, the correlation between maternal anxiety and infants' pain responses is presented as well as mothers' perceptions of kangaroo care during venepuncture. In the discussion, the results obtained in response to the research questions are summarized and interpreted according to the state of the art. Strengths and limitations of the study are disclosed, unresolved theoretical issues are put forward and implications are drawn for clinical practice and research.

The report concludes with an overall evaluation of the research process taking into consideration the objectives of the study, and opens the way for knowledge translation of these results into practice as well as for near-future research.

PART I
Theoretical background: The neonate and pain

CHAPTER 1.
The preterm neonate, a new paradigm

PART I - Theoretical background: The neonate and pain

CHAPTER 1. The preterm neonate, a new paradigm

For many years, preterm infants were considered immature, incomplete babies as mirrored in the way they were named: "premature babies". Neonatal care was focused on compensating and treating the consequences of the immaturity of the different systems – respiratory, gastro-intestinal, immune and nervous, among others. Infants born prematurely are abruptly deprived of the intrauterine environment, which provides the most favorable conditions for their development.

The conceptualization of development as proposed by the Model of the Synactive Organization of Behavioral Development (Als *et al.*, 2005) offers a theoretical background for examining preterm neonates, their development and the aims of neonatal care in a different way. Under this model, preterm infants are not incomplete fullterms but rather, well-equipped, competently adapted fetuses that would function appropriately for their stage of development if they were in their natural environment: the maternal uterus. They are seen as the initiators of the interaction with others, whether these are professionals or parents.

The infants' functioning is viewed as continuous interactions between the intraorganism subsystems (autonomic, motor, state, attentional/interactive and self-regulating) and the environment. Once exposed to the aggressive environment of a neonatal intensive care unit, a great amount of energy is consumed by infants in stabilizing their subsystems and little is left to pursue their development in the right direction unless the care provided helps them to do so (Als *et al.*, 2004). A main goal of neonatal care, as important as to respond to physiological needs, is therefore to attend to the developmental and emotional needs of preterm infants by adjusting the environment (Sparshott, 1997).

The first decades of development of neonatology, in the 1960s and 1970s, were devoted to the survival of preterm infants (Kennell, 1999) by maintaining the autonomic functioning: respiratory, cardiac, digestive and temperature control functions. The dawn of mechanical ventilation revolutionized neonatal care which became more and more intensive and invasive. Handling for procedures such as endotracheal suctioning became a routine that had to be followed regularly.

It wasn't until technology allowed non-invasive constant monitoring of oxygen blood levels through transcutaneous pO2 devices that the impact of care came to light.

Although some expressed concern for the lack of sleep and rest of these infants in the late 1970s (Lucey, 1977), state organization, motor system and sensory functioning were secondary concerns at that time (Als *et al.*, 1986). It was in the 1980s, that considering the developmental and behavioral detrimental consequences of the aggressive environment of neonatal intensive care, a number of interventions termed 'Developmental Care' were suggested to improve the neurodevelopmental outcome of preterm infants (Als *et al.*, 1986). This broad group of interventions includes controlling external stimuli (vestibular, tactile, auditory, visual) during nursing routines, handling, feeding, pain management, adjusting these to the individual cues of the baby and involving parents in the care of their infant in the NICU in a family-centered approach (Aucott, Donohue, Atkins, & Allen, 2002). The idea of organizing care based on the individual behavioral cues of each baby, especially those who are very low birth weight (VLBW) was the basis of the Newborn Individualized Developmental Care and Assessment Program (NIDCAP). The impact of NIDCAP on neurodevelopment, maturation and morbidity remains controversial, some studies reporting clear positive effects (Kleberg *et al.*, 2008; Als *et al.*, 2003; Kleberg, Westrup, Stjernqvist, & Lagercrantz, 2002; Kleberg, Westrup, & Stjernqvist, 2000) and others not (Symington & Pinelli, 2006; Jacobs, Sokol, & Ohlsson, 2002; Ariagno *et al.*, 1997). Yet, its foundational model, the Model of the Synactive Organization of Behavioral Development, was innovative in stressing the competencies of preterm newborns, namely their capacity to interact with the environment, and the importance of modifying the physical and emotional environmental factors that can adversely affect the behavioral organization of these infants.

The refinement of neonatal care and the increasing use of high technology gradually pushed back the limits of viability of preterm infants in the past 40 years (Seri & Evans, 2008). While it was a common understanding in the 1970's that infants born less than 28 weeks were not able to survive, today's knowledge and technological resources in developed countries make it possible that infants born as early as 23 weeks of

gestational age are cared for in NICUs. This achievement however has not been without cost. Infants born very prematurely display an array of physical and psycho-behavioral consequences that are related to their early life events.

Studies about the effects of early experience on brain function and structure as well as on subsequent behavior (Als *et al.*, 2004; Anand & Scalzo, 2000; Gunnar & Barr, 1998) seem to confirm the need to reduce environmental stress factors in the neonatal period, namely pain exposure and maternal deprivation.

CHAPTER 2.
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Understanding pain in the neonate demands first of all that this concept is made clear. Knowledge of the peripheral, spinal and supraspinal processing of painful stimuli owes a great deal to animal studies, which is why the most common animal models of infant pain will be briefly reviewed. The rate of development of the nervous system, both in growth and differentiation (birth and migration of neurons; growth of axons; formation of dendrites and synapses, myelination, pruning, to mention only a few processes) is such, that it is not possible to discuss pain in the neonate without considering developmental issues. The capacity of preterm neonates to experience pain can be demonstrated by examining their immediate responses, measuring their intensity of pain and analyzing the long-term consequences of pain in early life.

2.1 The pain experience

The concept of pain as a multidimensional experience is fairly recent. Attempts to categorize pain as a sensation or as an emotion come from as far back as Aristotle (Melzack & Wall, 1987).

The International Association for the Study of Pain (IASP) defines pain as "An unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage" (IASP Taskforce on Taxonomy, 1994) thus recognizing the multidimensional character of the pain experience.

The recognition that the pain experience is far more than the activity induced in the nociceptor and nociceptive pathways by a noxious stimulus, always involving a psychological state, finds support in the Gate-Control Theory (Melzack & Wall, 1987). Published in 1965 by Melzack and Wall, the Gate-Control Theory moved away from the Specificity Theory, which since Descartes postulated that a specific pain system transported pain messages from specific centers in the skin to a specific centre in the brain. The mechanism could be compared to pulling a string to ring a bell. This mecha-

nistic view was refined during the 19th and early 20th centuries by scientists like Müller and von Frey, under the new developments of physiology and histology but kept the idea of a fixed nervous system and direct pain pathways. The main contribution of this theory was the recognition of the specialized role of skin receptors. The existence of a direct and invariable relation between a particular quality of the physical stimulus and the psychological and sensory dimension of the experience defended by this theory, though, was not supported by empirical evidence and opened the way to other theories. Some of these theories like the Pattern theory and the Summation theory brought up important contributions to the understanding of pain but none of them alone could consistently offer a comprehensive explanation for the complexity and variety of pain syndromes (Melzack & Wall, 1987).

The conceptual mode that underlies the Gate-Control Theory is based on the following propositions:

> 1. The transmission of nerve impulses from afferent fibers to spinal cord transmission (T) cells is modulated by a spinal gating (SG) mechanism in the dorsal horns.
> 2. The spinal gating mechanism is influenced by the relative amount of activity in large-diameter (L) and small-diameter (S) fibers: Activity in large fibers tends to inhibit transmission (close the gate) and small-fiber activity tends to facilitate transmission (open the gate).
> 3. The spinal gating mechanism is also influenced by nerve impulses that descend from the brain.
> 4. A specialized system of large-diameter, rapidly conducting fibers (the central control trigger) activate higher cognitive processes that then influence, by way of descending fibers, the modulating properties of the spinal gating mechanism.
> 5. When the output of the spinal cord transmission (T) cells exceed a critical level, it activates the action system – those neural areas that underlie the complex, sequential patterns of behavior and experience characteristic of pain. (Jeans & Melzack, 1992, p. 22)

This model acknowledges the fundamental role of the central nervous system (CNS) in filtering, selecting and modulating pain inputs from high-threshold small fibers (Melzack, 1999) and definitely breaks the Cartesian dichotomy between mind and body. Brain activities related to attention, emotion and memory exert control over the sensory input.

However, how the brain functions to produce the qualities of the experience in the absence of sensory peripheral inputs has not been explained by this theory. Analyzing the phantom limb phenomena, Melzack developed the Neuromatrix Theory of Pain (Melzack, 2001; Melzack, 1999). This new conceptual model of the nervous system proposes that a widespread network of neurons, called the neuromatrix, is the anatomical substrate of our experience of the body-self and the somatosensory qualities we feel. The neuromatrix consists of loops between the thalamus and cortex and between the cortex and the limbic system, the spatial distribution and synaptic links of the neuromatrix being initially determined genetically and later "sculpted" by sensory inputs. These loops are responsible for the sensory-discriminative, affective-motivational and evaluative-cognitive components of the pain experience. They diverge to permit parallel processing in different components of the neuromatrix and converge to permit interaction between the outputs of this processing. This cyclical processing and synthesis of nerve impulses has a characteristic output pattern named "neurosignature". The neurosignature for the pain experience is, again, determined by the synaptic architecture of the neuromatrix resulting from the genetic and sensory influences and is modulated by sensory and cognitive inputs, such as psychological stress, to produce the particular qualities and properties of the pain experience. Multiple inputs act on the neuromatrix and contribute to produce the output signature. Painful stimuli might then trigger the neurosignature output but do not produce it. The neurosignature pattern, a continuous outflow of nerve impulses from the body-self neuromatrix, is projected into areas in the brain, the sentient neural hub, where it is modulated by ongoing inputs producing a continually changing stream of awareness. In the same way, the activation of neural networks responsible for movement produces the movement itself while the projection to the sentient neural hub produces the experience of movement. The inputs to body-self neuromatrix include sensory (e.g. cutaneous, visceral, visual, vestibular inputs) as well as motivational-affective (e.g. hypothalamic-pituitary-adrenal system, noradrenalin-sympathetic system, immune system, cytokines, endogenous opiates) and cognitive-evaluative inputs (e.g. learning, past experience, personality, attention, anxiety). The outputs from body-self neuromatrix involve the pain perception in its sensory-discriminative, motivational-affective and cognitive-evaluative dimensions, patterns of action, communication and coping, and stress-regulation programs.

This theory proposes a model of brain functioning that reinforces the concept of pain as a multidimensional experience integrating the role of higher psychoneural processes in addition to the previous modulation and descending control of sensory nerve

inputs caused by injury, and therefore offers an explanation for the experience of pain in the absence of injury or peripheral sensory pathways as is the case in most chronic pain syndromes.

In neonates, the Gate-Control Theory is a useful framework for acute procedural pain and the interventions that can be used as pain gating mechanisms. Sensory and motivational-affective inputs are certainly present in preterm infants. While cognitive-evaluative inputs such as learning and culture might have a minor role, other inputs such as past experience, attention (state) and anxiety (distress), may play an important role in the experience of pain. The long-term consequences of pain in early life, however, may come to find some explanation under the Neuromatrix Theory of Pain.

The capacity of neonates to feel pain was challenged for a long time, given that the experience of acute pain requires the structures in the CNS to be connected in order that the sensory inputs reach the brain cortex. The full development of pain pathways and the activity of the cortex have been considered critical issues in recognizing that neonates feel pain. Research with animal models has been extremely useful in elucidating about the development of neuronal structures and functioning of sensory pathways.

2.2 Animal models of infant pain

Much of what we know about pain and development is inferred from animal studies. For ethical reasons, certain kinds of experiments are not justifiable in humans unless strong evidence suggests that the results may be beneficial in clinical practice. Given the amount of animal studies that have generated hypotheses about pain relieving interventions and their mechanisms in neonates, a brief overview of common outcomes of experiments in animal models of infant pain may help understand the rationale of such studies.

Rodents represent a useful model for the investigation of human neonatal pain for three main reasons: it is possible to parallel the stages of neurosensory development of rat pups and human infants; the quick rate of maturation allows the study of long-term consequences of neonatal pain in a short time; and the control of extraneous variables is easier than in human research. Although the developmental timetables are different in rats and in humans, the basic sequence of events in the maturation of sensory systems is the same in both species (Fitzgerald & Anand, 1993). Rat pups are born fairly immature compared to fullterm infants and their neurological maturation stage at birth, in terms of somatosensory and motor development, is comparable to the human infant development around 24 weeks of gestational age. Studies of the developmental neuroanatomy and neurophysiology of pain as well as studies of pain behaviors relate

data obtained from newborn rats in the first week of life to preterm infants at the second trimester of gestation. By postnatal day 10 (P10) the stage of development is related to that of fullterm infants; data from 2-3 week-old rats corresponds to infants during the first years of life (Sternberg & Al-Chaer, 2007; Johnston, Walker, & Boyer, 2002; Fitzgerald & Anand, 1993). In addition, laboratory rodents have a short gestation (approximately 3 weeks) yielding large litters of pups. These have a rapid rate of postnatal maturation: they are weaned at around 20 days, reach sexual maturation around 6-7 weeks of age and are adults near the 10th week (P60). It is therefore possible to study the long-term consequences of neonatal pain in only a few months (Sternberg & Al-Chaer, 2007). Animal models offer the possibility to control the timing, frequency and intensity of the pain stimulation in a way that is not possible in the clinical environment where pain and the outcomes studied are related to clinical care. Genetic factors responsible for individual variability can also be controlled in animal studies, by using selected strains of rats. Smaller samples can be big enough to show small differences in effect sizes (Johnston *et al.*, 2002a). The clinical relevance of animal studies though, has some limitations and Johnston and colleagues articulate the questions that can be validly answered by animal studies, considering the asynchronous development of the various brain regions and the higher complexity of human behavior compared to rodent behavior: "The specific questions relate to the effects of peripheral injury of differing types and magnitude on the central nervous system (CNS), how long the effects last, how widespread the changes are (peripheral, spinal, supraspinal), and what mechanisms can block the change" (Johnston *et al.*, 2002a, p. 397).

Studies that have examined the immediate responses and long-term consequences of early exposure to pain use the paradigm of acute needle pain (Johnston & Walker, 2003; Anand, Coskun, Thrivikraman, Nemeroff, & Plotsky, 1999), persistent inflammatory pain caused by chemical agents or cutaneous tissue injury (Ririe, Bremner, & Fitzgerald, 2008; Ruda, Ling, Hohmann, Peng, & Tachibana, 2000; De Lima, Alvares, Hatch, & Fitzgerald, 1999; Reynolds & Fitzgerald, 1995) and nerve injury (Lee & Chung, 1996).

Acute pain can be elicited by single or repeated needle stick in the dorsum or plantar surface of the hindpaw (Johnston *et al.*, 2002a; Anand *et al.*, 1999) or by repeated footshock (Sternberg & Al-Chaer, 2007). Inflammatory pain is frequently obtained through injections into paws of formalin, a mild inflammatory agent producing short-lasting local inflammation (30 to 60 minutes) while carrageenan, capsaicin, bee-venom or complete Freund's adjuvant (CFA) are stronger inflammatory agents causing long-

lasting pain and in the case of CFA[1], long-term activation of immune responses more suitable to mimic chronic pain (Johnston *et al.*, 2002a).

Outcome measures used to examine the effect of single or repetitive pain and the modulating effect of interventions on different types of pain include pain thresholds to thermal or mechanical stimuli as well as pain behaviors, stress responsiveness, changes in tissue innervation and pain circuitry, and peripheral, spinal and supraspinal activity of neurons and neurotransmitters.

Pain thresholds

Thermal sensitivity threshold is measured using the Hargreaves test, the hot plate test or the tail flick test. In the Hargreaves test the rat is placed in an acrylic box and a beam of light is directed to the footpad of one paw. The temperature of the beam rises rapidly and latency to nociceptive behavior i.e., the time elapsed until the rat displays behaviors such as paw lifting, licking, shaking or flicking, is considered to be the pain threshold (Johnston *et al.*, 2002a). The hot plate test is similar but the surface of the box in which the rat is placed is at a constant temperature of 50 to 60 degrees Celsius. In the tail flick test a heated beam of light set at a certain temperature is directed onto the tail of the animal placed in a narrow acrylic box not allowing him to move, and the latency to flick the tail out of the heat source is the pain threshold.

Mechanical sensitivity is most commonly measured through stimulation with von Frey hairs, nylon filaments of graded calibrated diameters. Von Frey hairs are applied sequentially in increasing diameters on the animal's paw, until the cutaneous flexor reflex is elicited. The end of the filament is pressed against the skin requiring a precise force to form a buckle, the caliber of the filament in grams being considered the mechanical threshold. The cutaneous flexor reflex is a protective response and depends upon the development of spinal sensory processes (Johnston *et al.*, 2002a).

Variations in thermal and mechanical sensitivity in inflamed versus non-inflamed animals allow the study of analgesic agents as well as and the modulating effect of certain Non-pharmacological interventions such as non-nutritive suckling (Anseloni, Ren, Dubner, & Ennis, 2004) or sucrose.

The mediating role of maternal rearing on adult pain thresholds as a consequence of neonatal repetitive pain has recently been explored testing thermal sensitivity (de Medeiros, Fleming, Johnston, & Walker, 2009).

[1] Complete Freund's Adjuvant (CFA) is a mineral oil emulsion containing heat-killed tuberculosis bacteria.

Pain behaviors

Specific pain behaviors of the animal when inflammatory pain is inflicted include ultrasonic vocalizations, licking or shaking the paw, lifting the paw and protecting the paw. The formalin test, consisting of a formalin injection in the paw, is a well validated method of testing used as a model of inflammatory pain (Johnston & Walker, 2003; Teng & Abbott, 1998; Abbott & Guy, 1995). It involves supraspinal mechanisms and allows the understanding of maturation processes and consequences of repeated, long-lasting or severe pain in higher structures of the central nervous system. Recently, this model has been used to examine maternal behavior (grooming) in the presence of repeated neonatal pain in offspring (Walker, Kudreikis, Sherrard, & Johnston, 2003).

Stress responses

Behavioral responses such as rats' exploratory activity in an open field or in a new environment are used as a measure of discomfort associated with ongoing pain and to study the long-term consequences of neonatal pain on the responses to distress, anxiety and agoraphobia under the assumption that early pain experiences will interfere with adult stress responsiveness (Sternberg & Al-Chaer, 2007; Anand *et al.*, 1999). Social discrimination, i.e. time spent investigating a novel juvenile has been used as a measure of chemosensory memory (Anand *et al.*, 1999). Since pain is a stressor, hormones such as ACTH, cortisol or corticosterone are used to measure the activation of the hypothalamic-pituitary-adrenal axis (Walker *et al.*, 2003). Alcohol preferences of adult rats have also been studied as a consequence of repetitive neonatal pain by measuring the intake of solutions of sucrose and sucrose with alcohol and comparing rats exposed to repetitive neonatal pain to rats exposed to non-noxious touch stimulation (Anand *et al.*, 1999), although this was not replicated in a subsequent study (Bhutta *et al.*, 2001).

Structural and functional changes in nervous tissue and neurons

Inflammatory pain elicited by inflammatory agents such as formalin or CFA or by skin wound is used as a stimulus to identify the structural and physiological changes that occur during tissue insult and in the long-term. Skin injury causes inflammatory pain as a result of sensitization of peripheral nociceptors and central neuronal pathways followed by sprouting of sensory nerve terminals and hypersensitivity (Sternberg & Al-Chaer, 2007).

Innervation of the skin, dorsal horns and root ganglia as well as nociceptive pathways, are studied through immunocytochemistry and immunohistochemistry techniques. These methods use antibodies to target components of cells or tissues, re-

spectively. Components identified include neurotransmitters such as glutamate or γ-aminobutyric acid (GABA), neuropeptides such as substance P and enkephalins, neurotransmitters and neuropeptides receptors such as N-methyl-D-aspartate (NMDA), GABA and opiate receptors, immediate early genes such as c-fos expression (Johnston *et al.*, 2002a).

Examining the responses to tissue injury at different ages, it is possible to identify which populations of sensory fibers are more sensitive to nerve sprouting, playing a more significant role in skin hyperinnervation, and which are the critical stages of development (Reynolds & Fitzgerald, 1995). The effect of interventions such as nerve blocks before skin wound in young animals can be studied using cutaneous hyperinnervation and sensory thresholds as outcomes (De Lima, Alvares, Hatch, & Fitzgerald, 1999).

Measurements of electrophysiological activity in the dorsal horn of rats of different ages elucidate the postnatal development of spinal cord mechanisms of inflammatory pain, changes in receptive fields at the dorsal horn as well as the disruption of structural and functional organization of nerve connections at the spinal level as consequence of inflammatory pain and the role of neurotransmitters in fiber connectivity (Peng, Ling, Ruda, & Kenshalo, 2003; Torsney & Fitzgerald, 2002; Beggs, Torsney, Drew, & Fitzgerald, 2002).

To summarize, several models of neonatal pain are used in animal studies to understand basic pain mechanisms related to development, long-term consequences of early exposure to pain and factors that can mediate or block those effects. Acute needle pain and inflammatory pain by chemical agents or tissue injury are the most common ones. Outcomes of studies using these paradigms alone or combined include thermal and mechanical sensitivity, pain and stress behaviors, structural and functional changes in nervous tissue, neurons and nociceptive pathways, measured through a variety of methods. The number of possible combinations of model, studied outcomes and measurement techniques is such that nearly each study reaches findings that are difficult to compare with others.

Furthermore, the parallel between pain inflicted in these experimental conditions and pain experienced by human neonates under clinical care is hard to establish, requiring caution when drawing clinically useful conclusions from animal studies. The findings in animal studies about the development of pain circuitry, widening receptive fields and decreased threshold following injury have led to studies showing similar results in humans (Andrews, Desai, Dhillon, Wilcox, & Fitzgerald, 2002; Fitzgerald & De Lima, 2001; Andrews & Fitzgerald, 1999; Andrews & Fitzgerald, 1994; Fitzgerald, Millard, & McIntosh, 1989; Fitzgerald, Shaw, & MacIntosh, 1988)

Understanding the immediate and long-term effects of different types of pain on the developing peripheral and central nervous system of animals and the factors that can mediate theses consequences increases clinicians' awareness of the detrimental effects of pain in human infants and generates hypotheses regarding interventions to be tested in the clinical environment. As Johnston and colleagues point out: "Interaction between clinicians and basic scientists, with an understanding of the domain in which each group is working, is critical to the meshing of efforts from these domains. With collaboration between these groups, more relevant research can be conducted that can lead to the decrease in pain and its consequences in neonates." (Johnston *et al*., 2002a, pp. 411-412).

2.3 The capacity of preterm neonates to experience pain

The requirements for the occurrence of pain are the existence of functioning peripheral, spinal and supraspinal anatomic structures related to the pain/tactile system as well as the neurochemical system associated with pain transmission and modulation. Some have argued that the development of the mind to allow consciousness of pain is also needed for the pain experience (Derbyshire, 2006). While it is clear that fetuses in the second trimester of gestation have endocrine and reflex responses to noxious stimulation (Glover & Fisk, 1999), it is controversial whether this can be considered pain or just nociception. It is accepted, however, that the interaction with the outside world that occurs at birth marks the beginning of consciousness and is the key to consider that very preterm neonates are able to feel pain. Consciousness may be defined by sensory awareness of the body, the self and the world (Lagercrantz & Changeux, 2009). Early preterm neonates exhibit sensory awareness when they react to sound, smell, touch and taste. In responding to painful stimuli through both behavioral and physiological signs, they express emotions, differentiate self and non-self touch and show signs of shared feelings (Lagercrantz & Changeux, 2009). Yet, these authors argue, they are present-oriented and self-awareness is limited, which is why they can be considered in a minimal level of consciousness that will increase with age.

Regarding the peripheral anatomical and functional requirements for the pain experience, it is known that nociceptive neurons are specified in early fetal life (Fitzgerald, 2005). Sensory neurons in the dorsal root ganglia begin to grow towards the skin and towards the spinal cord by 6 weeks of gestation. Specialized sub-populations of these sensory neurons reach all the cutaneous and mucosal surfaces by 20 weeks. The final density of nociceptive nerve endings in the skin of newborns is at least the same as in adults (Anand & Hickey, 1987) and is a result of the balance between cell growth and cell death (Fitzgerald, 2005).

The synapses between the cells in the dorsal horn of the spinal cord and peripheral sensory neurons are formed by 19 weeks of gestation and, at the spinal level, the organization of the laminar structure of the cells in the dorsal horn and their synaptic connections are completed by 30 weeks (Anand & Hickey, 1987). The size of peripheral cutaneous receptive fields in the dorsal horn of preterm infants is larger than at term. These large receptive fields, dominated by inputs from low-threshold mechanoreceptors in the skin, overlap more than in adults increasing the chances of activation by peripheral skin stimulation (Fitzgerald & Jennings, 1999).

The appearance of specific neurotransmitter vesicles in the dorsal horn begins at 13 weeks but concentrations of neurotransmitters are very low in early fetal life. Substance P appears in the dorsal horn at 8-10 weeks gestation and enkephalin at 12-14 weeks. The high density of receptors with a widespread distribution seems to be compensating the low levels of neurotransmitters (White & Wolf, 2004).

Incomplete myelination of ascending pathways before 30 weeks gestational age implies a slower conduction velocity but does not prevent the nociceptive information from travelling from the dorsal horn to the brainstem and the thalamus (Hall & Anand, 2005a). The thalamus is the main regulator of sleep and arousal states and it integrates sensory inputs before relaying them to different regions of the cortex, namely the somatosensory cortex and the anterior insula, the hippocampus and temporal lobe where memory and learning occur and the fontal lobe, involved in the associative functions and the limbic system emotional dimension of the pain experience. An increase in heart rate and respiratory rate in response to noxious stimulation show that autonomic responses are present at a very early gestational age (Hall & Anand, 2005a).

The nociceptive fibers that link the thalamus to the cortex reach the cortical subplate by 20-22 weeks and are in place by 24-26 weeks gestational age, thus completing the anatomic connection needed for pain perception and pain facial behavior (Hall & Anand, 2005a).

Supraspinal processing of pain has only been demonstrated recently, although suggested long ago by somatosensory evoked potentials in neonates 25 weeks, in favor of the ability of peripheral and spinal cord sensory pathways to conduct peripheral inputs to the cortex (Anand & Hickey, 1987). Activation of the somatosensory cortex in response to noxious stimulation can be detected in human neonates 25 to 45 weeks gestational age, by measuring changes in cerebral oxygenation, using real-time near-infrared spectroscopy (NIRS) (Slater *et al.*, 2006). Preterm infants' specific hemodynamic responses in the somatosensory cortex to tactile and painful stimuli imply conscious sensory perception (Bartocci *et al.*, 2006).

As for the descending inhibitory system, descending axons from the brainstem, although present in early fetal life, do not become functional until P10 in the rat (approximately at term in the human neonate) (Fitzgerald & Anand, 1993). Endogenous opioids are released in the human fetus at birth and in response to distress, but their levels are not high enough to produce analgesia. In preterm infants, the levels of neurotransmitters involved in descending control, such as serotonin and norepinephrine, are also very low. The relatively delayed development of this descending inhibitory mechanism contributes to increased pain sensitivity in preterm newborns (Fitzgerald & Howard, 2003).

Infants who are premature and very low birth weight (< 1500g) have the capacity to differentiate between painful and non-painful stimulus (Johnston, Stevens, Yang, & Horton, 1995): in a cross-over design study, infants (n= 48) between 26 and 31 weeks gestational age at the time of the study who were exposed to a real versus a sham heel lance displayed a differential behavioral and physiological response.

In conclusion, the nociceptive system of the preterm infant is not just an immature version of an adult system: it is structurally and functionally different (White & Wolf, 2004). Not only is there evidence that preterm infants experience pain but also that they lack the endogenous analgesic system and are therefore more sensitive to noxious stimuli than older children and adults.

2.4 Short term responses to painful stimulation

Newborns respond to stimuli that are tissue-damaging in many different ways and in different time epochs. It is possible to differentiate an immediate response from a medium and a long-term response to pain (Fitzgerald & Anand, 1993) both through physiological and behavioral changes.

These changes are not all pain specific but are an index of infants' reactivity. As autonomic functioning regulates internal (hunger, for example) and external demands (such as environmental temperature). The paradigm to study these responses is that an undisturbed baseline condition regulated by the autonomic system would be disturbed by a stressor, producing an acute response phase during which reactivity could be measured. A recovery phase would then follow during which the response could be more or less regulated (Stevens, Pillai Riddell, Oberlander, & Gibbins, 2007).

Studies on neonates' short term responses to noxious stimuli have focused on behavioral cues such as grimacing, cry and body movement; and on physiological changes such as endocrine and autonomic responses. New technologies to examine brain activity also show significant responses at cortical level.

2.4.1 Behavioral cues

The capacity to communicate distress and attract the attention of carers to fundamental needs as well as the capacity of adults to recognize and attend those needs is the result of a long evolutionary process (Craig, 1992). Human infants are born with the capacity to communicate distress as this is essential for the survival of organisms that are dependant from others. Cry, facial activity, posture, limb movement and torso activity can be recognized by adults as signs of distress (Craig, 1992). Although many of these actions are not specific of pain and may be provoked by fatigue, hunger or discomfort, pain consistently triggers some of these signs.

Facial activity

Grimacing corresponds to changes in facial activity. The patterns of display of facial actions in response to pain are consistent across age, from newborns to adults. Facial actions like lowering of the brow, eyes tightly squeezed, deepening of the naso-labial furrow, mouth stretched open, and a taut tongue have been identified in preterm, fullterm and two- and 4-month-old infants during painful procedures, although with differences in the incidence of actions across age groups, preterm infants having a less vigorous facial activity than term and older infants (Gibbins *et al.*, 2008a; Johnston *et al.*, 1993). In infants with the same gestational age but different postnatal age, facial activity was dampened in those who were born earlier and had undergone a greater number of painful procedures (Johnston & Stevens, 1996). Sleep-awake state is also a modifier of facial behavior, the likeliness to show a response being significantly lower in sleeping preterms (Grunau & Craig, 1987). Given that the pain signaling system is in place by mid-gestation, granting preterm infants the capacity to experience pain, it is important to realize that a less robust response related to lower gestational age, previous painful procedures, severity of illness and sleep does not reflect a lower intensity of pain but it is most likely the result of a diminished capacity to communicate pain, related to the immaturity of the motor system and to the need of these vulnerable infants to conserve energy in the strive for survival (Johnston *et al.*, 1999; Craig, Whitfield, Grunau, Linton, & Hadjistavropoulos, 1993).

Facial activity has been shown to be more pain-specific than body movement (Grunau & Craig, 1987; Grunau, Johnston, & Craig, 1990). Stimulation of specific areas in the brain is responsible for behavioral responses to noxious stimulation. Changes in facial expression reflecting pain, anxiety and fear result from the stimulation of the periaqueductal gray area in the midbrain by receiving A-beta, A-delta and C fiber input (Hall & Anand, 2005a).

Cry

Crying is also a generalized signal of distress. Vocalization is ontogenetically aimed at signalling a threat. It is a call signal to draw the attention of others from a distance. Although not specific to pain, cry features, namely the fundamental frequency (pitch), seems to change with the amount of tissue damage in fullterm infants. In preterm infants though, studies about the differences in cry duration and features between painful and less painful conditions have not been conclusive (Johnston *et al.*, 1999). Also, a large number of preterm infants may not cry at all during a tissue-damaging procedure even though this cannot be interpreted as absence of pain (Johnston *et al.*, 1995; Stevens & Johnston, 1994).

Body movement

The immediate visible pain response to a noxious stimulus in the heel of a newborn is a reflex muscle contraction and withdrawal of the limb. Withdrawal reflex thresholds increase during gestation and with post natal age, suggesting lower pain thresholds in earlier stages of development (Fitzgerald & Beggs, 2001). However, "Stronger nociceptive reflexes in infants should not be interpreted as a greater pain experience, but might be protective and beneficial to an organism that is unable to perceive and organize a more directed response to pain" (Fitzgerald, 2005, p. 517). This motor response, involving spinal cord functions, is followed by grimacing or crying which reflect the transmission of the pain information to higher centres in the brain. In the hours or days after the noxious event, especially if the stimulus is repeated, as it is often the case in neonatal intensive care, sensitization of local nociceptors results in primary hyperalgesia at the site of injury; and increased excitability of the cells of the dorsal horn, i.e., central sensitization, is responsible for secondary hyperalgesia and allodynia in areas adjacent to the injury site (Fitzgerald & Beggs, 2001; Fitzgerald & Anand, 1993). The already lower pain threshold in preterm infants up to 32 weeks is further decreased after exposure to repeated painful stimulation and the mechanical sensory reflex threshold in an area of local tissue damage caused by repeated heel lances can reach half the value of that on the contralateral intact heel (Fitzgerald, Shaw, & MacIntosh, 1988).

Other than reflexes, gross motor activity has been examined in search of potential pain cues. There seems to be a differential behavioral, as well as physiological, response between tactile (stressful) and noxious (painful) stimulation, neonates displaying different behaviors and in a different time course during routine care involving handling and a skin-breaking procedure (Holsti, Grunau, Oberlander, Whitfield, & Weinberg, 2005). Comparing clustered care (changing the diaper, measuring girth, taking the ax-

illary temperature, and cleaning the mouth with gauze and sterile water) with pain (heel lance), the authors concluded that changes in facial activity and heart rate remain the most sensitive markers of pain in preterm infants. Yet, finger splay and limb extension have been significantly related to handling for routine neonatal intensive care (Grunau, Holsti, Whitfield, & Ling, 2000) and may therefore be useful to supplement the assessment of pain responses (Morison *et al.*, 2003).

As mentioned before, several factors have been shown to have an influence on these behavioral responses: age/stage of development of the preterm infant, older infants showing more robust responses (Gibbins *et al.*, 2008a; Johnston *et al.*, 1993); behavioral state, alert infants being more responsive in facial actions than infants in the sleep state (Grunau & Craig, 1987); severity of illness, which is related to the characteristics of cry, although not to the incidence of cry (Stevens, Johnston, & Horton, 1994); number of previous painful procedures, which predicts a decrease in behavioral response (Johnston & Stevens, 1996).

2.4.2 Physiological responses

Physiological responses include endocrine and autonomic responses as well as cortical activity.

Endocrine responses

Stress hormones are released in mammalians in response to conditions that threaten homeostasis. Activation of the hypothalamic-pituitary-adrenocortical (HPA) axis results in the release of cortisol. This glucocorticoid and catecholamines (adrenaline and noradrenaline) released in the brain and peripherally, are critical in mounting a defensive response: glucocorticoids help to mobilize and distribute energy stores, influence the activity of the immune system and coordinate adaptive behaviors (Gunnar & Barr, 1998). The hormonal-metabolic response of preterm and term infants to stress caused by surgery has been reported as similar to, but greater than, that observed in adults (Anand, Hansen, & Hickey, 1990). The immediate release of stress hormones (catecholamines, cortisol, growth hormone, and glucagon) is one component of a global response directed to facilitate wound repair. The endocrine-metabolic response to stress caused by surgery can be blocked by anesthesia (Anand *et al.*, 1990; Anand & Hickey, 1992; Anand, Sippell, & Aynsley-Green, 1987). Persistence or severity of these metabolic changes, however, may result in increased morbidity and mortality (Fitzgerald & Anand, 1993).

Salivary cortisol has been used to evaluate stress produced by painful procedures and by routine care to preterm neonates in the NICU (Morelius, Hellstrom-Westas, Carlen, Norman, & Nelson, 2006; Gunnar, Hertsgaard, Larson, & Rigatuso, 1991). The use of salivary cortisol to measure pain reactivity poses several problems though: the amount of saliva is very small in preterm neonates and substances used to increase salivary secretion may act as potential confounders when studying the effect of interventions involving oro-gustatory stimulation. On the other hand, cortisol production follows a diurnal pattern and therefore, the time of day used for obtaining the samples has to be considered in the interpretation of the results when using cortisol as an acute pain marker (Stevens *et al.*, 2007).

In healthy newborns, repeated exposure to stressful stimulation modifies the functional activity of the HPA axis. The direction of this change (increase or decrease of the response) seems to vary with the aversive stimulus: a repeated medical discharge examination causes habituation, no elevation of cortisol being observed in the second examination, while a repeated heel lance produces the same or an increase in cortisol levels (Gunnar, Hertsgaard, Larson, & Rigatuso, 1991). On the contrary, in infants born at 29 weeks gestational age or less, higher procedural pain exposure was related to a lower cortisol response to the stress of nursing procedures at 32 weeks postconceptional age (Oberlander, Grunau, Fitzgerald, & Whitfield, 2002).

The release of neuropeptides such as β-endorphin has also been examined as part of the biochemical response to a painful event. β-endorphin is known to be a part of the descending inhibitory pain system. However, the interpretation of the presence of β-endorphin in the plasma, in cerebral spinal fluid and in the brain stem remains controversial and needs to be further explored until it can give a clear picture of its modulating role in pain perception (Bach, 1997).

Autonomic responses

As a result of the activation of the autonomic nervous system following a painful stimulation, an increase in heart rate, respiratory rate, blood pressure, palmar sweating and intracranial pressure, and a decrease in oxygen saturation and vagal tone is often found (Sweet & McGrath, 1998).

Heart rate. Most often measured by the number of heart beats per minute, heart rate (HR) is a result of autonomic activity. It is higher in awake states than in sleep states (Stevens & Johnston, 1994). Age is also a significant factor: in preterm infants, heart rate decreases as gestational age approaches term and continues to decrease until the age of 10.

Because the control of the cardiovascular function in the brainstem is closely

linked to systems that modulate pain reactivity, changes in cardiorespiratory parameters can be observed in infants undergoing a painful procedure. Heart rate was found to significantly increase during the most aggressive phases of circumcision (Marchette, Main, Redick, Bagg, & Leatherland, 1991).

Although heart rate is commonly used as a pain indicator it should be regarded as an index of reactivity and not as a specific measure of pain response (Oberlander & Saul, 2002).

Heart rate variability. Heart rate is under the influence of the balance between sympathetic and parasympathetic systems. Sympathetic fibers action over the sinoatrial node accelerates heart rate while parasympathetic action (through the vagus nerve) decelerates it. The dominance of sympathetic and vagal systems on heart rythmicity varies during the respiratory cycle: during inspiration, there is a decrease in vagal influence, thereby increasing heart rate; and during expiration, vagal influence is predominant, decelerating heart rate. In the presence of pain or other stressful stimuli, parasympathetic influence on heart rate decreases in favor of sympathetic activity, reducing heart rate variability as part of a global response of arousal that would permit escape from threat.

This heart rate variability (HRV), also known as respiratory sinus arrhythmia (RSA), is too subtle to be detected by counting the heart beats per minute on the pulse, with a stethoscope or with a cardiac monitor. On the electrocardiogram though, this variability can be analyzed by measuring the R to R intervals (time in milliseconds between successive R waves in the electrocardiogram).

There are several approaches to the analysis of HRV. The variance associated with RSA, after filtering to remove aperiodic trends and periodic heart rate patterns slower than the respiratory frequency, followed by logarithmic transformation, has been termed Vagal Tone index by Porges (1995). Increased vagal tone, measured in log units, is considered a sign of CNS integrity, as the capacity to modulate heart rate in response to a variety of stimuli is a predictor of better developmental outcomes in very low birth weight infants at 3 years of age (Doussard-Roosevelt, Porges, Scanlon, Alemi, & Scanlon, 1997).

Two other approaches have been commonly used in studies of interventions to reduce pain: time domain and frequency domain analysis (Oberlander & Saul, 2002). Time domain analysis considers the total population of RR intervals and yields descriptive data such as the mean, standard deviation and variance of RR intervals. It is appropriate for analyzing long periods of time. Frequency domain analysis or spectral analysis of heart rate variability can be used to quantify changing levels of cardiac autonomic

modulation by quantifying the characteristic fluctuations of periodic rhythms of HR as a function of the frequency of these fluctuations (Morison, Grunau, Oberlander, & Whitfield, 2001). Two frequency ranges are of importance in examining the influence of vagal activity: frequencies> 0.15 Hz (high frequency, HF) are the result of vagal dominance; whereas frequencies< 0.15 Hz (low frequency, LF) are under the influence of both sympathetic and vagal activity. The HF component of HRV is therefore accepted as an index of parasympathetic activity (Oberlander & Saul, 2002). The link between LF and sympathetic activity has not been demonstrated. Yet, the ratio LF/HF is used as an index of "sympathovagal balance" (Oberlander & Saul, 2002, p. 430), an increased ratio suggesting an increased sympathetic cardiac modulation, decreased parasympathetic modulation or both. Dissimilar experimental designs and methods of analysis as well as lack of standardized outcomes make it difficult to compare between studies using HRV as a pain indicator, therefore preventing the use of HRV as a bedside pain indicator in clinical settings.

A number of variables have been associated with HRV, such as very low birth weight, gestational age, behavioral state and health status. Healthy infants diagnosed as small for gestational age were found to have lower HRV than appropriate-for-gestational age controls in one study (Galland, Taylor, Bolton, & Sayers, 2006) and the predominance of sympathetic influence on heart rate persisted in infants 11-12 weeks old born with intrauterine growth retardation (Massin, Withofs, Maeyns, & Ravet, 2001). With increasing GA, there seems to be a shift toward increasing parasympathetic modulation (Harrison *et al.*, 2006a; Morison *et al.*, 2001), although when reaching the age of term infants, preterm infants still have a lower HRV (Rosenstock, Cassuto, & Zmora, 1999). Behavioral state is another factor that has been studied in relation to cardiac modulation, a stronger vagal influence being observed during quiet sleep (Rosenstock *et al.*, 1999). Respiratory distress syndrome, birth asphyxia, intra-ventricular hemorrhage and patent ductus arteriosus have also been related to attenuated HRV (Rosenstock *et al.*, 1999). These factors need to be taken into consideration when using HRV as a measure of pain reactivity in preterm and ill neonates.

Respiratory rate. The number of breaths taken in a given time period obtained through direct observation or cardio-respiratory monitoring is the usual measure of respiratory rate. In the presence of pain, an increase in respiratory rate is reported in some studies (Stevens, Johnston, & Grunau, 1995) and a decrease is reported in others (Craig *et al.*, 1993). As an autonomic response, respiratory rate increases in wake states but the freezing reaction to a strong stimulus perceived as threatening may explain the decrease

of respiratory rate. Studies of analgesics and comforting measures to reduce pain have produced mixed results or have found no difference in respiratory rate between interventions (Abad, Diaz, Domenech, Robayna, & Rico, 1996), therefore making it difficult to use respiratory rate as a pain indicator (Sweet & McGrath, 1998).

Blood pressure. Blood pressure is also related to autonomic functioning and has been reported to increase during pain episodes (Sweet & McGrath, 1998). Although non-invasive methods are available and commonly used in clinical settings to measure blood pressure as a vital sign, they have not been frequently used for research on pain responses. A possible explanation is that the inflation of the cuff is in itself a stimulation that might alter blood pressure values.

Oxygen Saturation. Like heart rate, oxygen saturation is considered an index of reactivity, not a specific indicator of pain (Stevens *et al.*, 2007). In the presence of a painful stimulus, a decrease in oxygen saturation may be found compared to baseline values. While some studies of pain relieving interventions like Codipietro and collaborators study on breastfeeding (Codipietro, Ceccarelli, & Ponzone, 2008) report a significantly smaller decrease in oxygen saturation in the experimental group, other studies, like sucrose studies analyzed in a systematic review (Stevens *et al.*, 2010) have found no significant variations. Yet, changes in oxygen saturation are included in several composite measures of neonatal pain as will be described below.

Palmar sweating. The activity of the sympathetic nervous system on sweat glands of the hand palm and foot sole is influenced by changes in arousal and by emotions. A release of acetylcholine, which acts on muscarine receptors of sweat glands, causes a burst of sweat, increasing skin conductance. Palmar sweating, also known as skin conductance activity, has been reported as an objective response to heel lance in infants 29 weeks gestational age and more (Storm, 2000). Palmar or plantar sweating is currently measured through fluctuations in skin conductance. Although still limited to research, its use as a potential clinical indicator of pain is regaining increasing interest (Harrison *et al.*, 2006a; Eriksson, Storm, Fremming, & Schollin, 2008; Storm, 2008).

Intracranial pressure. In neonates, intracranial pressure can be measured non-invasively by placing a probe on the anterior fontanel (Stevens & Johnston, 1994) but it is not currently assessed for clinical purposes. It has been reported to increase during the painful phases of heel lance and to correlate significantly with other measures such as maximum heart rate and minimum oxygen saturation (Johnston, Stevens, Yang, & Horton, 1995).

Cortical activity

Painful stimuli are associated with circulatory and metabolic changes in specific areas of the cortex and subcortex (Bartocci, Bergqvist, Lagercrantz, & Anand, 2006). This response is measured as an increase in total hemoglobin concentration in the somatosensory areas of the brain cortex (Slater *et al.*, 2006) and can now be measured using near-infrared spectroscopy, a recent non-invasive method. Major hemodynamic-oxygenation changes in the brain also occur during routine caregiving procedures in critically ill preterm infants, even though they are not detected by usual bedside monitoring (Limperopoulos *et al.*, 2008). Tactile and noxious stimulation elicit specific changes in infants as young as 28 weeks gestational age (Bartocci *et al.*, 2006).

Several factors seem to affect the cortical response to noxious stimuli: awake infants have larger cortical responses to noxious stimulation (Slater *et al.*, 2006) which may help explain why behavioral responses are dampened during sleep states; early gestational age infants and boys show a stronger response while postnatal age is correlated with pain-induced cortical activity (Bartocci *et al.*, 2006).

In summary, neonates of any gestational age are able to mount and regulate an immediate or short-term response to stressful and painful stimuli. This response can be seen at different levels: in behaviors, changes in autonomic functioning and biochemical processes and in the activity of the cortex. The main factors that affect these responses have been mentioned: gestational age, postnatal age, clinical condition, behavioral state and the extent of exposure to stress and pain. The reactivity to painful stimuli also seems to be related to individual characteristics that will later in life be expressed by temperamental features (Klein, Gaspardo, Martinez, Grunau, & Linhares, 2009).

2.5 Neonatal pain assessment tools

A systematic assessment approach with valid and reliable tools is needed both for research purposes and for the clinical management of pain. Measurement of the intensity is just one component of assessment, which is more global and requires tools that are valid, reliable and sensitive (Stevens, Johnston, & Grunau, 1995).

Because the above-mentioned definition of pain presupposes the capacity for self-report, Anand and Craig (1996) have suggested that a broader definition of pain should be considered for people who cannot communicate verbally, such as young children, who are unable to describe their experience although they seemingly have pain. They propose that "the behavioral alterations caused by pain are the infantile forms of self-report and should not be discounted as 'surrogate measures' of pain" (Anand & Craig,

1996, p. 5). Again, the need to respect the infants' development-related capacities to communicate is stressed.

Considering the pain responses described above which are exhibited by infants under a painful condition, efforts have been made to identify indicators that are sensitive and specific when proposing measures of pain intensity. Facial actions seem to be more useful than cry or body movement, and are more specific than physiologic changes which are general stress responses and therefore cannot, in an isolated way, be used as specific indicators of pain. Importantly, consistency between behavioral and physiological responses is limited (Lucas-Thompson *et al.*, 2008; Stevens *et al.*, 1995).

The pertinence of combining behavioral and physiologic indicators to measure pain intensity is sustained by the fact that the pain response of infants is also multidimensional in nature. A review of 46 research reports between 1941 and 2001 contributing to knowledge about pain measurement was conducted by Warnock and Lander (2004). The authors found that these studies used one or two of the following categories of indicators of neonatal pain: physiological, biochemical and behavioral. Behavioral indicators were used in 38 studies, physiological indicators in 23 studies and biochemical indicators in 8 studies. Only one study used all three categories of indicators.

Composite measures, i.e. multidimensional measures that combine more than one type of indicator, are therefore considered to be more reliable than the use of unidimensional indicators (cry only or even multidimensional tools using only one type of indicator (e.g., several indicators of facial action) (Stevens *et al.*, 2007; Franck & Miaskowski, 1997) and an effort has been made to develop such measures. In addition, contextual factors that may modify the response (e.g. gestational age, behavioral state, severity of illness) need to be considered when measuring pain (Johnston, Stevens, Craig, & Grunau, 1993).

In a systematic review of infant pain assessment tools conducted in 2004, seventeen unidimensional and 18 multidimensional tools were found in the literature (Duhn & Medves, 2004). The unidimensional tools tended to focus on infant behavior and movement. The multidimensional pain assessment tools combined behavioral, physiological indicators and sometimes contextual indicators. Psychometric property testing of these tools, however, was found to be variable with some tools having no testing at all.

A presentation of infant pain assessment tools is beyond the scope of this review but the tool that has been used most often in studies on neonatal pain, the Premature Infant Pain Profile (PIPP), will be addressed.

The Premature Infant Pain Profile (PIPP) is a composite measure of pain that

consists of three behavioral indicators (brow bulge, eye squeeze, nasolabial furrow), two physiological indicators (heart rate and oxygen saturation), and two contextual indicators (gestational age and behavioral state) (Stevens *et al.*, 1996). All the indicators are assessed at baseline, through observing the infant for 30 seconds. During the procedure, the PIPP score is computed by blocks of 30 seconds. In each block, heart rate is scored for the increase of maximum heart rate from baseline in beats per minute, oxygen saturation is scored for the decrease from baseline, and facial actions are scored for the percentage of time they are present. Each indicator is scored on a 4-point scale (0-3), to obtain a total pain score between 0 and 21. Scores of 6 or less indicate minimal or no pain, scores between 6 and 12 indicate mild pain and scores over 12 indicate moderate to severe pain. The PIPP was developed as a measure of pain in preterm infants. Its initial validation was performed in a large sample of preterm infants of various gestational ages, undergoing heel stick in three settings. The development process included selecting physiological, behavioral and contextual indicators, evaluating their sensitivity and specificity, determining the factor structure of the indicators and establishing internal consistency and construct validity. Fifteen indicators were selected from previous studies about infants' responses to pain (Johnston *et al.*, 1995; Stevens & Johnston, 1994; Craig, Whitfield, Grunau, Linton, & Hadjistavropoulos, 1993; Grunau, Johnston, & Craig, 1990). Each of these indicators was evaluated for sensitivity (indicator is present in painful situation) and specificity (indicator is not present in non-painful situations). Indicators were considered sensitive if present at least 50% of time during a tissue-damaging stimulus and specific if present less than 20% of time during non-painful situation such as baseline or heel warming. Based on these criteria, the 15 indicators were reduced to 7. Principal components analysis demonstrated a three factor structure of the indicators. The physiological and behavioral indicators were then categorized according to their distribution in the data set, to facilitate scoring by clinicians. Contextual indicators (gestational age and sleep state) were categorized based on the results of studies describing the pain responses of preterm infants to painful stimuli and the modifying factors of these responses. Cronbach's Alpha coefficients were calculated and item-total correlations ranged from 0,59 to 0,76 showing a moderate internal consistency and suggesting that the indicators were related but not redundant. Finally, construct validity was established by using the PIPP to measure pain in painful and non-painful situations (Stevens *et al.*, 1996). Further studies have demonstrated the clinical utility of this scale (McNair, Ballantyne, Dionne, Stephens, & Stevens, 2004; Ballantyne, Stevens, McAllister, Dionne, & Jack, 1999) and the PIPP has been widely used in studies of pain

in infants (Freire, Garcia, & Lamy, 2008; Johnston *et al.*, 2008a; Johnston *et al.*, 2008b; Carbajal *et al.*, 2005; Johnston *et al.*, 2003; Gibbins *et al.*, 2002; Stevens *et al.*, 1999; Johnston *et al.*, 1999),

A more recent study analyzing the structure of pain response in vulnerable infants confirms the consistency of the underlying structure of the PIPP (Stevens *et al.*, 2007).

When trying to quantify the patterns of response to a painful event, it is useful to examine the different components of this response: the reactivity (change from baseline), the intensity (magnitude of the change), the direction (increase or decrease of the variable under study), the regulation (change from pain to recovery) and the slope (tendency to up regulate or down regulate the response) (Stevens *et al.*, 2007). The consideration of these different components is of utmost importance to understand and quantify the responses when several interventions to control pain are being compared (Figure 3).

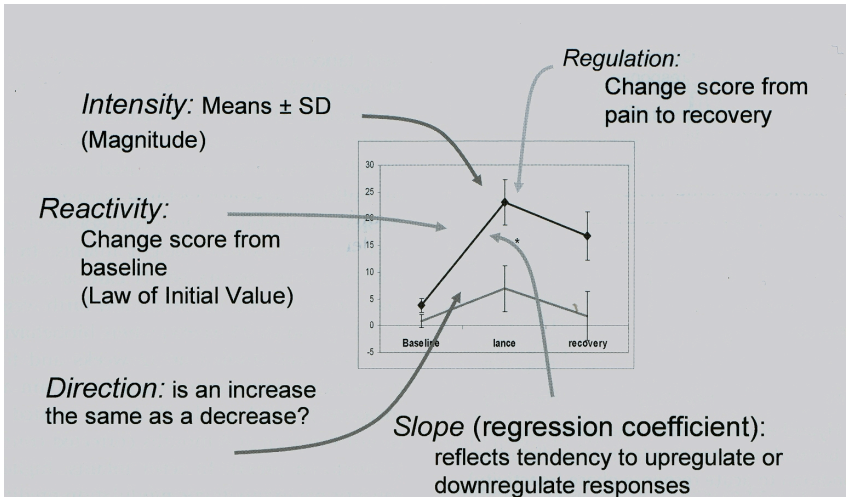

Figure 3. Hypothetical reactivity patterns comparing responses in Group A vs Group B. Patterns include measures of: Intensity (magnitude), Reactivity, Regulation, Direction and Slope (Oberlander, 2005, not published). Note: From *Pain in neonates and infants* (p. 78), by KJS Anand, B. Stevens, & P. McGrath (Eds.), 3rd edition, 2007, Edinburgh: Elsevier. Copyright 2007 by Elsevier BV. Reprinted with permission.

2.6 Long-term consequences of early pain exposure

A vast amount of literature has examined the health and social outcomes of former preterm infants in early adulthood. It is well established today that preterm neonates, especially those with low birth weight, are at risk for long-term alterations in cognitive, motor and social development, self-regulation of stress-arousal systems, pain sensitivity and pain-related behaviors. These consequences are related to stressful events in the neonatal period that include, among others, exposure to the environment of

Neonatal Intensive Care Units, maternal separation, and pain. These stressful experiences occur in a critical period of time for brain development and therefore shape the central nervous system both structurally and functionally (Fitzgerald & Anand, 1993).

Grunau (2002) proposes a model for the long-term effects of pain that takes into consideration the neonate in the NICU and the way pain is managed which influences its reactivity and arousal in the NICU and pain in infancy and childhood; these will have a mutual influence with neurodevelopment. Important factors in this model are parent/caregiver interaction with the child, parent and family context as well as medical complications in the pre and postnatal period and later impairments.

Neurodevelopment

The impact of prematurity on later cognitive performance and behavior of children has been under analysis for the past 30 years. In a meta-analysis including 1556 formerly preterm school-aged children and 1720 controls (Bhutta, Cleves, Casey, Cradock, & Anand, 2002) the authors found that controls had significantly higher cognitive scores compared with children who were born preterm. The mean cognitive scores of preterm-born cases and term-born controls were directly proportional to their birth weight and gestational age. Preterm-born children had more than twice the relative risk for developing attention deficit and hyperactivity disorder (ADHD). The authors concluded that children who were born preterm are at risk for reduced cognitive test scores and their immaturity at birth is directly proportional to the mean cognitive scores.

Factors suggested to underlie these developmental differences include the higher risk of preterm infants for postnatal complications such as sepsis, lung disease, metabolic complications, exposure to multiple painful procedures and maternal separation for long periods. Combined evidence from animal models and clinical studies suggest that all these factors can promote increased neuronal cell death and volumetric losses in critical brain regions that would explain poor cognitive and behavioral outcomes during childhood and adolescence. Perinatal traumatic events have also been identified as risk factors related to adult self-destructive behavior (Anand & Scalzo, 2000).

Self-regulation of stress-arousal systems

Repeated exposure to stressful situations causes repetitive activation of the HPA axis and above normal levels of plasma cortisol. Animal models support the notion of lifelong influences of early experience on stress hormone reactivity (McEwen, 2000). High levels of cortisol in rats have shown to cause hypervigilance, fear behaviors and activation of the production of catecholamines. These are accompanied by structural

changes in the hippocampus, shrinking of dendrites and facilitation of processes that lead to cell death, thus impairing future regulation of the HPA axis and cognitive functions that depend on the hippocampus (Gunnar & Barr, 1998).

Animal studies also suggest changes in stress-related behavior in relation to neonatal pain. Adult rats exposed to repetitive neonatal needle-pain show defensive withdrawal, with increased latency for exploration of an open field and spend more time in the shelter (Anand, Coskun, Thrivikraman, Nemeroff, & Plotsky, 1999); adult rats exposed to neonatal visceral pain have decreased exploratory activity, confining themselves to a limited area (Sternberg & Al-Chaer, 2007).

In humans, increased prevalence of withdrawal, social difficulties, anxiety and depression among very low birth weight children suggests that, as adults, they may be at increased risk for psychopathology although for the moment, this remains an hypothesis only (Grunau, 2003). Human studies have examined basal functioning and responses of the HPA axis to stress situations, in order to determine the influence of neonatal experiences on stress-arousal systems. At 3 months, levels of basal cortisol were lower in extremely low gestational age infants (23-28 weeks) and in very low gestational age infants (29-32 weeks) compared to term infants; conversely, at 8 and 18 months corrected age, levels of basal cortisol of extremely low gestational age infants (ELGA) were higher than those of the other two groups ($p = .016$ and $p = .06$, respectively) (Grunau *et al.*, 2007). The authors suggest that this reflects a "resetting" of endocrine stress systems and that the elevation of the cortisol "set-point" may have negative implications on later developmental and health outcomes (Grunau *et al.*, 2007, p. 155). Arousal is seen as a state of central nervous system regulation (Grunau, 2003) and the capacity to regulate arousal varies with individual and contextual factors. Reaction to novelty can be used as a marker of arousal regulation. At 8 months, ELGA preterm infants show a different pattern of cortisol levels before and after positive stimulation of visual novelty than very low gestational age preterm and term-born infants and display poorer self-regulatory behaviors during novel tasks during standardized cognitive assessment, compared with term-born children. Again, exposure to high numbers of skin-breaking procedures is presented as a possible explanation for the "resetting" of basal arousal systems in preterm infants (Grunau, Weinberg, & Whitfield, 2004).

Pain sensitivity

Long-term alterations in pain sensitivity are induced by the need of continual adaptation of a physiological and neurobehavioral immature system to repeat-

ed stress. Changes in pain sensitivity during the first year of life have been examined (Abdulkader, Freer, Garry, Fleetwood-Walker, & McIntosh, 2008). The threshold for the flexion withdrawal reflex to cutaneous mechanical stimulation of the heel remained significantly lower in infants born preterm throughout the first year following birth compared to fullterm infants. The preterm infants showed no apparent change in sensitivity, but the fullterm infants showed a reducing level of sensitivity during the first year (Abdulkader *et al.*, 2008).

In a cohort study of children 9 to 14 years old who were born preterm (< 31 weeks) or fullterm and had received neonatal intensive care for three or more days, Hermann and colleagues found that compared to their fullterm counterparts with no intensive care experience, these children displayed enhanced perceptual sensitization to prolonged painful stimulation and hypoalgesia to brief heat pain stimuli, suggesting beyond infancy changes in the functioning of pain pathways (Hermann, Hohmeister, Demirakca, Zohsel, & Flor, 2006).

In a longitudinal study, former preterm children 7 to 11 years old had different physiological responses and thermal sensitivity to conditioning cold stimulation, compared to their fullterm counterparts, suggesting that their endogenous pain modulatory mechanisms were not as well developed as those of children not exposed to noxious stimuli at birth. Greater frequency of painful procedures seemed to be related to a smaller rise in heart rate than is normally observed (Goffaux *et al.*, 2008).

Neurobiological studies on the other hand, free from many of the confounding variables encountered in behavioral studies, confirm that at cellular level, there are changes in sensory connections which are not evident in behavior tests (Fitzgerald & Walker, 2009).

Pain-related behaviors

One of the first studies to examine the effects of pain in the neonatal period on later pain responses was conducted by Taddio and associates (Taddio, Goldbach, Ipp, Stevens, & Koren, 1995). Observing the responses to immunization at 4 or 6 months of age of boys that had circumcision without analgesia and comparing them to infants uncircumcised and circumcised with topical analgesia, the authors concluded that circumcised infants displayed a stronger pain response than uncircumcised infants and that among the circumcised group, those that had preoperative topical analgesia showed attenuated pain responses to immunization (Taddio *et al.*, 1995; Taddio, Katz, Ilersich, & Koren, 1997). This suggests that neonatal pain impacts on later pain response, namely facial action and cry duration, and that appropriate pain management may reduce this

impact. The same conclusion emerges from the study of a cohort of 50 infants that had undergone major surgery in combination with an appropriate and standardized analgesic protocol within the first 3 months of life. Compared to controls at 14 and 45 months of life, the pain responses to immunization of these infants treated for pain were no different (Peters *et al.*, 2003).

Contrasting with the studies that point to a stronger pain response from children with early pain experience, parents of extremely low birth weight toddlers at 18 months rated the pain sensitivity of their children to everyday bumps and scrapes lower than parents of former heavier preterms and fullterm controls (Grunau, Whitfield, & Petrie, 1994).

Yet, greater somatisation (unexplained stomach aches, headaches, leg pains, and other somatic concerns) occurred in former preterm infants at 4 ½ years of age (Grunau, Whitfield, Petrie, & Fryer, 1994). The combination of family relations, neonatal intensive care experience, poor maternal sensitivity at 3 years of age and child avoidance of touch or holding at age 3 were predictive of somatisation scores prior to school entry. However, at 9 years (Grunau, Whitfield, & Petrie, 1998) and 17 years (Grunau, Whitfield, & Fay, 2004) the prevalence of somatization at clinically significant levels did not differ among ELBW children compared with term-born children.

Comparing pain catastrophizing of former preterms and fullterms with NICU experience to fullterm controls (Hohmeister, Demirakca, Zohsel, Flor, & Hermann, 2009) the scores of preterms but not fullterms were significantly higher ($p = .02$ and $p = .69$, respectively). In children with NICU experience, pain catastrophizing was significantly correlated with illness severity/mortality risk ($r = .39$, $p = .02$) and duration of hospitalization ($r = .33$, $p = .048$).

Children's judgements about pain have also been examined. Former extremely low birth weight (ELBW) infants 8 to 10 years old and full birth weight (FBW) controls were asked to rate pictures of children in pain situations related to medical, recreational, daily living, and psychosocial events, rated pain intensity using the Color Analog Scale and pain affect using the Facial Affective Scale. The two groups of children did not differ overall in their perceptions of pain intensity or affect, but within-subject differences were found: the ELBW children rated medical pain intensity significantly higher than psychosocial pain, unlike the FBW group (Grunau *et al.*, 1998).

The findings of these studies must be read taking into consideration multiple possible confounders: infants born preterm may have conditions requiring later hospitalizations during infancy that may account for their overall pain experience, neonatal experiences being impossible to isolate. Also, parents' ratings may be influenced by their experience of previous pain in their infants, leading them to undervalue daily life pain.

Maternal factors

Caregiver-infant interaction seems to play a very important role in mediating consequences of early exposure to adverse events later in life.

In former preterm infants at 8 months corrected age, maternal factors such as self-reported stress and interactive behaviors buffered the relationship between high neonatal pain-related stress exposure and poorer focused attention, frequently found in these infants, while in infants exposed to high concurrent maternal stress and overwhelming interactive maternal behaviors, higher basal cortisol levels were associated with poor focused attention (Tu *et al.*, 2007). These findings suggest the importance of maternal factors in shaping the cognitive outcomes of preterm infants.

Mothers of 9 to 14 year-old preterm-born children, who had been more severely ill and had been hospitalized longer than fullterm NICU children, were more likely than mothers of fullterms with no NICU experience to engage in solicitous pain-related behavior that reinforced the child's pain response (Hohmeister, Demirakca, Zohsel, Flor, & Hermann, 2009). In the same study, heat pain thresholds and perceptual sensitization to tonic painful heat obtained in the presence versus absence of the mother showed that maternal presence was associated with increased heat pain thresholds in all three groups. Regarding habituation, control children habituated significantly more to tonic heat when their mother was present but NICU children showed overall significantly less habituation than controls and no modulating effect of maternal presence. The authors suggest that neonatal pain exposure and prolonged hospitalization may, aside from neuronal plasticity, promote maladaptive pain related cognitions, namely perceptual sensitization (Hohmeister *et al.*, 2009).

Mechanisms

At the neuronal level, the long-term permanent response of newborns to peripheral injury is thought to be similar to that observed in animal studies: a structural and functional reorganization of the central nervous system that alters the final adult pattern of connections resulting in permanent altered sensation (Fitzgerald & Anand, 1993). Anand and Scalzo (2000) suggest that there are complex interactions between early neonatal experience and the gene products that control cellular and neurotransmitter development in the brain. This may alter the structure of receptors and the biochemical mechanisms responsible for the capacity for learning, memory and vulnerability to psychiatric disorders. The authors stress that exposure to repetitive neonatal pain may promote an increased susceptibility to chronic pain states. The plasticity of the brain in the neonatal period is such that "disruptive experiences at this time may have a greater impact on subsequent neurobiological and behavioral development" (p.7).

Considering the normal processes that regulate early brain development and the plasticity of the neonatal brain, Anand and Scalzo (2000) propose mechanistic hypotheses that provide a rationale for these phenomena (Figure 4). Maternal separation and lack of appropriate stimulation, on one hand, lead to a lack of N methyl D-aspartate (NMDA) activity, consequently increasing programmed brain cell death, which in turn will have an impact on cognitive and behavioral development; repetitive or prolonged pain, on the other hand, produces excitotocic damage from increased NMDA activity, that will also affect behavior and cognition. Both groups of factors lead to long-term adverse neurological outcomes.

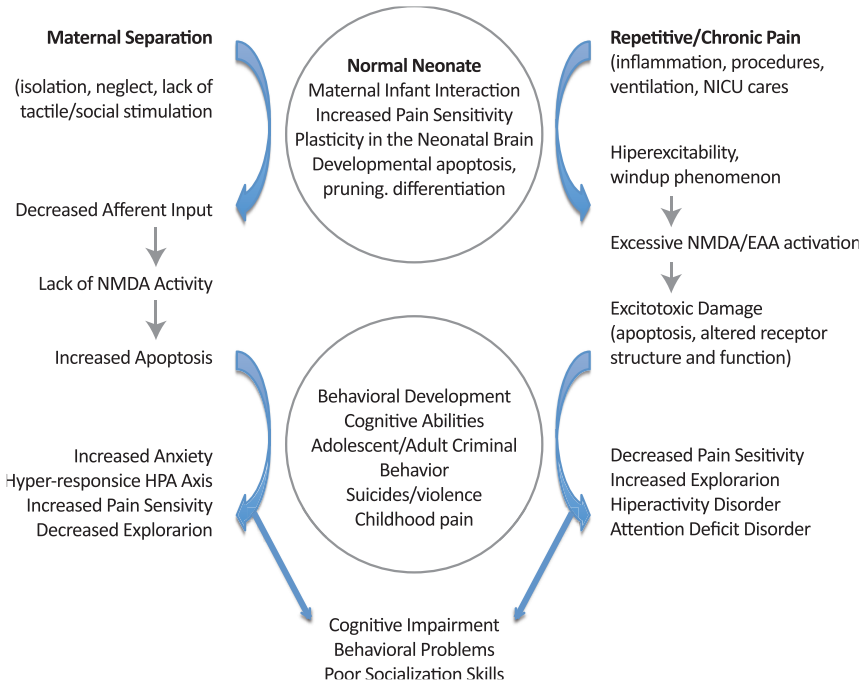

Figure 4. Neonatal factors leading to long-term adverse neurologic outcomes. NMDA: N methyl D- aspartate; NICU: Neonatal Intensive Care Unit; EAA: Excitatory Amino Acid; HPA: Hypothalamic-Pituitary-Adenocortical axis. Note: From "Can adverse neonatal experiences alter brain development and subsequent behavior?" by Anand, K. J. & Scalzo, F. M., 2000, *Biol.Neonate, 77,* 69-82, Copyright 2000 S. Karger AG. Reprinted with permission.

The type and extent of the consequences of early exposure to pain will depend on genetic variability as well as the developmental maturity of the infant at the time of the painful events, associated clinical factors, the length and extent of exposure to pain, and multiple environmental influences at the time of pain exposure and ongoing during development (Grunau & Tu, 2007; Anand & Scalzo, 2000). To determine the contribution of early pain exposure and pain management to later outcomes, the roles of these multiple interacting factors must be examined (Grunau, 2002).

CHAPTER 3.
Pain exposure in the NICU environment

CHAPTER 3. Pain exposure in the NICU environment

During admission in a NICU, infants are exposed to a hostile environment in terms of light and noise as well as to diagnostic and treatment procedures that require handling and often cause stress and pain. The more sick and young the infants the more intensive is the care they get (Barker & Rutter, 1995).

In spite of great improvements in making the intensive care environment more suitable to the developmental and emotional needs of sick infants, the picture presented 30 years ago by Lucey (1977) has not totally disappeared:

> Picture yourself in a brightly lit room, nude, defenseless, and your eyes hurting from silver nitrate. You are blindfolded, chilly, and surrounded by a tepid fog. You're gasping for air fighting to breathe, and choking and gagging every so often on mucus. You're unable to clear your throat or cough. A mask is placed over your face, and blasts of air are forced into your lungs. Somebody sticks a catheter into your mouth, occasionally too far, causing you to retch or vomit. You're startled and frightened by loud, strange noises (beepers, voices, roaring respirators, telephones, radios, incubator noise). Some giant is pouring food into a tube which has been forced through your nose or throat into your stomach. It's uncomfortable and obstructs your nasal airway. You're probably nauseated; you're certainly not hungry, but you are expected to eat - and soon.
>
> You have a headache, probably the worst one of your life. You are sleep deprived. Every time you doze off, somebody gets worried about you. They think you're in a coma. You have to be very careful to breathe very regularly. You're not allowed the multiple long pauses (15 seconds or more) of a sleeping, dreaming adult. If you do pause, a bell goes off, waking you up, and somebody slaps your feet or pulls your hair to see if you will or can cry. If you're exhausted or unresponsive, you're in trouble. If you have any jerky

movements, you're suspected of having a convulsion.

Every few hours somebody cuts your foot or sticks a needle into your scalp or one of your arteries. Your arms and legs are taped down to boards. Electrodes are attached to your chest. You're immobilized. You may even have an itch, but you can't scratch. Cool, rude hands probe your abdomen every so often, feeling for your liver, kidneys, or bladder. After a few days of this "intensive" care you're exhausted and you may need assistance to continue breathing just because you're too tired to do it on your own. (Lucey, 1977, p. 1064-1065)

Pain can also occur as a result of certain clinical conditions that develop at birth such as fractures or cephalematome and during the course of the weeks of hospitalization such as necrotizing enterocolitis (NEC). These conditions, as well as surgery and mechanical ventilation can cause persistent pain that requires appropriate assessment and management. In this review however, we shall focus only the frequency and management of procedures that are time defined.

3.1 Epidemiology of pain in the NICU

A few studies have examined the frequency of painful procedures in infants admitted to NICUs and the interventions most commonly used to reduce pain. However, several difficulties arise in trying to compare the results of these studies, related to differences in definitions, design of studies, reported outcomes and sample characteristics.

First of all, among the vast number of procedures performed in neonatal intensive care, which are indeed painful? Procedures that are invasive are usually considered to be painful (Porter & Anand, 1998; Barker & Rutter, 1995), which is probably why Johnston and colleagues have only reported invasive procedures (Johnston, Collinge, Henderson, & Anand, 1997). However, Simons and associates (2003) have included invasive and non-invasive procedures on their checklist of 34 painful procedures, based on experts' opinion. Stevens and colleagues too, have included tissue-damaging and non tissue-damaging procedures as painful procedures (Stevens *et al.*, 2003). Carbajal and colleagues give a conceptual definition, reporting that they considered a procedure was painful if it "invaded the neonate's bodily integrity, causing skin injury or mucosal injury from the introduction or removal of foreign material into airway or digestive or urinary tract" (Carbajal *et al.*, 2008, p. 61).

Nonetheless, Carbajal and team (2008), like Simons and colleagues (2003) and Stevens and colleagues (2003) chose to extend that definition and include in their study

procedures that were deemed painful by the staff, such as physiotherapy. Barker and Rutter (1995) enhance that most invasive procedures they surveyed involved tissue injury and Carbajal and colleagues (2008) refer that 83.4% of painful procedures were invasive. In summary, there seems to be a consensus that the concept of invasive procedure includes tissue damage as well the penetration of body cavities (e.g. gastric tube insertion, bladder catheterization) but that the concept of painful procedure is wider, including invasive procedures and procedures that are potentially skin-breaking, like adhesive removal, or require the infants manipulation, such as physiotherapy or positioning for X-ray.

The total number of procedures per infant is most often reported as a mean per day. It varies between 2 per infant per day (Johnston *et al.*, 1997a) and 14.78 per infant per day (Stevens *et al.*, 2003). Maximum number of procedures endured in average by one infant during one day has been reported as diverse as 6.9 (Benis & Suresh, 2001), 8 (Johnston *et al.*, 1997a) and 53 (Simons *et al.*, 2003).

The procedures more frequently performed are heel lance (7.1% - 87%), endotracheal suctioning (23% - 26%) and IV cannula insertion (1.4% - 21%). Together, these three interventions represent 90.25% of all studied procedures in one study (Barker & Rutter, 1995) and 33.9% in another (Simons *et al.*, 2003), and ten years after Barker and Rutter (1995), they correspond to 44.5% of all procedures in the study of Carbajal and team (2008).

Tracheal suctioning, one of the procedures often performed, has a similar incidence across studies. It represents 26% in Barker & Rutter (1995), 23,3% in Carbajal and associates (2008) and 23% in Simons and collaborators (2003). Adding nasal suctioning, suctioning accounts for more than half of the procedures in three studies: 56.9% (Batalha, Santos, & Guimarães, 2007), 54,2% (Simons *et al.*, 2003) and 52.2% (Carbajal *et al.*, 2008). Benis and Suresh (2001), on their side, report suctioning without specifying the type as 51% of all procedures This similarity is curious though, given that the percentage of ventilated babies is very different among these studies: 24% in Barker, 49.6% in Simons, and 70.5% in Carbajal, and it would be expected that ventilated babies are suctioned more often that non-ventilated babies.

Of particular interest is the fact that the incidence of heel lance, the most frequent procedure in the studies of the 1990's, representing 87% of the procedures in a US study (Porter & Anand, 1998), seems to have fallen in this decade to 46% in Canada (Johnston, Barrington, Taddio, & Carbajal, 2008), 39% in Brazil (Prestes *et al.*, 2005), 20% in France (Carbajal *et al.*, 2008), 7% in The Netherlands (Simons *et al.*, 2003) and 5% in Italy (Cignacco *et al.*, 2009). This may reflect an increased aware-

ness that a judicious prescription of procedures is the first step to reduce pain, and that international recommendations about pain management in neonates (American Academy of Pediatrics, Committee on Fetus and Newborn and Section on Surgery, Canadian Paediatric Society, & Fetus and Newborn Committee, 2006) as well as evidence produced since 1998 about venepuncture being less painful than heelstick (Shah & Ohlsson, 2007) are being followed. The lower incidence of heel lance in European compared to North and South American studies may instead represent a different professional culture regarding routine heel lance.

A summary of the studies examining the frequency of procedural pain in neonates is presented in Table 1.

Table 1 - Studies examining the frequency of procedural pain in neonates

Study	Country Number of sites	Nº of Infants	Gestacional age (weeks)	Duration of observation	Total nº painful procedures	Nº painful procedures (mean)	Most frequently performed
Barker & Rutter (1995)	United Kingdom Single centre	54	23-41	Total NICU stay	3283	60.8 per infant	Heel lance (56%)
Johnston *et al.* (1997)	Canada Multicentre	239	23-42	First 7 days	2134	2/infant/day	Heel lance (60,8%)
Porter & Anand (1998)	United States Single centre	144	32-38	Total NICU stay	7672	53.3/infant	Heel lance (87%)
Benis & Suresh (2001)	United States Single centre	15	24-32	Total NICU stay	5663	332/infant *MD*= 6/ infant/day	Suctioning (51%) Skin puncture (38%)
Stevens *et al.* (2003)	Canada Multicentre	194	30-41	First 7 days	N.R.	5.22– 14,78 per infant/ day*	Suctioning
Simons *et al.* (2003)	The Netherlands Single centre	151	25-42	First 14 days	19674	14.3/infant/ day	Suctioning (63.6%)
Prestes *et al.* (2005)	Brazil Multicentre	91	25-41	1 month observation	3663	3,57/infant/ day	Heel lance (39.3%)
Carbajal *et al.* (2008)	France Multicentre	430	24-42	First 14 days	42413	12/infant/ day	Nasal suctioning (28,9%)
Batalha *et al.* (2007)	Portugal Single centre	170	25-41	1-15 observations per infant	770	0-15/8 hours	Suctioning (56.9%)
Cignacco *et al.* (2009)	Switzerland Multicentre	120	24-37	First 14 days	38626	22.9/infant/ day	CPAP prongs insertion (24.27%)

Md: median; N.R.: not reported; * Depending on the cohort of infants and day of admission considered.

Failed procedures are yet another dimension of the problem. The number of attempts needed to successfully perform a procedure has to be added to the actual number of procedures required. Two studies have looked at this. Simons and colleagues (2003)

found 1/3 of IV cannulations and 21% of venepunctures were not successful at first attempt and Carbajal and team (2008) report that overall, 18.9 % of procedures were not successful.

Regarding the factors behind the number of procedures, gestational age and day of admission have been identified to be related, although with mixed results. Barker and Rutter (1995, p. F48) report that the number of procedures "increased dramatically in infants below 30 weeks gestation", 74% of procedures being performed on the 30% of infants below 31 weeks. Conversely, Simons and colleagues (2003), through a random regression model, found that the frequency of procedures was not predicted by gestational age (p= .51). As for the day of admission, the first study day versus the second to fourteenth day recorded a significantly higher number of procedures in Simons and team (2003) study, and Stevens and collaborators (2003) found that infants at higher risk of neurological impairment received the most painful procedures on day 1. On the contrary, Carbajal and colleagues (2008) report that the number of procedures decreased over the NICU stay.

Caregivers' beliefs have been examined as a possible influence on the incidence and management of painful procedures (Porter & Anand, 1998; Simons *et al.*, 2003). The authors of these studies found that most professionals rate procedures as causing moderate to severe pain concluding that there is a discrepancy between what is considered optimal and the actual practice.

The results of these studies have to be read with caution, though. If there is, indeed, a decrease in the number of procedures as time goes by, wide variations in the incidence of painful procedures may be explained by the duration of the study period, from 7 days to the total stay in the unit. Differences in inclusion criteria may also account for different results: consecutive inclusion of all admitted babies in a period of time has been used in some studies (Carbajal *et al.*, 2008; Prestes *et al.*, 2005; Johnston *et al.*,1997a) but others like Barker and Simons excluded babies that were admitted less than 24 hours, likely to be healthier babies with no need for intensive care (Simons *et al.*, 2003; Barker & Rutter, 1995). So much as one study excluded healthy preterm and small for gestational age babies (Johnston *et al.*, 1997a). Benis & Suresh (2001) studied VLBW only (Mean GA= 27,1 weeks; mean birth weight= 899g) and Stevens and colleagues (2003) studied only infants at risk of neurological impairment, which might have increased the incidence of procedures per baby since in both cases, smaller and sicker babies usually require more interventions.

The list of procedures reported varies in number and in type. It is likely that the number of painful procedures under analysis, from 7 (Prestes *et al.*, 2005) to 44 (Carbajal *et al.*, 2008) reflects on the total number of procedures, a wider list increasing

the number of procedures per baby. Yet, there is a core group of procedures reported in most studies that are still very frequent, although with wide variations.

Overall differences in practice across countries and continents can also account for this difference. Out of four more recent studies, three (Simons *et al.*, 2003; Carbajal *et al.*, 2008) are European.

Having reviewed the studies that report the frequency of painful procedures in NICUs, we will now focus on the studies that have looked at pain management, especially for minor painful procedures.

3.2 Pain management in the NICU

Two types of studies have been conducted to examine pain management in neonatal units: prospective studies and surveys. The former are based on the records of the actual pain management provided, and will be reviewed first, while the latter are based on the respondents' views about current practice.

In their study following 151 neonates during the first 14 days of admission in one unit, Simons and team (2003) report that more than 39,7% of patients did not receive any analgesic therapy. This number is 72% in the study of Prestes and colleagues (2005), in which 91 neonates from four units were included. Stevens and team (2003) report a similar result: no analgesic was administered to 70–87% of the 194 infants across days 1–7, depending on whether it was a cohort of high, moderate or low risk for neurological impairment.

Infants who are ventilated seem to be an exception, receiving more analgesics than other infants (Prestes *et al.*, 2005; Johnston *et al.*, 1997a; Anand, Selanikio, & SOPAIN study group, 1996). Indeed, in one study examining ventilated babies only, (Cignacco *et al.*, 2009) 99,2 % of babies had received at least one dose of analgesia. The prescription of opioids was found to be positively correlated to illness severity and negatively correlated to birth weight and day of admission (Kahn *et al.*, 1998).

Regarding specific analgesia for painful procedures, two studies from the late 1990s show it was provided in 0.79 % of 2134 procedures (Johnston *et al.*, 1997a), and in 3% of around 7000 procedures (Porter and Anand, 1998). Recently in this decade, it was provided in 20,8% of the 42413 procedures in one study (Carbajal *et al.*, 2008).

Specific analgesia for heel lance and venepuncture varied from 0% in both procedures (Prestes *et al.*, 2005; Johnston *et al.*, 1997a) to 44% and 71,9% (Carbajal *et al.*, 2008), respectively. Simons and team report that "although some nurses used pacifiers and tried to comfort infants during and after procedures by holding, non-pharmacological analgesic treatment was not given routinely for any of the procedures" (Simons *et al.*, 2003, p. 1062). Specific analgesia for peripheral insertion of a central catheter var-

ied in these studies from 8% (Prestes *et al.*, 2005) to 71.7% (Carbajal *et al.*, 2008) and to 100% (Johnston *et al.*, 1997a). The use of non-pharmacological strategies was reported as 87.7% of 844 observations and consisted mainly of positioning, massage and comforting techniques (Batalha *et al.*, 2007).

Among the factors associated with greater use of specific pre-procedural analgesia, type of procedure, parental presence, surgery, daytime, and day of procedure after the first day have been identified (Carbajal *et al.*, 2008) .

Considering all painful procedures together, there was some non-specific pain management, i.e., medications that help alleviate pain but are given for other purposes such as sedation, in 6,8% (Johnston *et al.*, 1997a), 28% and 50,9% of the times (Carbajal *et al.*, 2008;Porter & Anand, 1998; Johnston *et al.*, 1997a). However, since non procedural-specific pain therapy, namely morphine and sedatives have been shown not to be effective for procedural pain (Carbajal *et al.*, 2005), this situation can only be considered unsatisfactory.

Observational studies aside and looking at surveys about pain management practices, it is striking that there are wide variations among units responding to the same survey and between surveys in the use of analgesia and in the guidelines followed. In 116 units surveyed in France (Debillon, Bureau, Savagner, Zupan-Simunek, & Carbajal, 2002), 77% of units reported using analgesia during venous catheter insertion. Lago and colleagues (2005) collected data from 90 of 102 level II and III units in Italy and found that the percentage of units using some pain reduction strategy was 44% for heel lance, 25% for injections and 50% for venepuncture. In the Nord-Pas-de-Calais, France, 7 of 11 units reported using sucrose for blood draw, and for a peripherally inserted central catheter, fentanyl, nalbuphine or propacetamol were systematically given (Klosowski, Morisot, Truffert, Storme, & Lequien, 2003). EMLA™ cream was used for lumbar puncture but also for venous and capillary blood draw and peripheral insertion of central catheters.

Harrison, Loughnan and Johnston (2006) conducted a postal survey and obtained responses from 105/181 intensive care and special care units in Australia. This was one of the few studies to look at minor painful procedures only. The authors report that non-nutritive sucking (NNS) alone followed by comforting measures was the most frequent strategy used for pain reduction in minor painful procedures. Sweet tasting solutions were used for procedures in 23% units but were used infrequently. For heel lance, NNS alone was the most frequently used pain reduction strategy, sucrose alone or combined with NNS was reported as never used by over 80% of the units, and breastfeed-

ing was reported as occasionally or often used by most units. For venepuncture, intra-venous cannulation and arterial line insertion, the use of NNS was frequent, sucrose use was reported low, and breastfeeding was used occasionally. Breastfeeding was offered more often during injections than any other procedure.

In 225 units representing 61% of all Austrian, German and Swiss neonatal units, 22% reported using some strategy for venepuncture, 16% for heel lance, 50% for central lines and 64% for lumbar puncture (Gharavi, Schott, Nelle, Reiter, & Linderkamp, 2007).

The wide variation in the use of analgesia can be seen with the pain management of tracheal intubation. This is a frequently studied procedure and might be an estimate of what is happening with other procedures. Premedication for non-emergent tracheal intubation has been reported as being used by 16% of 26 level III Canadian units (Fernandez & Rees, 1994), 100% of 109 of NICUs in the USA (Porter & Anand, 1998), 38% of the French units (Debillon *et al.*, 2002), 2/3 of 90 Italian units (Lago *et al.*, 2005), 98% of Neonatal units in Portugal (Eusébio & Fernandes, 2008) and 80% of Austrian, German and Swiss units (Gharavi *et al.*, 2007). Some of these studies, though, do not specify whether this medication is for analgesia, sedation or both.

Comparing the studies from the late 1990s to the studies over the last 5 years, there seems to be a clear improvement in the use of analgesia for painful procedures in neonates although it may still be considered far from evidence-based recommendations.

In most of the studies reported above, the use of opioids and sedation has been given most of the attention but Non-pharmacological interventions for minor painful procedures have been overlooked.

CHAPTER 4. Non-pharmacological interventions to reduce procedural pain in the NICU

CHAPTER 4.
Non-pharmacological interventions to reduce procedural pain in the NICU

Several interventions have been studied and have been recommended to reduce the pain of neonates during painful procedures. Evidence points to the fact that even though they are within the reach of health professionals they are seldom used (Anand *et al*., 2005). These interventions include pharmacological and non-pharmacological approaches.

Potent pharmacological approaches like morphine and fentanyl have been used successfully for surgery, reducing mortality and complications since the late eighties (Anand, Sippell, & Aynsley-Green, 1987). In ventilated babies however, continuous opioid infusions are controversial, especially after the results of the NEOPAIN study that raise issues regarding respiratory outcomes (Anand *et al*., 2004).

For procedural pain such as related to heel lance, intra-venous cannulation and endotracheal suctioning, the use of these pharmacological agents is not an option given the high frequency of those procedures and the potential of these agents for adverse effects. Morphine has not consistently been reported to be effective for acute procedural pain (Carbajal *et al*., 2005) and neither has Paracetamol (Anand *et al*., 2005). Lidocaine-prilocaine cream, known as EMLA™ (Eutectic Mixture of Local Anesthetics), has been reported to be safe but not effective in preterm infants in reducing pain from heel lance (Larsson, Jylli, Lagercrantz, & Olsson, 1995; Stevens *et al*., 1999) and its beneficial effects for venepuncture are unclear (Taddio, Ohlsson, Einarson, Stevens, & Koren, 1998).

This obviously limited choice of pharmacological agents for common procedures warrants research for Non-pharmacological interventions.

Among the Non-pharmacological interventions studied in Neonatal Intensive Care Units to reduce the pain responses of babies during heel lance and, we can count

developmental care, containment, swaddling, positioning, rocking, auditory and olfactory stimulation, breastfeeding, non-nutritive-sucking, oral sweet solutions such as glucose and sucrose, and maternal skin-to-skin contact. Combinations of these interventions have also been studied. After a brief review of the efficacy of these interventions in both term and preterm infants, oral sweet solutions, non-nutritive-sucking, and skin-to-skin contact will be addressed in more detail since they were used in the present study.

When looking at possible ways to reduce procedural pain in neonates, the method used for blood sampling deserves some consideration. Heel lance has been the usual way to collect blood samples in NICUs, and outside hospitals for neonatal screening. It is, however, more painful than venepuncture. A recently updated Cochrane review of five trials enrolling 457 neonates (Shah & Ohlsson, 2007) found that pain scores were significantly lower in the group using both the Neonatal Infant Pain Scale (NIPS) and the Premature Infant Pain Profile (PIPP). The number of neonates who cried within 60 seconds after skin puncture was lower in the venepuncture group compared to the heel lance group. The percentage of the first crying time relative to the total procedure time was shorter in the venepuncture group. One important secondary outcome of these studies was the need for more than one skin puncture. Four trials in this review reported the number of neonates who required additional skin puncture, which was significantly lower in the venepuncture group. The reviewers conclude that when performed by a trained phlebotomist, appears to be the method of choice for blood sampling in term neonates (Shah & Ohlsson, 2007). Recently, a new device has been experimented for collecting blood from the forearm of healthy term infants (Sato *et al.*, 2007). Compared to heel lance, it was found to be less painful, infants crying less and scoring less on the Neonatal Facial Coding System (NFCS) and the Neonatal Infant Pain Scale (NIPS). The comparison with venepuncture however, was not explored. Although both these studies refer to term infants, there is reason to believe that the pain responses of preterm infants will follow the same trend.

Developmental Care

Preterm birth and subsequent admission into a Neonatal Intensive Care Unit leads to the sudden deprivation of the infants' intrauterine world and disrupts the normal environment in which the preterm infant should mature and develop. The extreme contrast between the womb and the harsh neonatal intensive care context create numerous challenges to these at-risk infants. As described above, developmental care is a concept that encompasses a family-centered nursing care philosophy and multiple strategies designed to minimize the stress of the NICU environment for both the infant

and his/her family (Symington & Pinelli, 2006; Aita & Snider, 2003; Als *et al*., 1994) . The interventions provided may include elements such as control of external stimuli (vestibular, auditory, visual, tactile), clustering of nursery care activities, and positioning or swaddling of the preterm infant (Symington & Pinelli, 2006). The Newborn Individualized Care and Assessment Program (NIDCAP), the global developmental care program most studied, has shown that preterm infants have improved respiratory outcomes, requiring less oxygen and less mechanical ventilation (Jacobs, Sokol, & Ohlsson, 2002), a lowered incidence of grade III or grade IV intraventricular hemorrhage (Symington & Pinelli, 2006), higher mean mental developmental index (MDI) scores at 9-12 months (Jacobs *et al*., 2002) and, improved survival without severe disability (Westrup, 2007). Consistent findings were also reported in a study examining the effect of developmental care practices for very low birth weight infants (Tyebkhan, Peters, McPherson, Cote, & Robertson, 1999).

With regard to its efficacy as a pain relieving strategy, NIDCAP has been associated with reduced stress and pain expression during routine non tissue-breaking procedures considered to be stressful to neonates and fewer episodes of cardio-respiratory instability and hypoxia (Sizun, Ansquer, Browne, Tordjman, & Morin, 2002) and less usage of sedatives and opioids (Heller, Constantinou, Vandenberg, Benitz, & Fleisher, 1997). In the first study to examine the effects of NIDCAP on neonatal pain expression, 19 stable preterm neonates (mean gestational age= 29 ± 1.8 weeks, birth weight= 1212 ± 255 g, and post natal age= 21 ± 15 days) were randomly assigned to undergo a routine diaper change with or without developmental supportive strategies using a crossover design (Sizun *et al*., 2002). Acute pain scores (PIPP) measured during the procedure and chronic pain scores measured during and after the procedure using the Échelle Douleur et Inconfort du Nouveau-Né (EDIN) (Debillon *et al*., 2001) were significantly decreased with developmental care versus without developmental care. In a later study using a similar design, 45 stable neonates with a gestational age range of 29-40 weeks underwent a weighing procedure with and without developmental care, namely, attenuated noise and light with closed doors and covered incubator, lateral posture with head, back, and feet contacting supportive bedding, swaddling and opportunity for grasping or sucking (Catelin, Tordjman, Morin, Oger, & Sizun, 2005). Pain scores using the NIPS and the EDIN were significantly lower during weighing when developmental care was provided.

Physiological parameters were also found to be more stable during developmental care. The lowest oxygen saturation observed during the PIPP measure was significantly higher with developmental care compared to control during diaper change (Sizun *et*

al., 2002). No significant difference was observed for mean oxygen saturation during weighing, although the heart rate was significantly lower with developmental care versus control (Catelin *et al.*, 2005).

It is still unknown which specific aspect or which combination of NIDCAP interventions contributes to diminishing stress and pain in newborns nor if this benefit would occur during tissue-breaking procedural events or ongoing pain. However, elements of developmental care such as containment, swaddling, positioning, and rocking, non-nutritive sucking, sweet taste, music, exposure to familiar sound or smell, and skin-to skin contact, used alone or in combination, have been shown to provide comfort.

Containment/facilitated tucking

Containment refers to restricting the premature infant's motions by holding or using an arm to place the neonate's arms and legs near its trunk to maintain a flexed in-uterus posture with limbs placed in body midline (Huang, Tung, Kuo, & Chang, 2004). It is also referred to as facilitated tucking, in which a nurse or a parent holds the infant in the side-lying, flexed fetal-type position (Axelin, Salantera, & Lehtonen, 2006).

The effects of facilitated tucking have been examined in both preterm and very preterm infants undergoing commonly performed tissue-breaking procedures in the NICU and have been shown to diminish the magnitude of physiological and behavioral pain response. Facilitated tucking provided to preterm neonates (n= 30; 25-35 weeks) when compared to no tucking during heel lance lowered mean heart rate 6-10 minutes post-stick ($p< .04$), and resulted in less crying time ($p< .001$) and more stability in the sleep-wake cycles post heel lance ($p= .003$) (Corff, Seideman, Venkataraman, Lutes, & Yates, 1995).

A randomized placebo-controlled crossover trial (Axelin, Salantera, Kirjavainen, & Lehtonen, 2009) was conducted with 20 preterm infants (28 to 32 weeks gestational age) to compare the effectiveness of facilitated tucking by parent with oral glucose, oxycodone and water in reducing the pain response to heel lance and pharyngeal suctioning. During the 30 seconds after heel lance, mean Premature Infant Pain Profile score was significantly lower with facilitated tucking by parent when compared to placebo and oxycodone, and similar to the score with oral glucose. The Neonatal Infant Pain Scale score too was significantly lower with facilitated tucking by parent, compared to placebo ($p= .001$). For pharyngeal suctioning, mean PIPP score was lowest with oral glucose (11.05, $p= .014$) and facilitated tucking (11.25, $p= .034$) compared with placebo (12.40). In both heel lance and pharyngeal suctioning, oxycodone equaled placebo. The authors found less adverse effects per administration with facilitated tuck-

ing (5%), compared to glucose (21.25%) and water (12.5%) concluding that efficacy and safety considered together, facilitated tucking is preferable to the other pain management interventions.

Similar findings had been previously reported for pharyngeal/endotracheal suctioning in very preterm infants (24-33 weeks gestational age)(Axelin *et al.*, 2006). Median NIPS score was 3 for facilitated tucking and 5 for standard care (p= .001). Infants took less time to calm down (5 seconds after facilitated tucking vs 17 seconds after control care (p= .024). Facilitated tucking for endotracheal suctioning was also studied in the same age group by Ward-Larson, Horn and Gosnell (2004) who found a significantly lower PIPP score after facilitated tucking (8.95) compared to standard care (13.75, p= .001).

Facilitated tucking may therefore be used to effectively reduce pain in preterm and very preterm infants during heel lance and pharyngeal/endotracheal suctioning.

Swaddling

Similar to facilitated tucking in respect to containment and midline positioning, swaddling consists of wrapping the infant in a sheet or blanket, limbs flexed, head, shoulders and hips neutral, without rotation, and hands accessible for exploration (Aucott, Donohue, Atkins, & Allen, 2002). In many cultures, this is a traditional way to care for infants. Under this form of containment, term infants arouse less and sleep longer. Its use in preterm infants improves neuromuscular development and motor organization, and reduces physiologic distress (van Sleuwen *et al.*, 2007). Neonates' swaddled during the weighing procedure show less stress behaviors than when weighed without being swaddled (Fernandes, Miranda, Campos, & Camarneiro, 2006) confirming previous results by Catelin, Tordjman, Morin, Oger, & Sizun (2005).

A systematic review of swaddling in preterm infants () has identified 3 studies that looked at swaddling for pain in neonates. The first study was published 20 years ago (Campos, 1989). Infants two weeks and two months-old were studied for heel lance and immunization, respectively. Swaddling and pacifier were used for three minutes after the painful procedure. Infants in the swaddling group spent less time in alert state (22%) than did infants with pacifier (59%, p< .01). After termination of the soothing intervention, cry and heart rate tended to rebound more in infants that had a pacifier than in those who had been swaddled. In another study, maturation appeared to affect the efficacy of swaddling used during heel lance. In preterm infants with a postconceptional age over 31 weeks, swaddling improved recovery from heel lance (decrease in HR, p< .01 and increased arterial oxygen saturation, p< .01); whereas in infants with

a postconceptional age below 31 weeks, although oxygen saturations did increase faster ($p < .06$) while swaddled, heart rate did not vary between groups (Fearon, Kisilevsky, Hains, Muir, & Tranmer, 1997). Finally, compared to containment, swaddling from five minutes before to eleven minutes after heel lance was found to reduce recovery time, i.e. the time needed for infants to go back to their baseline heart rate and oxygen level (5 minutes in swaddling and 8 minutes in containment) and infants exhibited lower pain scores although significant differences were found only at the 3rd ($p < .05$) and 7th minute ($p < .05$) after heel lance (Huang *et al.*, 2004). The authors conclude that there is little difference between the effects of swaddling and containment on attenuating physiological and behavioral stress caused by acute pain and therefore these interventions can be used interchangeably.

A meta-analysis of four studies in Thailand reports that the effect size of swaddling compared to no intervention on pain scores during heel stick in term infants was .79, 95% CI [0.53, 1.05] and in preterm infants was .53, 95% CI [0.27, 0.80] (Prasopkittikun & Tilokskulchai, 2003). The magnitude of the intervention was moderate for decreasing heart rate in term neonates ($MD = .64$, 95% CI [0.46, 0.81]) and small for preterm neonates ($MD = .23$, 95% CI [0.08, .38]) and so was the effect on increasing oxygen saturation in preterm neonates ($MD = .13$, 95% CI [0.06, 0.21]).

These studies suggest that swaddling in preterm infants above 31 weeks promotes physiological stability during heel lance and reduces recovery time.

Positioning

Prone positioning in preterm infants has been shown to improve ventilation and oxygenation (Martin, Herrell, Rubin, & Fanaroff, 1979; Hutchison, Ross, & Russell, 1979), increase time in quiet sleep (Brackbill, Douthitt, & West, 1973; Masterson, Zucker, & Schulze, 1987), lessen disrupted sleep (Goto *et al.*, 1999), decrease energy expenditure (Masterson, Zucker, & Schulze, 1987), and diminish crying (Brackbill, Douthitt, & West, 1973). Given these numerous benefits, two studies have examined the hypothesis that prone position provides comfort to infants during painful procedures.

In the first randomized controlled trial conducted in very low birth weight preterm infants (n= 122) to compare various methods of non-pharmacological pain relieving strategies, Stevens *et al.* (Stevens *et al.*, 1999) reported that prone positioning did not decrease pain scores measured using the Premature Infant Pain Profile ($F = 2.24$, $p = .137$) when compared to side lying or supine, and were higher than pain scores re-

ported when infants were provided with pacifier with or without sucrose. Another trial compared responses to heel lance of preterm infants in prone versus supine position (Grunau, Linhares, Holsti, Oberlander, & Whitfield, 2004). Although prone position was found to promote deep sleep, 44% of time compared to 6% of the infants in supine position, findings related to the possible comforting effect of prone position were consistent with the earlier study. Heart rate and total facial activity were not significantly different between groups ($p = .96$ and $p = .35$, respectively). In conclusion, none of these studies have produced evidence to support the use of prone, supine or side-lying position to alleviate pain from painful procedures.

Rocking

Vestibular stimulation through rocking has been a traditional way to promote sleep and comfort infants in many cultures. Rocking was compared to pacifiers and routine care after heel lance for neonatal screening (Campos, 1989). Rocking and pacifiers reduced crying but rocking promoted arousal levels while pacifiers promoted sleep. Heart rate was significantly reduced with pacifiers, compared to rocking. The authors concluded that both interventions can be useful as comforting methods. This study looked at the distress displayed after a painful procedure so it is unclear whether the results would be similar if the intervention was applied before and during the actual procedure. More recently, rocking was compared to expressed breast milk, 20% sucrose, distilled water, non-nutritive sucking and massage in term, stable neonates (Mathai, Natrajan, & Rajalakshmi, 2006). Neonates were rocked by lifting the baby's head off the cot on the palm of the hand but not the body, and making rocking movements in a gentle, rhythmic manner. The authors concluded that at 2 and 4 minutes after the heel lance, infants in the rocking group, as well as infants in the non-nutritive sucking group, had significantly lower Douleur-Aigue du Nouveau-Né (DAN) scores and cried significantly less.

Based on studies of rocking being effective in fullterm neonates and on studies of simulated rocking promoting quiet sleep (Campos, 1994; Barnard & Bee, 1983) simulated rocking was tested for pain in preterm neonates (Johnston, Stremler, Stevens, & Horton, 1997). Infants in supine or side lying position on an oscillating air mattress were compared during heel lance to infants given sucrose, usual incubator care with no intervention, or a combination of both simulated rocking and sucrose. Both sucrose conditions (with and without rocking) showed a decrease in facial expression of pain by 40% or more across the procedure although heart rate was similar in all four groups and simulated rocking was no better than incubator care (Johnston *et al.*, 1997a).

Maternal holding

During heel lance, being held by mother and breastfed was compared to being held by mother with pacifier and to being held by non-mother with pacifier. In the first two conditions infants cried significantly less (M= 33% and M= 45%) compared to being held by non-mother (M= 66%), p< .01 and p= .03, respectively (Phillips, Chantry, & Gallagher, 2005). Prasopkittikun and Tilokskulchai (2003) report four studies of pain interventions for heel stick, one of which examined holding and touching term babies. The effect sizes were moderate to large in behavioral pain scores (d= .73, 95% CI [0.41, 1.04]) but less robust for physiological variables, namely heart rate (d= .48, CI . 18 to .77) and oxygen saturation (d= .39, 95% CI [0.17, 0.60]).

Auditory recognition

The human fetus is thought to be capable of auditory perception by 29 weeks gestational age (Shahidullah & Hepper, 1994)f and have the ability to learn and remember auditory stimuli from their intrauterine environment. This early experience may have lasting effects on the developing brain and later self-regulation (Fifer & Moon, 1994). When exposed to voices, near term fetuses had an increased heart rate (Kisilevsky *et al.*, 2003) and more robust vagal tone (Smith, Dmochowski, Muir, & Kisilevsky, 2007) in response to the mother's voice and a decrease in response to a stranger's voice. Infants between 33 and 41 weeks gestational age were even able to distinguish language (English versus Mandarin) following recorded playing of passages in either language, further supporting early auditory attention and memory facility (Kisilevsky *et al.*, 2009). It has been known for more than two decades, that infants as young as three days recognize their mothers' voice (DeCasper & Fifer, 1980) and this memory has been shown to affect physiological and behavioral responses and have soothing effects (Kurihara *et al.*, 1996).

Numerous studies have determined that maternal heart beat and recorded voice or lullaby can be soothing to both fullterm and preterm newborns. Following birth, infants exhibited heart rate decelerations, increased non-nutritive sucking, more relaxed facial expressions, diminished crying and less body movements when hearing syllables that are paired with the maternal voice than when syllables are paired with another woman's voice or silence (Fifer & Moon, 1994; Nakajima, 1994). Exposure to familiar sounds has been positively associated with improved physiological stability (decreased heart and respiratory rate and an increase in oxygen saturations) (Collins & Kuck, 1991) less agitation (Standley & Moore, 1995) and more time in stable sleep or quiet alert state (Collins & Kuck, 1991). Maternal heart beat has also been shown to blunt the effects

of pain associated with a tissue-breaking procedure in a study in which 131 fullterm infants underwent a heelstick while being exposed to either maternal heart rate, Japanese drum with identical rhythm or no sound. Infants exposed to maternal heart beat had reduced facial response and crying and lower levels of cortisol and dehydroepiandrosterone (DHEA) following heelstick when compared to the other two groups (Kurihara *et al.*, 1996). In a crossover design study with infants 30 to 41 weeks gestational age, music therapy consisting of intrauterine maternal pulse sounds with soothing music, music therapy (intrauterine maternal pulse sounds) combined with non-nutritive sucking, non-nutritive sucking alone and no intervention were compared when used for five minutes after heel lance (Bo & Callaghan, 2000). Music therapy alone had the strongest effect on neonates' heart rate and the effect lasted after the intervention was withdrawn. Non-nutritive sucking with music therapy had the strongest effect on neonates' TcPaO2 levels and pain behavior. Butt & Kisilevsky (2000) exposed preterm neonates to vocal or instrumental music for 10 minutes after the end of a heel lance. During this period, infants above 31 weeks had a more rapid return of heart rate, behavioral state, and facial expressions of pain to baseline levels in the presence of music compared to the absence of music.

Similar findings were not observed in a recent study examining the soothing effect of maternal voice in infants between 32 and 36 weeks gestational age where no differences were seen between those infants exposed to a recorded and filtered maternal 'singsong' voice versus no voice during heelstick procedure (Johnston, Filion, & Nuyt, 2007). These results may have been affected by the high volume of the recorded sound (70 db) or may indicate that familiar sound alone in the absence of additional environmental context such as olfactory stimulus or proximity may not be sufficient to ameliorate the effects of a tissue-breaking procedure in younger more immature infants. Although the benefits of music and vestibular action may be less promising in isolation (i.e., without the mother), these results have helped us better understand the importance of maternal presence and relationship with respect to pain response (Johnston *et al.*, 2008a; Johnston *et al.*, 2007a).

Olfactory recognition/aromatherapy

There is now compelling evidence that both term and preterm infants remember, recognize and prefer smell that is associated with their intrauterine environment and their mothers, and that olfactory stimuli can provide infants with comfort and modulate pain response (Goubet, Strasbaugh, & Chesney, 2007; Goubet, & Bullinger, 2005; Goubet, Rattaz, Pierrat, Bullinger, & Lequien, 2003; Sullivan & Toubas, 1998;

Varendi, Christensson, Porter, & Winberg, 1998). Infants less than 4 days of age regardless of being formula fed (Marlier, Schaal, & Soussignan, 1998) or breastfed (Schaal, Marlier, & Soussignan, 1998) showed preference by head-turning towards familiar amniotic smell versus formula or an unfamiliar amniotic smell, and had decreased crying and increased sucking bursts when presented with maternal odor versus no odor (Sullivan & Toubas, 1998). Exposure to amniotic fluid smell also diminished stress and crying associated with maternal separation. Babies exposed to amniotic fluid smell cried significantly less (median= 29 seconds) than babies in the two other groups (maternal odor median= 301 seconds, no odor median= 135 seconds) (Varendi, Christensson, Porter, & Winberg, 1998).

Newborns also appear to have early learning and memory of olfactory stimuli and that this memory can affect both behavioral and hormonal response to a tissue-breaking procedure. To determine the effect of familiar, unfamiliar or no odor on infant pain response during heelstick, 44 breast-fed newborns were randomly assigned to one of four groups: Group 1 was naturally familiarized with their mother's milk odor, Group 2 was familiarized with a vanilla smell, and Groups 3 and 4 did not receive any familiarization. During and after the heel stick, Group 1 was presented with their familiar mother's milk odor, Group 2 was presented with the familiar vanilla, Group 3 was presented with an unfamiliar odor, and Group 4 was a control group. Results revealed that infants who smelled a familiar odor (their mother's milk or vanilla) cried and grimaced significantly less during the recovery phase compared with those infants exposed to a non familiar or no odor condition. Infants exposed to their mother's milk also exhibited significantly less motor agitation during and after the heelstick (Rattaz, Goubet, & Bullinger, 2005). These findings were also seen in studies in which fullterm infants (Goubet *et al*., 2007) as well as preterm infants (average 32.3 weeks gestational age) (Goubet at al., 2003) exposed to a familiar vanilla smell during heelstick had significantly less crying and grimacing compared to infants exposed to an unfamiliar odor.

Massaging the baby's face and back with baby-oil scented hands was used as part of an intervention designated as sensorial saturation, which also included placing the baby in a flexed position with limbs brought to midline, talking to the baby while face-to-face and orally administering 10% glucose (Bellieni *et al*., 2001). In comparison to no treatment control, 10% glucose by mouth, sucking, and combination of sucking and glucose, on decreasing pain scores in preterm neonates using a standardized scale, sensorial saturation lowered PIPP scores to no pain (3/21).

These studies provide clear evidence that neonates, even those born preterm, have

some ability for auditory and olfactory processing of familiar sound and smell, not just perception. This memory recognition is associated with diminished pain response and may have the potential when combined with other familiar context to help the infant modulate pain experiences.

The mechanism underlying the comforting effects of intrauterine, maternal and familiarized smell remain unknown although it has been postulated that it is an opioid mediated system. The rationale behind this hypothesis is twofold and is derived from animal and human studies: 1) Animal models have demonstrated that the opioid system modulates olfactory learning, odor preference and nociceptive responses in rats (Jahangeer, Mellier, & Caston, 1997; Roth & Sullivan, 2005; Shide & Blass, 1991); and 2) in humans, gustatory systems encompassing the beneficial effects of sweet tasting solutions are known to be opioid mediated and are strongly linked with the olfactory system (Stevens, Yamada, & Ohlsson, 2004).

Breastfeeding and breastmilk

Animal studies in the late eighties showing the stress-reducing effects of ingesting milk, sugar and fats (Blass & Fitzgerald, 1988) aroused a growing interest on the role of milk, breast milk and breastfeeding in reducing stress and pain in human infants.

Newborns have the capacity to distinguish between substances with different flavors (Blass & Smith, 1992). The calming properties of formula milk and of 12% sucrose solution against those of water were shown by a significant reduction in spontaneous cry in fullterm infants in the first three days of life who were delivered 0.1 ml of one of the fluids over 10 seconds every minute during five minutes. Reduction in crying started in the first minute of milk delivery and persisted after the end of milk treatment, contrary to water. Compared to sucrose however, milk, like sterile water, did not elicit hand-in-mouth behavior (Blass, 1997) .

A recently updated systematic review of clinical trials (Shah, Aliwalas, & Shah, 2008) has examined the effects of breastmilk (six studies) and breastfeeding (five studies) to alleviate procedural pain in neonates. The painful procedure examined was heel lance except in three studies looking at venepuncture (Upadhyay *et al.*, 2004; Gradin, Finnstrom, & Schollin, 2004; Carbajal, Veerapen, Couderc, Jugie, & Ville, 2003). Only one of these studies included preterm (30 or more weeks gestational age) as well as term neonates (Skogsdal, Eriksson, & Schollin, 1997).

The effects of breastmilk placed in the neonates' mouth in reducing heart changes provoked by a painful procedure do not appear to be any better than placebo (Uyan, Ozek, Bilgen, Cebeci, & Akman, 2005; Upadhyay *et al.*, 2004; Bucher, Baumgartner,

Bucher, Seiler, & Fauchere, 2000; Ors *et al.*, 1999; Skogsdal, Eriksson, & Schollin, 1997), no treatment and 10% glucose, artificial sweetener and glycine (Bucher *et al.*, 2000) since no significant difference was found comparing breastmilk to these interventions. It seems that the effect is not as potent as sweet taste: compared to 25% sucrose, fullterm infants that had 2ml of human milk syringed into the mouth had a significantly higher increase in heart rate (Ors *et al.*, 1999). Compared to infants who had 1ml of 30% glucose, infants who had 1ml of expressed breastmilk had a significantly higher increase in heart rate (Skogsdal *et al.*, 1997). Colostrum delivered by pacifier but not by syringe, significantly reduced the increase in heart rate compared to water by syringe or pacifier (Blass & Miller, 2001) suggesting that the pacifier played an important role. Changes in oxygen saturation were not significantly different between neonates given breastmilk and those given placebo (Upadhyay *et al.*, 2004). As for cry, two studies reported a reduction of time spent crying in infants who received breastmilk versus placebo (Upadhyay *et al.*, 2004; Blass & Miller, 2001) but the meta-analysis from the other four studies that looked at cry (Ors *et al.*, 1999; Uyan, Ozek, Bilgen, Cebeci, & Akman, 2005; Bucher *et al.*,2000; Skogsdal *et al.*, 1997) found no statistically significant difference in the duration of crying in seconds between the breastmilk and the placebo group (Weighted Mean Difference= -6, 95% CI [-16, 3]). A difference between hindmilk and foremilk, hindmilk known to contain more fat than foremilk, was not found, although the sample size might have been too small to detect an effect (Uyan *et al.*, 2005). Median recovery time in the human milk group (112 s) was significantly longer than in the sucrose group (72 seconds), p= .007, but not different from the water group (124 s), p= .44. Pain scores using the NFCS showed no significant difference between breastmilk and placebo in two studies (Uyan *et al.*, 2005; Bucher *et al.*, 2000) and a significant reduction in one study (Upadhyay *et al.*, 2004). Heterogeneity in the data collection of the NFCS prevented the combination of data from these studies (Shah, Aliwalas, & Shah, 2008) to reach a clearer conclusion.

As for breastfeeding, the results of clinical trials seem quite different from those described for breastmilk. Heart rate increase was significantly lower in the breastfeeding group compared to swaddling (*MD*= -23, 95% CI [-35, -11]) (Gray, Miller, Philipp, & Blass, 2002) and to being held by mother along with pacifier use (*MD*= -11, 95% CI [-21, -1]) (Phillips, Chantry, & Gallagher, 2005). No significant differences were found in changes in oxygen saturation and in blood pressure between the breastfeeding group, the group of infants held by mothers holding a pacifier in the infant's mouth and the group of infants held by research assistant holding a pacifier in the infant's mouth

(Phillips *et al.*, 2005). Percentage of time crying was significantly lower in the breastfeeding group compared to the swaddled group (*MD*= -39, 95% CI [-55, -23]) (Gray *et al.*, 2002) and to infants held by research assistant (*MD*= -33, 95% CI [-50, -13]) (Phillips *et al.*, 2005). In a four-group trial, Gradin *et al.* (2004) report that the duration of crying in seconds during was lower in infants breastfed for 45 minutes before the procedure and given 1ml of 30% glucose (18 seconds) or placebo (63 seconds) immediately prior to the procedure, compared to infants that had fasted for at least 2 hours and were given 1ml of 30% glucose (93 seconds) or placebo (142 seconds) (*MD*= -50, 95% CI [-79, -22]). Compared to infants given 30% glucose, breastfed infants did not cry significantly less (93 vs 63 seconds). A cumulative effect of breastfeeding and glucose is suggested by less time crying and lower PIPP scores in the breastfed plus glucose group (Gradin *et al.*, 2004). During breastfeeding, infants cried significantly less during heel lance compared to infants held in mothers' arms or held in research assistants' arms ($p < .01$). Validated pain scores used in these studies were the PIPP and the DAN. PIPP scores were significantly lower in the breastfeeding group compared to placebo group (*MD*= -6, 95% CI [-7, - 4]) and no treatment (*MD*= 0, 95% CI [-2, 1]) (Shah *et al.*, 2008). Compared to 30% glucose, PIPP scores were statistically significantly higher when two studies (Gradin *et al.*, 2004; Carbajal *et al.*, 2003) were combined (*MD*= 1.30, 95% CI [0.05, 2.56]) (Shah *et al.*, 2008). The DAN scores were significantly lower in the breastfeeding group compared to placebo and being held by mother but were not different from the glucose group (Carbajal *et al.*, 2003). Using a composite measure of pain, Shendurnikar and Gandhi (2005) found a lower score in the breastfeeding group compared to the swaddled group (*MD*= -3, 95% CI [-4, -2]).

Evidence from these studies points out that breastfeeding is more efficacious than placebo or no treatment, but compared to sweet solutions, the results are mixed. A recent trial (Codipietro *et al.*, 2008) comparing breastfeeding to 1ml sucrose solution during heel lance found that PIPP scores were lower in the breastfeeding group and physiological parameters and crying were also improved by breastfeeding suggesting that breastfeeding might be superior to sucrose in decreasing pain.

Although the systematic review recommends that breastfeeding and breastmilk be used for painful procedures in neonates (Shah *et al.*, 2008), from our point of view, these studies suggest that breastfeeding has an analgesic effect, comparable to that of sweet solutions, but the effects of expressed breastmilk are less clear. More studies using comparable interventions and the same outcomes and involving preterm infants are needed to produce evidence to guide clinical decisions.

There is evidence that milk analgesia is opioid-mediated. The decrease in distress vocalizations and increase in paw-lift latency during intraoral infusions of milk in 10 day-old rat pups was similar to that produced by low doses of morphine injections (Blass & Fitzgerald, 1988). Furthermore, in the same study, the analgesic effects of oral milk were blocked by the use of low doses of naltrexone.

Sucking action may also be an important contributor to pain relief during breastfeeding. Fullterm infants provided with a pacifier when compared to controls lying supine without pacifier exhibited an increase in peripheral somatosensory threshold at which both the flexion withdrawal reflex (p= .042) and the occurrence of gross body movements (p= .027) were elicited (Abdulkader, Freer, Fleetwood-Walker, & McIntosh, 2007). Interestingly, suckling at the breast significantly increased the threshold at which both elicitation of the flexion withdrawal reflex (p= .001) and manifestation of gross body movements (p= .001) occurred. It is uncertain whether proximity to the mother or ingestion of the breastmilk contributes to these differences.

Oral sweet solutions

Sweet taste has always been known as a source of pleasure. Before sugar had reached Europe in the Middle Ages, coming from Asia and the Middle East where it was known since at least 300 years BC, honey was used to change the flavor of food and beverages, showing that preferences for sweet taste are very ancient and are present across cultures. Although there are several types of sugars, like sucrose, glucose, fructose, lactose and galactose varying in their chemical composition and level of sweetness, the common table sugar is sucrose. Sucrose, a simple carbohydrate, is a disaccharide that is broken down into two molecules: glucose and fructose.

The calming effects of sugar were known among lay-people and were used as a part of traditional care of infants. It is not uncommon today, in Portugal, to hear 50 year-old people born in the countryside describe in their memories from infancy, when pacifiers were not available, how sugar was delivered to babies to keep them calm while parents left the house to work in the fields. Sugar was wrapped in a small piece of cloth and carefully tied with thread to form a dummy that was placed in the infants' mouth and could last for weeks as a source of comfort.

Research in animals and human infants has shown that intra-oral sweet solutions have an analgesic effect. Studies using sucrose started in the late eighties (Blass & Hoffmeyer, 1991; Blass, Fitzgerald, & Kehoe, 1987) and since have included term infants as well as preterm and very preterm infants. Other sweet solutions besides sucrose that have been investigated against a placebo or no intervention are glucose (Akcam &

Ormeci, 2004; Eriksson, Gradin, & Schollin, 1999; Akcam & Ormeci, 2004; Carbajal, Lenclen, Gajdos, Jugie, & Paupe, 2002; Carbajal, Chauvet, Couderc, & Olivier-Martin, 1999; Gradin *et al.*, 2004; Gradin, 2005; Isik, Ozek, Bilgen, & Cebeci, 2000; Okan, Coban, Ince, Yapici, & Can, 2007), fructose (Akcam, 2004) and artificial sweeteners (Bucher *et al.*, 2000; Ramenghi, Griffith, Wood, & Levene, 1996). The capacity of infants to distinguish between flavors, namely sucrose, quinine and corn oil has been demonstrated (Graillon, Barr, Young, Wright, & Hendricks, 1997). Animal studies (Blass & Shide, 1994) reinforce the evidence from studies in human infants (Blass & Smith, 1992)that sucrose, glucose, fructose but not lactose have a calming and pain-reducing effect increasing the latency to withdraw from a heated surface in rat pups.

Studies of the effects of sucrose and glucose will be reviewed next, as well as comparisons between sucrose on one hand and glucose, breastfeeding and EMLA™ on the other hand. The additive effects of these interventions and issues like concentration, volume, mode of delivery and adverse effects will be addressed.

Sucrose. Research on the use of oral sucrose in human neonates has been examined in two systematic reviews (Gaspardo, Linhares, & Martinez, 2005; Tsao, Evans, Meldrum, Altman, & Zeltzer, 2008) and one recently updated systematic review including 44 studies and the meta-analysis of twelve studies (Stevens *et al.*, 2010). All these reviews agree on the efficacy of sucrose to reduce procedural pain in term and preterm neonates.

The Cochrane updated review of Stevens and colleagues (2010) adds twenty-three more studies to the twenty-one studies included in their review published in 2004. Besides heel lance (26 studies) and venepuncture (3 studies), the procedures now examined were subcutaneous injections (2 studies), circumcision (3 studies), bladder catheterization (1 study), eye exam for retinopathy of prematurity (5 studies) and nasogastric tube placement (1 study). Three studies examined more than one procedure. Given the large number of studies included and excluded from the Cochrane review, only reports on needle procedures (heel lance, venepuncture and subcutaneous injections) included in the Cochrane review will be reviewed here.

Infants' responses to interventions have been assessed using both behavioral (cry and facial action) and physiological indicators as well as composite measures of pain.

Cry during heel lance was examined in thirty studies. Mean duration of first cry was not significantly different but the mean duration of total cry, in seconds, was in favor of 2 ml of 20-30% sucrose versus sterile water for heel lance when two studies (N=

88 infants) (Mathai, Natrajan & Rajalakshmi, 2006; Isik *et al.*, 2000) were combined for meta-analysis. At venepuncture, two studies in preterm infants (Annamali, Taub, & Field, 2004 ; Abad *et al.*, 1996) reported that 24% sucrose significantly reduced cry duration while 12% sucrose compared to water did not (Abad *et al.*, 1996). One study in term infants comparing 0.1 ml of 50% sucrose with water reported no difference (Ogawa *et al.*, 2005). In subcutaneous injections, 2 ml of 12% sucrose significantly reduced crying time compared to water and no treatment (Allen, White, & Walburn, 1996).

The quality of sucking was reported to be significantly more intense (p= .04) in one study comparing 1ml of 25% sucrose to 1ml of water two minutes before heel lance (Ramenghi *et al.*, 1996a).

Display of facial actions was measured during heel lance and was significantly reduced with 2ml of 12% sucrose with or without pacifier compared with water (Blass & Watt, 1999). Composite measures of pain were used to assess pain from heel lance and venepuncture. The PIPP was used in five out of six studies at heel lance (Codipietro *et al.*, 2008; Stevens *et al.*, 2005; Gibbins *et al.*, 2002; Johnston *et al.*, 1999; Stevens *et al.*, 1999) Sucrose doses ranged from 0.05 ml to 2 ml of a 24% or 25% solution. In all five studies, sucrose significantly reduced PIPP scores. The meta-analysis of three of these studies (N= 220 infants)(Gibbins *et al.*, 2002; Johnston *et al.*, 1999; Stevens *et al.*, 1999) comparing sucrose with or without pacifier with pacifier and water, water or positioning and containing intervention, showed a significant reduction of PIPP scores at 30 seconds (*WMD*= -1.64, 95% CI [-2.47, - 0.81]) (N= 220 infants) and 60 seconds (*WMD*= -2.05, 95% CI [-3.08, -1.02]) (N= 195) after heel lance (Stevens *et al.*, 2010). When the NFCS or a modified version of the NFCS was used to assess pain at heel lance, pain scores were significantly lower in the sucrose groups compared to other groups (Gaspardo, Miyase, Chimello, Martinez, & Martins Linhares, 2008; Okan *et al.*, 2007; Ogawa *et al.*, 2005; Harrison, Johnston, & Loughnan, 2003; Johnston *et al.*, 1997b). At venepuncture, the NFCS was used in four studies (Gaspardo *et al.*, 2008; Ogawa *et al.*, 2005; Acharya *et al.*, 2004; Abad *et al.*, 1996), and the DAN was used in one study (Carbajal *et al.*, 1999). Pain scores were significantly reduced in preterm infants with 2 ml of 25% sucrose compared to water (Acharya *et al.*, 2004; Abad *et al.*, 1996) and with 0.5 ml/kg f 25% sucrose compared to water (Gaspardo *et al.*, 2008), as well as in term infants with 2 ml of 30% sucrose with or without pacifier compared to water (Carbajal *et al.*, 1999). One study with fullterm infants (Ogawa *et al.*, 2005) did not find any significant differences between 1 ml of 50% sucrose and water groups. During subcutaneous

injections, the DAN and the NFCS scores were lower in the groups receiving 0.2-0.5 ml of 30% sucrose compared to the group receiving pacifier alone (Mucignat *et al.*, 2004).

Physiological indicators have also been examined in the studies included in the Cochrane review. At heel lance, sucrose significantly reduced heart rate in eight studies (Codipietro *et al.*, 2008; Okan *et al.*, 2007; Gormally *et al.*, 2001; Blass & Watt, 1999; Ors *et al.*, 1999; Ramenghi *et al.*, 1996a; Bucher *et al.*, 1995; Haouari, Wood, Griffiths & Levene, 1995). However, when results from some of the studies were pooled, no significant differences were found between sucrose and sterile water (Stevens *et al.*, 2010). Regarding vagal tone, one study (Gormally *et al.*, 2001) reported no difference whereas another study (Greenberg, 2002) found a significantly lower vagal tone at heel lance in infants given sucrose and pacifier compared to sucrose alone and no intervention groups, which was an unpredictable finding. The author suggests that rather than indicating higher pain levels, the results may indicate that the double impact of sugar taste and sucking focused the infants' attention, thereby decreasing vagal tone (Greenberg, 2002). Blood oxygen and respiratory rates assessed at heel lance were no different in infants given sucrose compared to infants given water or other interventions (Okan *et al.*, 2007; Mathai *et al.*, 2006; Harrison *et al.*, 2003; Overgaard & Knudsen, 1999; Bucher *et al.*, 1995). Salivary cortisol was also measured to evaluate stress and in neonates given sucrose before painful procedures during the first week of life compared to neonates given sterile water (Boyer *et al.*, 2004). Low gestational age of the subjects (< 31 weeks) may have been responsible for the absence of any significant difference between the groups since saliva is scarce in this age group. At venepuncture, heart rate was assessed in two studies (Acharya *et al.*, 2004; Abad *et al.*, 1996) and a significant reduction was found in groups receiving 2 ml of 25% sucrose. Regarding oxygen saturation, none of the two studies (Acharya *et al.*, 2004; Abad, Diaz, Domenech, Robayna, & Rico, 1996) assessing this outcome during and after venepuncture found significant differences between sucrose and control groups. At subcutaneous injections (Mucignat *et al.*, 2004), there was no difference in heart rate between 0.2-0.5 ml of 30% sucrose and pacifier alone but oxygen saturation was significantly lower in the pacifier alone group.

These results reveal dissociation between behavioral and physiological indicators of pain, the former being far more sensitive than the latter. Furthermore, changes in heart rate may be caused by the administration of sweet solutions in the absence of any painful procedure (Gradin, 2005) which may account for the fact that differences in heart rate between infants receiving sweet solutions and placebo are more difficult to find.

Two particular factors were examined in one study (Taddio *et al.*, 2008): the first two days of life and being born from a diabetic versus non-diabetic mother. Term newborns of diabetic and non-diabetic mothers, receiving 2ml of 24% sucrose solution or placebo solution during vitamin K intra-muscular injection and venepuncture during the first two days of life were compared. In newborns of diabetic mothers only, the comparison between sucrose and placebo was also made for 3 heel lances. Mean differences in the PIPP score between sucrose and placebo were significant for venepuncture in newborns of non-diabetic mothers (*MD*= -3.2, 95% CI [-4.6, -1.8]) and in newborns of diabetic mothers (*MD*= -2.4, 95% CI [-3.8, -1.0]) but not for intra-muscular injection in both neonates of diabetic and non-diabetic mothers. For neonates of diabetic mothers, there was no significant difference between the sucrose and the placebo group for pain from heel lance. The authors suggest that the effectiveness of sucrose in the first two days of life is limited to venepuncture (Taddio *et al.*, 2008).

The effect of sucrose analgesia for procedural pain on infant pain responses during a subsequent caregiving procedure has also been examined (Taddio, Shah, & Katz, 2009). During diaper change after venepuncture for neonatal screening, newborns treated with sucrose had lower PIPP scores (*M*= 5.1, 95% CI [4.4, 5.7]) than those that received a placebo (*M*= 6.5, 95% CI [5.8, 7.2]). The conclusion drawn is that the benefits of sucrose analgesia extend beyond the painful event to the routine care that follows. Studies examining the effects of sucrose to reduce pain from venepuncuture in term and preterm infants are displayed in Table 2.

Table 2 - Studies examining the effects of sucrose to reduce pain from venepuncture in term and preterm infants

Study	Participants	Design and Interventions	Outcome measures	Results
Abad 1996	28 preterm GA: 29- 36 weeks Postnatal age: 1-26 days	Randomized, double-blind, controlled trial 1) 2ml of 12% sucrose 2) 2ml of 24% sucrose 3) 2ml of spring water Administered 2 minutes prior to venepuncture via syringe	Time crying for 3 minutes after	Cry duration for 3 minutes after was significantly reduced in the 24% sucrose group (*M*= 19.1 sec) compared to water (*M*= 72.9 sec), *p*< .05.
			Heart rate	Significant group effect for HR, *F* (2, 25)= 6.37, *p*= .006. Post hoc Tukey test showed that group receiving 2 ml of 12% sucrose (0.24 g) had lower HR compared to the 2 ml of 24% sucrose group (0.48 g) or water group at all three time points (pre solution, *p*= .048; post solution, *p*= 0.010; 5 minutes after, *p*= 0.007).
			Mean 0^2saturation and respiratory rate pre solution, post solution, 5 minutes after	No significant differences noted between groups over time for oxygen saturation and respiratory rates.

Study	Participants	Design and Interventions	Outcome measures	Results
Acharya 2004	39 healthy preterm neonates Mean GA: 30.5 weeks Mean postnatal age: 27.2 days	Randomised, double blind, placebo controlled, crossover trial 1) 2ml of 25% sucrose 2) 2ml of water	Duration of first cry (beginning to end of first cry);	Mean duration of first cry lower in infants who received sucrose (18.6 seconds) compared to infants who received water (52.3 seconds) (estimated treatment effect= 33.7, *p*< .001).
		Administered 4 minutes prior to venepuncture, over 2 minutes into front of infant's mouth	Total duration of crying (onset of first cry to cessation of all crying)	Mean total duration of crying was significantly lower in infants who received sucrose (31.9 seconds) compared to infants who received water (72.5 seconds) (estimated treatment effect= 40.6, *p*< .001).
			NFCS	Changes in mean NFCS scores were significantly lower in the sucrose group compared to water group from pre-procedure to procedure phase (estimated treatment effect= 1.08), *p*= .013 and between the pre-procedure and post-procedure phase (estimated treatment effect= 2.39, *p*< .001).
			Rise in heart rate	Mean rise in heart rate from pre procedure to procedure was lower in the infants receiving sucrose compared to water (estimated treatment effect= 7.5, *p*= .003). Mean rise in heart rate from pre procedure to post procedure was lower in the infants who received sucrose compared to water (estimated treatment effect= 4.16, *p*= .036).
			O^2 saturation	No significant differences between groups with respect to changes in oxygen saturation from pre procedure to procedure phase, *p*= .17.
			$TcpO^2$ and $tcpCO^2$	No significant differences between groups with respect to measures for tcP02 (*p*= .05) and tcPC02 (*p*= .21)
Carbajal 1999	150 term newborn infants Postnatal age: 3-4 days	Randomized, single-blind, controlled trial: 1) no treatment 2) 2 ml sterile water via syringe; 3) 2 ml 30% glucose via syringe; 4) 2 ml 30% sucrose; 5) pacifier; 6) 2 ml 30% sucrose via syringe followed by a pacifier	DAN scale	Median pain scores with interquartile ranges were: 1) No treatment group 7 (5-10); 2) sterile water group 7 (6- 10); 3) 30%glucose group 5 (3-7); 4) 30% sucrose group 5 (2-8); 5) pacifier alone group 2 (1-4); 6) 30% sucrose with pacifier group 1 (1-2). All groups had significantly lower pain score compared to sterile water. Pacifier alone group had significantly lower pain score compared to infants receiving 30% glucose or 30% sucrose

Study	Participants	Design and Interventions	Outcome measures	Results
Gaspardo 2008 a)	33 preterm infants GA: 25-33 weeks	Randomized, double-blind, placebo-controlled trial. 1) 0.5 ml/kg of sterile water 2) 0.5 ml/kg 25% sucrose	Neonatal facial coding system (NFCS) score	A lower percentage on infants in the sucrose group had NFCS scores> 3 points compared to infants in the water group during the puncture phase but the difference was significant only on the 1st day of intervention (p= .05)
		Administered 2 minutes prior to all procedures performed during three days	Activated Behavioral State (ABS) score	Less infants were in active states in the sucrose group compared to the water group but differences were significant only in the puncture phase on the 1st and 3rd day of intervention.
			Cry	Less infants cried in the sucrose group. The difference during the puncture phase was significant on the 1st and 3rd day of intervention.
			Heart Rate	No differences between groups.
Ogawa 2005 b)	50 fullterm infants GA: 37- 41 weeks	Randomized, double-blind, placebo-controlled trial: 1) 0.1 ml of sterile water 2) 0.1ml of 50% sucrose	NFCS	NFCS score was not significantly different between the intervention groups.
			Duration of first cry	Duration of first cry was significantly lower in the sucrose group (p< .01)
		Administered on infants' tongue via syringe 2 min before procedure	Percentage of time crying Ratio of crying to no crying Total procedure time	No difference in other outcomes.
Taddio 2008 b)	120 infants born to nondiabetic mother 120 infants born to diabetic mother GA:> 36 weeks Postnatal age: 0-2 days	1) 2 ml 24% sucrose 2) 2 ml sterile water Administration on the anterior surface of the tongue, starting 2 minutes before the procedure over a period of about 60 seconds	PIPP	In newborns of nondiabetic mothers: significantly lower PIPP score in sucrose group (M= 5.7) compared to placebo (M= 8.9), p< .001 In newborns of diabetic mothers: significantly lower PIPP score in sucrose group (M= 6.8) compared to placebo (M= 9.2), p< .001

a) Other procedures were included in the study and the results presented do not discriminate venepuncture
b) Although other procedures were examined in the same study, data presented in the table refer to venepuncture only.

Glucose. Oral glucose has also shown to have an effect on behavioral indicators of pain. For venepuncture in preterm neonates, duration of first cry after glucose solu-

tion was significantly reduced (M= 4.50, SD= 38.58 seconds) compared to sterile water (M= 85.5, SD= 44.15 seconds), p= .002; but no effect was noted on heart r′ate, respiratory rate and oxygen saturation (Deshmukh & Udani, 2002). In 60 term infants enrolled in a cross over trial, 0.5ml of 30% glucose compared to the same volume of sterile water 2 minutes before heel lance reduced the DAN pain score (M= 4.3 for glucose and M= 6.1 for water), p< .001 (Akcam & Ormeci, 2004). In a trial with 20 very preterm infants (28-32 weeks gestational age) pain score using the PIPP after heel lance was lower with oral glucose (M= 4.85) than with water (M= 7.05), p= .001 (Axelin *et al.*, 2009). For subcutaneous injections, very preterm neonates (23-31 weeks gestational age) receiving 0.3ml of 30% oral glucose for one injection and 0.3ml of water for another showed a lower pain score measured with the Douleur-Aigue Nouveau-Né scale in the glucose condition (median= 4.5) compared to the water condition (median= 7), p= .033) (Carbajal *et al.*, 2002).

Sucrose versus other pain relieving interventions. Studies comparing sucrose and glucose solutions report similar effects in reducing the pain response of term infants and preterm infants. In a randomized controlled trial, crying time and pain scores on the NFCS showed a significant difference (p< .00) between 5 interventions: sterile water; dextrose (D-glucose) 12.5%; sucrose 12.5%; dextrose 12.5% with pacifier; and sucrose 12.5% with pacifier. The median crying time in the groups was 132, 102, 92.5, 55, and 16.5 seconds, and mean pain scores were 6.2, 5.5, 3.4, 2.6 and 1.7 respectively. Sucrose and pacifier had the shorter crying time and lowest pain score followed by dextrose and pacifier, with no significant difference between the two (Akman, Ozek, Bilgen, Ozdogan, & Cebeci, 2002).

A randomized controlled trial measuring heart rate before, during and three minutes after heel lance in healthy term infants, could not find a statistically significant difference between no treatment, water, glucose at 5%, 33% and 50%, and sucrose at 33% and 50% (Guala *et al.*, 2001). A similar result was reported in another study where no difference was found between 2ml of 30% solution sucrose, 10% glucose, 30% glucose and water, regarding maximum heart rate after heel lance (p= .71) and mean recovery time (p= .09) (Isik *et al.*, 2000). In this study, a borderline significance was found only at 2 minutes after heel lance in percent change in heart rate, favoring sucrose (p= .05).

Comparing breastfeeding to 1ml of 24% sucrose via syringe before heel lance, infants who were breastfed showed significant less crying, lower median PIPP scores, lower median increase in heart rate and lower median decrease in oxygen saturation (Codipietro *et al.*, 2008) suggesting that breastfeeding is more effective than sucrose.

Glucose has also been compared to EMLA™ cream (Gradin, Eriksson, Holmqvist, Holstein, & Schollin, 2002). In a randomized controlled trial, fullterm infants given 1ml 30% glucose for venepuncture had a lower mean PIPP score compared to 0.5g of EMLA™ cream (4.6 vs 5.7, $p = .0314$). The median for duration of crying in the first 3 minutes was also significantly shorter (1 second vs 18 seconds), $p < .00$. Sweet-taste solutions can therefore be considered more efficacious than EMLA™ cream for venepuncture in fullterm neonates.

Combining sweet solutions to other interventions. The additive effect of sucrose and local anesthetic cream (EMLA™) was studied in fullterm infants during venepuncture (Abad *et al.*, 2001). Both 2ml of 24% sucrose alone and the same amount of sucrose with 1g of EMLA™ cream reduced crying time significantly ($p = .008$) but the use of EMLA™ did not enhance the analgesic effect of sucrose.

In preterm infants during subcutaneous injections of erythropoietin, a crossover trial with four groups of interventions: 1) pacifier alone; 2) pacifier and 0.2 - 0.5ml 30% sucrose solution; 3) pacifier and 0.5g of EMLA™ cream; 4) pacifier, 0.2 - 0.5 ml 30% sucrose and 0.5g of EMLA™ cream showed that the analgesic effect of sucrose is greater than the effect of EMLA™, and that the combination was more efficacious than sucrose or EMLA™ alone (Mucignat *et al.*, 2004).

In fullterm neonates, Bellieni and colleagues (2002) used 33% glucose in combination with sensorial saturation and essentially obliterated the pain response according to the DAN pain scale (Carbajal *et al.*,1997) and the amount of crying, which was negligible. When glucose was not a component of the sensorial saturation, the effect was not significant compared to control. Finally, this group tested the feasibility of training mothers to use sensorial saturation with the removal of baby-oil to scent the hands, and found that they were as effective as highly trained staff and more effective than glucose and pacifier by two points on their pain score based on crying (Bellieni *et al.*, 2007).

Concentration, volume and mode of delivery. Concentrations of sucrose have varied from 12% to 50%. Although one study reports a flat dose-response function (Blass & Shah, 1995), there seems to be a dose-response effect in the reduction of crying with increasing concentration of sucrose. In the 3 minutes after venepuncture, a significant difference in duration of cry was found between preterm infants given water (72.9 seconds) compared to 2ml of 24% sucrose (19.1 seconds, $p < .05$) but not compared to those given 2ml of 12% sucrose (63.1 seconds) (Abad *et al.*, 1996). In term neonates, concentrations on 12,5%, 25% and 50% sucrose were compared to water (Haouari *et al.*, 1995). In the first minute after heel lance, time crying was significantly less in the 50% sucrose

group (35 secs) compared to water (60 secs, p= .02); in the second minute, the difference was significant for the 50% sucrose group (0 secs, p= .003) and for the 25% sucrose group (18 secs, p= .02). Difference in total time crying was significant for the 50% sucrose group only (p= .02). On the Cochrane review no statistically significant benefit in concentrations higher than 0.50g (2ml of 25% solution) were found (Stevens *et al.*, 2010).

Different concentrations of glucose have also been tried although the most common is 30%. Compared to a 10% solution, a 25% solution significantly reduced duration of first cry (Deshmukh & Udani, 2002).

Small volumes of sucrose administered in a single dose were 0.05ml (Johnston *et al.*, 1999; Johnston *et al.*, 1997b), 0.5ml (Gibbins *et al.*, 2002), 1ml (Storm & Fremming, 2002; Ramenghi, Wood, Griffith, & Levene, 1996) and 2ml in most other studies.

The time needed to obtain the effect was studied by Blass & Shah (1995) by imposing a delay of 30, 60, 90, 120 and 240 seconds between sucrose intake and the initiation of blood collection. The 2 minutes interval was the most effective time delay which according to the authors corresponds to the endogenous opioids release triggered by sweet taste. Studies using glucose have used the same interval assuming that the mechanism of sweet-taste is the same.

The delivery methods studied include giving the solution via syringe into the baby's mouth, specifically on the anterior surface of the infant's tongue, followed or not by offering a pacifier, or through offering a pacifier dipped in the sweet solution. The use of a pipette has also been reported (Gormally *et al.*, 2001). Duration of administration reported has varied between 15 seconds to two minutes. One study has used 30% glucose on a spray bottle, each puff delivering 0,17ml of solution. No difference in pain scores was found between the use of this method and the use of a syringe. The authors argue that it is well accepted by the neonates and that it is an easier way to deliver sweet solutions (Akcam & Ormeci, 2004).

Sucrose has also been used in 3 aliquots of 0.05ml (Johnston *et al.*, 1999) and 0.1ml (Boyer *et al.*, 2004; Johnston *et al.*, 2002b) two minutes before heel lance, just prior to lancing and two minutes after lancing; and in doses of 0.25ml given three times at 30 seconds intervals (Gormally *et al.*, 2001). In one study a pacifier dipped in a 24% sucrose solution, estimated to deliver 0.1ml, was given 5 and 2 minutes prior to heel lance (Stevens *et al.*, 1999). These small volumes in repeated aliquots appear to be effective.

The recommendation in the Cochrane review (Stevens *et al.*, 2010) is that 0.012 to 0.12 g of sucrose should be administered approximately two minutes prior to single heel lances and considered for use with venepunctures for pain relief in neonates. Since sucrose was found to reduce composite measures of pain in approximately 20%, addi-

tional pain relief measures, including non-nutritive sucking, are recommended to significantly reduce or eliminate pain in this population (Stevens *et al.*, 2004). As will be described below, adding a pacifier improves the effect of sweet solutions.

Adverse effects. A few studies have evaluated the occurrence of immediate adverse effects of sweet-solutions.

Blood levels of glucose were monitored in infants of diabetic mothers that had 2ml of 24% sucrose for three consecutive heel lances and no difference was found when compared to infants given a placebo (Taddio *et al.*, 2008).

Minor side effects of sucrose, such as retching, gagging and oxygen desaturation occurred in one study (Gibbins *et al.*, 2002), which were considered not clinically significant because they resolved in seconds, with no need for intervention. In another report, when the following adverse events were considered: choking, coughing, or vomiting, sustained tachycardia (heart rate>200) or bradycardia (heart rate< 80) for longer than 15 seconds; sustained tachypnea (respiratory rate> 80) or dyspnea (respiratory rate< 20) for longer than15 seconds; or sustained oxygen desaturation of< 80% for longer than 15 seconds, the youngest infants (27 to 32 weeks gestational age) did have a higher incidence of immediate adverse effects compared to infants 32 weeks and more but none of the adverse events occurred in the infants given sucrose (Gibbins & Stevens, 2003).

The effects of using sucrose routinely on consecutive days have been addressed and need further investigation. While one study of sucrose for all painful procedures in the first week of life in the NICU reported poorer neurodevelopmental scores with high doses of sucrose (Johnston *et al.*, 2002b), a secondary analysis found that this was the case in infants who had received more than 10 doses over 24 hours (Johnston *et al.*, 2007b). Of note, the infants in this study were less than 31 weeks gestational age on entry into the study. Another study evaluating the routine use of sucrose over four weeks in the NICU did not find a higher incidence of intraventricular hemorrhage, nor neurobiological risk (Stevens *et al.*, 2005).

One of the most feared adverse effects of sucrose is necrotizing enterocolitis. None of the studies examining adverse outcomes (Gaspardo *et al.*, 2005; Stevens *et al.*, 2005; Acharya *et al.*, 2004; (Ramenghi *et al.*, 1996b) has reported this.

Sucrose seems to maintain its efficacy after repeated use (Boyer *et al.*, 2004; Mucignat *et al.*, 2004; Stevens *et al.*, 2005; Taddio *et al.*, 2008).

tion of the substance in the gut that produces analgesia, since the administration of sucrose in the stomach does not reduce pain responses (Ramenghi, Evans, & Levene, 1999). It is therefore considered a pre-absorptive rather then a post-ingestion mechanism.

The hypothesis that it is mediated through opioid systems is strongly supported by the reversibility of the analgesic effect of sucrose and glucose by naltrexone in animal studies (Blass & Ciaramitaro, 1994) although this blocking effect has not been found in infants given 1ml 30% glucose orally and 0,01mg/kg of naloxone intravenous (Gradin & Schollin, 2005). Sweet taste solutions seem to trigger the release of beta-endorphin in the brain which plays an important role in nociception (Bach, 1997). In human neonates whose mothers were treated with methadone and therefore did not have functional opioid systems, sucrose did not produce a calming effect suggesting that sucrose analgesia is mediated by the same pathway (Blass & Ciaramitaro, 1994). Also, like opioid analgesia, sucrose has a slow onset and a slow offset of the effect that remains after the administration, when compared to other stimuli like oro-tactile stimulation. More recently, using immunohistochemistry for the protein product of the immediate early gene c-fos, to identify sites involved in the analgesic effect of sucrose in neonate rats, Anseloni and team (2005) have demonstrated that sucrose-induced analgesia is mediated by circuitry within the brainstem and does not require involvement of forebrain structures. Infusion of small, analgesic volumes of sucrose activated brainstem neurons in several areas implicated in centrally mediated analgesia, including opiate receptor-dependent analgesia.

Arguments against an opioid-mediated mechanism would be that serum levels of beta-endorphin have not increased 2 to 5 minutes after the administration of 0.1 to 0.15g/kg of a 30% sucrose solution (0.2 to 0.75ml) in preterm infants (26 to 29 weeks gestational age), in the absence of a painful procedure (Taddio, Shah, Shah, & Katz, 2003). Several explanations for this result are put forward by the researchers: the absence of a painful stimulation; improper timing of blood sampling, being insufficient to detect concentration changes in the peripheral circulation although this was the time window where the analgesic effect would be present; a lack of correlation between the concentration of beta-endorphin in the blood and in the receptors located in the central nervous system; the mediation of other endogenous opioids than beta-endorphin. To these explanations, we could add that the very low gestational age of the infants influencing the maturity of the descending inhibitory system may hinder their capacity to produce a significant amount of beta-endorphin.

In animal models, analgesia elicited by intra-oral sucrose as well as milk, is age-dependent and limited to the pre-weaning period in rats. As if, from a developmental perspective, the analgesia induced by sucrose and sweet components of milk were a natural defense mechanism ceasing to be functional when the animal's locomotor activity enables him to escape by himself from threatening or tissue-damaging situations (Anseloni *et al.*, 2002).

Non-nutritive sucking

Among Non-pharmacological interventions, non-nutritive sucking (NNS) was the first to be studied in the mid 1980s. It refers to the placement of a pacifier in the infant's mouth to promote sucking behavior in the absence of breast or formula milk. Its use in term and preterm infants treated in neonatal intensive care and in minimal care shows that behavioral distress, namely percent time spent in fussing and crying state, is reduced during and after heelstick (Field & Goldson, 1984).

The same findings were obtained in a crossover trial enrolling 26 term and preterm neonates in intensive care. The percentage of time crying before, during and after heel lance was reduced by using a pacifier compared to no pacifier ($p< .00$) (Corbo et al., 2000).

Compared to no intervention, pacifier with sucrose was found to be more effective using the PIPP score (MD= 1.92, $p< .0001$) and so was pacifier with distilled water (PIPP score MD= 1.37, $p< .0006$) (Stevens et al., 1999).

The effect on oxygen levels are contradictory as they were noted to have no change by Corbo et al., (2000) but considered to be improved by Shiao (1997). A meta-analysis (Shiao et al., 1997) of studies from the past 30 years on the effects of NNS on heart rate and transcutaneous oxygen tension (TcPaO2) found that NNS significantly reduced heart rate both in the presence and absence of painful stimulation and significantly increased TcPaO2. The total weighted effect size for heart rate during the painful stimulation was large and the effects were larger in preterm than in term neonates.

One of the first studies to compare pacifiers to another intervention, namely swaddling, was Campos (1989) in a previously cited study in two week-old infants. Used after heel lance, infants with pacifier interrupted crying earlier (23.2 seconds) than swaddled infants (58.7 seconds, $p< .1$). The decline in heart rate was also faster in the pacifier group.

Compared to glucose, pacifiers in term infants during heel lance seem to be more effective (Carbajal *et al.*, 1999). In a randomized controlled trial in term newborns (n= 150) undergoing venepuncture comparing water, 30% glucose, 30% sucrose, pacifier and 30% sucrose with pacifier, the median pain scores with the DAN were, respectively, 7, 5, 5, 2 and 1. While the difference between glucose and pacifier (MD= 3, 95% CI [2, 5), and between sucrose and pacifier (MD= 3, 95% CI [1, 5]) were significant in favor of pacifier (p= .0001 and p= .001, respectively), the addition of sucrose to pacifier slightly reduced the pain score but not significantly (MD= 1, 95% CI [0, 2]), p= .06.

Contrariwise, in a small sample of very preterm babies (n= 15), adding a pacifi-

er to glucose for subcutaneous injection did not reduce pain score compared to glucose alone (Carbajal *et al.*, 2002).

Bellieni and colleagues (2001) compared the effect of a pacifier with 10 % oral glucose to no intervention, sensorial saturation, 10% glucose, and pacifier alone for heel lance in preterm neonates. Sensorial saturation is an intervention that includes touch, massage, taste, voice, smell, sight and glucose with pacifier. The pacifier with 10% glucose was more effective than no intervention ($p< .001$), with no significant difference compared to glucose alone or pacifier alone. The effect of pacifier with 10% glucose though, was exceeded by that of sensorial saturation, which obtained the lowest PIPP scores ($p< .01$).

Conflicting results between these studies regarding the additive effect of sweet solutions and pacifiers may be due to different populations studied and different concentrations of sweet solutions.

Unlike oro-gustatory stimulation by sweet taste, the mechanism behind the calming effect of non-nutritive sucking, an oro-tactile stimulation, seems to be non-opioid mediated. The immediate onset of the effect and its rapid decay, infants resuming crying in the seconds following the removal of the pacifier, are in favor of this hypothesis (Blass & Ciaramitaro, 1994). During heel lance, the analgesic effect of sucking an unflavored pacifier was reported to occur only when the rate of sucking was over 30 sucks/minute (Blass & Watt, 1999). It is possible that sucking is such a strong source of sensory stimulation that it blocks the perception of pain. Another hypothesis is that sucking promotes self-regulation, allowing the infants to regulate the pain response through regulation of their own activity of sucking (Carbajal *et al.*, 1999). As evidenced by several studies described above, adding sweet taste to pacifier, i.e., an orogustatory to an orotactile stimulus, although involving different pathways, has an additive effect in human fullterm infants (Akman *et al.*, 2002; Carbajal *et al.*, 1999; Blass & Hoffmeyer, 1991) as well as in preterms infants (Stevens *et al.*, 2005; Gibbins *et al.*, 2002; Stevens *et al.*, 1999) although in this age group the effect might be less evident (Carbajal *et al.*, 2002).

Kangaroo Mother Care

While Non-pharmacological interventions like non-nutritive sucking and sucrose have been known for a long time (Field & Goldson, 1984; Blass & Hoffmeyer, 1991) the interest in Kangaroo Care for pain is recent.

Originating in Colombia, in 1978, kangaroo mother care (KMC), described as mother holding the baby naked with only a diaper in prone up-right position against her bare breasts, was used to improve the survival of premature babies in the context

of scarce technological resources for neonatal care and it was first described by Rey & Martinez in 1983 (Charpak *et al.*, 2005).

This low cost alternative to conventional care originally consisted of keeping low birth weight neonates (LBW), i.e. infants born with less than 2500g irrespective of gestational age, in skin-to-skin contact with their mothers' chest for 24 hours a day if possible. It could start at birth or on the moment the infants were stabilized and it would last until 40 weeks of gestational age or as long as the infant would tolerate it (Cattaneo, Davanzo, Bergman, & Charpak, 1998). The new method reduced major problems of LBW infants such as difficulty in keeping body temperature, need for respiratory stimulation, infection, and it facilitated exclusive breastfeeding. Initiated in hospital it could continue at home allowing for an earlier discharge.

Once the question of safety was established, kangaroo care (KC) expanded to Neonatal Intensive Care Units in developed countries in the 80's and early 90's. It was adjusted to meet the specific needs encountered by stable preterm infants and their parents, in settings with ample resources. As a result, the term KC became common to name any skin-to-skin chest contact independently of its duration (1 to 24 hours/day) and length of use (daily or intermittent). Fathers and even the staff from the NICU became involved in performing KC, which is now widely used in Neonatal Intensive Care Units across the world in both developing and developed countries.

Stability of cardiac, respiratory and thermal function as well as oxygen consumption, metabolic rate, energy balance and weight gain have been shown to be some of the benefits from KC. Chwo and colleagues (2002) found higher temperature, more quiet sleep and less crying in preterm infants assigned to KC the day after birth although there was no difference in weight gain nor in length of stay.

Mörelius team (2005) examined the effect of KC on 17 mother/preterm infant dyads, namely mothers' salivary cortisol, heart rate, stress and mood and infants' salivary cortisol, heart rate and pain scores. During and after KC, mothers' salivary cortisol, heart rate and stress measured on a visual analogue scale (VAS) decreased significantly compared to pre skin-to-skin contact, while mood increased. As for the effect on the infants, heart rate and pain scores decreased during KC while variations in salivary cortisol were inconclusive, probably due to an immature control of HPA axis.

The effects of KC on infants' pain response were first studied in fullterm infants (Gray, Watt, & Blass, 2000). In a randomized controlled trial, 10-15 minutes KC reduced crying by 82%, and grimacing by 65% compared to infants who stayed in the crib during heel lance. Significant differences persisted in the 3-minute recovery period. The increase of heart rate in beats per minute, although present in both groups, was smaller in the KC group (8-10 vs 36-38). In another recent trial, ten minutes of KC

prior to intramuscular Vitamin K injection was shown to effectively reduce behavioral pain response in healthy fullterm newborns less than 2 hours old (Kashaninia, Sajedi, Rahgozar, & Noghabi, 2008). Neonatal Infant Pain scores measured immediately following the injection were significantly lower ($p< .001$) in the KC group compared to controls. Pain scores>3, representative of moderate to severe pain, were recorded 98% of the time in the control infants compared to 38% for KC infants. Mean duration of crying was also longer, 24.61 in the control group versus 14.55 seconds in the KC group.

The first study in preterm infants 32 to 36 weeks gestational age was in 2003 (Johnston *et al.*, 2003) and other studies followed. These studies consistently show that KC significantly reduces PIPP scores during and after the painful procedure. A 2 point reduction was found at 30, 60 and 90 seconds after heel lance in one study (Johnston *et al.*, 2003) and at 90 seconds ($p< .001$) in another study with very preterm infants (Johnston *et al.*, 2008b). An even larger difference was found by Akcan, Yigit, & Atici (2009) in preterm infants at 1, 2 and 3 minutes after heel lance or venepuncture ($M= 7$, 4 and 4 in the KC group and $M= 15$, $p< .001$, 15.5, $p= .001$ and 15, $p= .047$ in the control group).

The effect on facial actions is also clear. Significant lower scores were found in the NFCS at heel lance (difference= -1.140, $p= .023$) and heel squeezing (difference= -1.872, $p= .001$) (Castral, Warnock, Leite, Haas, & Scochi, 2008).

Crying length during the lance and after the lance was less in infants in KC than in controls ($p= .003$ during and $p= .02$ after) (Ludington-Hoe, Hosseini, & Torowicz, 2005) and in another study it was 55 seconds vs 96.2 seconds during heel lance ($p= .001$) and 5.8 versus 25.5 during recovery ($p< .01$) (Kostandy *et al.*, 2008). The mean duration of cry was reduced by 37.4% in infants after 15 minutes in KC ($M= 2.5$ minutes) compared to infants swaddled in the crib ($M= 4.8$ minutes, $p= .024$) (Castral *et al.*, 2008).

As in studies of other interventions, the effect on heart rate did not always reach statistical significance in spite of a lower mean in the KC group (Castral *et al.*, 2008). However, in very preterm infants in KC, average heart rate was significantly lower at 30, 60, and 90 seconds post-heel lance and average oxygen saturation levels were significantly higher at 60 and 90 seconds post-heel lance compared to infants in the incubator (Johnston *et al.*, 2008b). In this trial, the time for heart rate to return to baseline after the end of the procedure was shorter ($M= 123$ seconds, 95% CI [103,142] for the KC condition and $M= 193$ seconds for the incubator condition, 95% CI [158, 227]), $p< .001$, showing that in the context of stress caused by pain, KC contributes to energy conservation and homeostasis maintenance which are of major importance for preterm infants' growth and development. Recently, in a study examining the effect of KC

on autonomic stability during heel lance in very preterm infants (30-32 weeks) (Cong, Ludington-Hoe, McCain, & Fu, 2009), heart rate variability was significantly more stable in infants in the KC condition compared to infants in the incubator. HRV differences between KC and incubator were that LF was higher in KC at baseline ($p< .01$) and at heel lance ($p< .001$), and HF was higher in KC condition than in the incubator condition ($p< .05$). The LF/HF ratio had less fluctuation across the periods in KC than in incubator condition and was significantly lower during recovery in KC than in incubator ($p< .001$) (Cong *et al.*, 2009).

Neurobehavioral assessment of preterm neonates (28-34 weeks gestational age) using the naturalistic behavioral observation proposed by the NIDCAP, has shown that neonates in KC during heel lance exhibit less motor disorganization and extension movements and an increase in attention signs (Ferber & Makhoul, 2008). These changes were sustained for at least one hour after cessation of the KC.

In a trial comparing placebo, oral glucose and KC for heel lance (Freire *et al.*, 2008), there was a significant difference between the three groups in PIPP scores for facial action, heart rate changes and oxygen saturation changes with lower scores on the KC group. The difference between KC and glucose, however, was not examined in the report.

The addition of rocking, singing and sucking in infants 32 to 36 weeks gestational age, did not prove better than KC alone (Johnston *et al.*, 2008a). However, in term newborns (N= 640) for intramuscular injection of Hepatitis B vaccine, the combination of kangaroo care with 1ml of 25% Dextrose was found to significantly reduce NFCS and NIPS scores compared to either intervention alone or no intervention, and PIPP scores were lower in KC regardless of whether dextrose was administered (Chermont, Falcao, Silva, Balda, & Guinsburg, 2009).

A summary of the studies examining KC for pain can be found in Table 3.

Table 3 - Studies examining the effect of Kangaroo Mother Care on pain responses of term and preterm infants during heel lance and venepuncture

Study	Participants	Design and Intervention	Outcome Measures	Results in KMC
Gray 2000	30 healthy fullterm infants>37 WGA	Randomized controlled trial. 1) KMC 10–15 minutes before and during heel lance 2) swaddled in crib during heel lance	Duration of cry Grimacing HR	Cry reduced by 82%. Grimacing reduced by 65%. Smaller increase in beats/minute during blood collection (8–10 versus 36–38).

Study	Participants	Design and Intervention	Outcome Measures	Results in KMC
Kashaninia 2008	100 healthy fullterm infants GA:>37 weeks	Randomized controlled trial. 1) KMC 10 minutes before and during intramuscular injection 2) cot before and during intramuscular injection	Duration of cry NIPS score	Mean duration of crying was longer, 24.61 in the control group versus 14.55 seconds in the KMC group. NIPS scores significantly lower immediately following injection in KMC, $p < .001$.
Johnston 2003	74 preterm infants GA: 32-36 weeks	Randomized, single-blind, cross-over trial. 1) KMC 30 minutes before and during heel lance 2) swaddled in incubator before and during heel lance	PIPP: facial actions, heart rate, oxygen saturation, gestational age, behavioral state	PIPP scores significantly lower by two points at 30, 60, and 90 seconds after lancing.
Ludington-Hoe 2005	23 preterm infants GA: < 37 weeks (mean= 31,4)	Randomized cross-over trial. 1) KMC three hours before and during heel lance 2) incubator before and during heel lance before and during heel lance	Heart rate Respiratory rate Oxygen saturation Length of crying Behavioral state	Lower mean rise in HR from baseline to lance, during lance, and post procedure. No differences in respiratory rate and oxygen saturation More time in quiet/sleep states.
Castral 2008	59 preterm infants GA: 30–37 weeks	Randomized controlled trial. 1)KMC 15 minutes before and during heel lance 2)swaddled in incubator or crib	NFCS score Behavioral state Duration of cry Heart rate	NFCS score significantly lower at heel lance (-1.140; $p = .23$) and squeeze phase (-1.872; $p < .001$). Cry reduction by 37.4%.No difference in HR.
Johnston 2008 b	61 very preterm infants GA: 28-31 weeks	Single-blind randomized cross-over trial. 1)KMC 15 minutes before and during heel lance 2)swaddled in incubator	PIPP Time to recover (heart rate return to baseline) Facial actions Heart rate Oxygen saturation	PIPP scores lower at 90 seconds (8.871 versus 1. 677; $p < .001$). Time to recover shorter ($p < .0000$). Facial actions significantly fewer across the procedure. HR lower across the first 90 seconds. Oxygen saturation higher at 60 seconds.
Kostandy 2008	10 preterm infants GA: 30–32 weeks	Randomized cross-over trial. 1) KMC 30 minutes before and during heel lance 2) nested in incubator	Audible and inaudible crying (Anderson Behavioral State Scoring system)	Less combined crying time during heel stick (55 versus 96.2 seconds; $p = .001$) and during recovery (5.8 versus 25.5 seconds; $p < .01$). Inaudible cry was minimal in each phase, in both conditions 0–1.34 seconds.
Freire 2008	95 preterm neonates GA: 28–36 weeks	Randomized controlled trial. KMC 15 minutes before and during heel lance oral glucose prone position in incubator and oral glucose	Behavioral state, HR variation, oxygen saturation, PIPP scores for facial actions	No difference in behavioral state, smaller variation in ($p < .0001$) and HR in oxygen saturation ($p < .0012$), lower scores for facial actions ($p < .0001$).

Study	Participants	Design and Intervention	Outcome Measures	Results in KMC
Johnston 2008 a	90 preterm infants GA: 32-36 weeks	Randomized, single-blind cross-over trial. 1)KMC with additional rocking, singing, and sucking 2)KMC without additional stimulation	PIPP, time to recover	No significant differences in PIPP scores or time to recover significant, differences across sites.
Cong 2009	14 preterm GA: 30-32 weeks	Single-blind randomized cross-over trial. KMC 60 minutes before and during heel lance incubator	Infant behavioral state, HR, HRV	No difference in behavioral state. HR lower in KC at baseline (p< .05) and heelstick (p< .05). HRV more stable in KM at baseline (p< .01) and at heel lance (p< .001).
Ferber 2008	30 preterm infants GA: 28 -34 weeks	Randomized, single-blind, cross-over trial. 1)KMC before and during blood stick 2)KMC without blood stick 3) incubator during blood stick 4) incubator without blood stick	NIDCAP	Less motor disorganization and extension movements and an increase in attention signs during KMC.
Akan 2009	GA: 26-36 weeks	Randomized, controlled trial KMC 30 minutes before, during and 10 minutes after venepuncture or heel lance incubator	PIPP	Lower PIPP scores at 1st (p< .001), 2nd (p= .001) and 3rd minute (p= .047) of the procedure. Lower PIPP scores at 1st (p< .001) and 2nd minute (p= .023) after the procedure.
Chermont 2009	640 healthy fullterm infants	Randomized, single-blind, controlled trial. Standard care KMC 2 minutes before and during intramuscular injection of vaccine 1ml Dextrose 25% KMC 2 minutes before and during intramuscular injection of vaccine and 1ml 25% Dextrose during injection	NFCS NIPS PIPP	During the procedure, the combination of KMC and Dextrose was more effective than either interventiion alone (p< .001). After the procedure, NFCS and NIPS lower in KMC compared to Dextrose (p= .045) PIPP scores lower in KMC with or without Dextrose

Abbreviations: WGA, weeks gestational age; HR, heart rate; KMC, kangaroo mother care; GA, gestational age; NIPS, Neonatal Infant Pain Score; NFCS, Neonatal Facial Coding System; PIPP, Premature Infant Pain Profile; HRV, heart rate variability; NIDCAP, Newborn Individualized Developmental Care Assessment Program.

The underlying mechanisms that support the effectiveness of skin-to-skin contact in reducing reactivity to painful stimulus are not yet fully understood. Contact with siblings or an anesthetized female increased thermal withdrawal latencies in rat pups compared to pups in isolation (Blass, Shide, Zaw-Mon, & Sorrentino, 1995). The protection from contact was not reversed by naltrexone in a dose previously shown to reverse morphine-induced behavioral changes.

There is no doubt that KMC combines several kinds of stimulation: containment in mother's arms, vestibular stimulation produced by mother's breathing movements, recognition of mother's voice and smell, tactile stimulation from contact with mother's skin.

A deactivation of the Hypothalamus-Pituitary-Adrenal axis and a release of oxitocyn might be consequence of maternal touch and proximity (Ludington-Hoe *et al.*, 2005; Johnston *et al.*, 2003). An increase of opioid peptide secretion may also be present linked to the stimulation of the olfactory system. Skin-to-skin contact is considered by Gray, Watt and Blass (2000) to be the third component of the nursing-suckling relationship together with suckling per se and taste/flavor of milk. Considering that the first two components have analgesic effects, it would not be surprising that the third component would too. Behavioral state regulation is improved in KC, which promotes the sleep state during which pain responses are blunted. This involvement of the serotoninergic system is also likely to play a role in pain modulation. Thus, different pathways, opioid and non-opioid mediated, might be involved in the overall effect of KMC.

Given the efficacy of numerous Non-pharmacological interventions (Cignacco *et al.*, 2007) for procedural pain in neonates and the difficulties with pharmacological agents in this population, for common painful procedures such as heel lance and venepuncture, Non-pharmacological interventions should be the first choice in uncompromised infants (American Academy of Pediatrics *et al.*, 2006). Within the framework of developmental care, we have described compelling evidence supporting the use of interventions that promote self-regulation and provide oro-tactile, oro-gustatory and touch stimulation, capable of reducing the pain responses of infants during the most common painful procedures in neonatal care. These interventions are focused on creating a favorable environment, promoting confort and are family-centered, since mothers are clearly implicated in breastfeeding and kangaroo care, but they can also be included in other interventions like facilitated-tucking, holding a pacifier in the infants' mouth or in sensorial saturation.

Parents find pain one of the most distressing aspects of the neonatal intensive care unit (Gale, Franck, Kools, & Lynch, 2004) and wish to actively participate in comforting their infants (Franck, Cox, Allen, & Winter, 2004). Using these strategies may empower parents and reduce their feelings of helplessness, while attaining the goal of reducing infants' procedural pain.

CHAPTER 5.
Parents and pain in the NICU

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It is known for several decades that mothers of preterm babies go through a complex process of adjustment to the new situation. Feelings of sadness, anger, fear and anxiety are experienced by parents of very low birth weight babies (Eriksson & Pehrsson, 2005). While concerns with infection kept parents away from the units for many years, today, a global family-centered philosophy of developmental care encourages parents to stay and care for their infants. But having their baby admitted to a NICU is, in itself, a stressful experience for parents: to be confronted to the baby's unexpected appearance and behavior, the exposure to the highly sophisticated equipment of the NICU environment, the inability to perform their expected role as parents, the communication with the staff are major sources of stress for mothers and fathers of preterm babies (Gale et al., 2004; Holditch-Davis & Miles, 2000; Shields-Poe & Pinelli, 1997; Miles, Funk, & Carlson, 1993).

Besides the appearance and behavior of the infant, an important source of stress is their inability to fulfill their parental role as primary care providers to the infant and particularly in protecting the child from pain (Franck et al., 2004). Forty-four mothers of three year-old children born prematurely could vividly report their memories of stress among which the pain and the procedures endured by the infant, as well as the alteration as their role as parents, were main sources of stress (Wereszczak, Miles, & Holditch-Davis, 1997).

In human infants, the effects of early separation from mothers are not well known but animal studies use maternal separation as a model of early stress and show an array of changes in gene expression and brain architecture, as well as in the development of peripheral immune systems, as a consequence of a daily separation of a few minutes to hours (Daniels et al., 2009). On the other hand, the deleterious effects of early stress, whether from separation or from pain, have been presented earlier in this review, and warrant the promotion of contact between preterm infant and mother.

Promoting maternal-infant attachment by encouraging early interaction between mother and infant can be done in a number of ways, which include participating in care, holding conventionally and holding in skin-to-skin contact (Franck, Bernal, & Gale, 2002). Parents' involvement in caring for their babies in the neonatal unit is thought to improve parent-infant attachment and to moderate the psychological stress for parents(Franck & Spencer, 2003; Browne & Talmi, 2005). Yet, emotional state as well as physical condition may not favor a positive interaction of the mother with her baby. A feeling of exclusion often dominates when the new mother feels a lack of interaction and a sense of not belonging to either the maternity care unit or the NICU (Wigert, Johansson, Berg & Hellstrom, 2006).

Holding the baby skin-to-skin is experienced by mothers as a way to complete the preterm newborn's growth and development, and a way to develop skills in caring for the child (Furlan, Scochi, & Furtado, 2003). Feelings of "being a mother" and "being able to care" illustrate the feeling of empowerment that kangaroo care may bring to mothers.

Later effects of providing maternal-infant body contact during the stay in a neonatal nursery on parent-infant and triadic interactions have also been examined (Feldman, Weller, Sirota, & Eidelman, 2003). At three-months, mothers and fathers who received kangaroo care were more sensitive and less intrusive, infants showed less negative affect, and family style was more cohesive. The authors highlight the role of touch as a constituent of the co-regulatory parent-infant and triadic systems and the effects of maternal contact on mothering and co-parenting.

A vast amount of literature points out the benefits of skin-to-skin care for mothers and infants, although very different outcomes make it difficult to reach a consensus in recommending its routine use in low birth weight infants (Conde-Agudelo, Diaz-Rossello & Belizan, 2003). Yet, in some developing and developed countries, kangaroo care is used in a continuous or intermittent way (Ruiz-Pelaez, Charpak, & Cuervo, 2004), combining the presence with the participation in care.

While the participation in tasks like feeding or cleaning is easily accepted by parents and staff, parental presence during painful procedures is controversial. When an invasive procedure on the baby is needed, namely heel lance or venepuncture, kangaroo care may have to be interrupted to spare the parents from seeing the procedure been done on the baby. It is often argued by the staff that parents do not wish to and should not attend their child during medical painful procedures because of concerns with safety, parents' emotions and performance anxiety. However, the opposite is suggested by studies that have investigated the parents' wish to be present.

Out of four-hundred parents inquired in the waiting area of an emergency department, 97.5% wished to be present if their child was having a venepuncture, 94% if it was a laceration repair, 86.5% if it was a lumbar puncture (Boie, Moore, Brummett, & Nelson, 1999). A majority of emergency physicians and nurses indicated parents should be present for some invasive pediatric procedures. However, as the invasiveness of the pediatric procedures increased, fewer physicians and nurses believed that parents should be present (Beckman *et al.*, 2002). Similar conclusions were presented in a study involving 104 clinicians from a pediatric emergency department (Fein, Ganesh, & Alpern, 2004), where parental presence was accepted for minor procedures but in highly invasive procedures, like chest tube placement and resuscitations, was supported by most attending physicians and nurses but not by residents. Another survey of clinicians, family members and patients, in a 300-bed urban academic hospital that included the neonatal intensive care unit, found that patients and families had a positive attitude toward family presence during invasive procedures and even resuscitation of their child/family member (Duran, Oman, Abel, Koziel, & Szymanski, 2007).

To our knowledge, the mothers' view on doing kangaroo care while the baby is having a painful procedure has not been explored at deserves to be so, in order to guide professional practice.

PART II
Empirical study: Kangaroo mother care, sucrose and pacifier vs sucrose and pacifier

CHAPTER 6.
Methods

PART II - Empirical study:
Kangaroo mother care, sucrose and pacifier vs sucrose and pacifier

CHAPTER 6. Methods

6.1 Research design

As stated earlier, the main objective of this research was to compare the efficacy of the combination of Kangaroo Mother Care, sucrose and pacifier, with that of sucrose and pacifier, in reducing the pain responses of preterm infants undergoing venepuncture in the Neonatal Intensive Care Unit. A randomized controlled trial was undertaken to respond to this main objective. In each data collection site, infants were randomly assigned to receive one of two interventions during venepuncture for blood draw:

1) 0.1 ml of oral sucrose with pacifier (Sucrose), or

2) 0.1 ml of oral sucrose with pacifier combined with maternal skin-to-skin contact or Kangaroo Mother Care (S+KMC).

Besides the main objective, two secondary objectives were devised: to identify the relation between maternal anxiety and the pain responses of preterm babies in KMC, for which a cross-sectional study was undertaken; and to explore mothers' perceptions of doing KMC during venepuncture, through content analysis of the interviews with mothers.

6.2 Research setting

The study was conducted in two level II/III Neonatal Intensive Care Units in Coimbra, Portugal, which will be referred to as Site 1 and Site 2. Each of these units is attached to a level III Hospital, one of them being a University Hospital, and together they cover a region with 2 383 284 inhabitants. Main health indicators related to birth are displayed in Table 4.

Site 1 has 9 intensive care beds and 6 special care beds. In 2008, there were 328 admissions with a mean duration of stay of 12.3 days. The occupation rate was 71%. Fifty-four infants (16.46%) were born at 30 weeks gestational age or less. Main diagnoses were prematurity, respiratory distress and hyperbilirubinemia. Thirty-two percent of the infants (108) were ventilated, 33 of which under conventional ventilation and the rest in continuous positive airway pressure (CPAP) (Hospitais da Universidade de Coimbra, EPE, 2009).

Table 4 - Main health indicators related to birth in 2008 in the Centro region of Portugal

Indicator	Rate
Birth rate a)	8.5%
Preterm rate	9.8%
Rate of low birth weight	7,5%
Perinatal mortality rate	3.6%
Neonatal mortality rate	2,1%

Note: a) INE, http://www.ine.pt/xportal/xmain?xpid= INE&xpgid= ine_mapa_portal. Source: Alto Comissariado da Saúde, http://www.acs.min-saude.pt/pns/page/2/?s= meta

In site 2 there are 4 intensive care and 8 special care beds. In 2008, there were 279 admissions. The occupation rate was 74% and mean stay was 11.7 days. Gestational age ranged between 23 and 41 weeks and fifty-nine infants (21.15%) were born at 30 weeks gestational age or less. Eighty-three infants (29.75%) were ventilated, of which 55 were under conventional ventilation. The main causes of admission were prematurity, respiratory distress and malformations (Centro Hospitalar de Coimbra, EPE, 2009).

Both units use sweet solutions for painful procedures and assess pain on a regular basis. Kangaroo care is performed in both units, being a regular practice in site 2.

6.3 Sample

The sample size was calculated according to a previous study on the efficacy of kangaroo care (Johnston *et al.*, 2003), who found a standard deviation of 3.5 on the main outcome (PIPP) in the experimental group. It was hypothesized in the present study that by adding kangaroo care to sucrose and pacifier, a mean difference of 2 points could be found compared to sucrose and pacifier only.

For sample size calculations, the following formula was used, in which n is the sample size, z is the value of an observation expressed in standard deviation units, α the level of significance, β the probability of a Type II error, σ the standard deviation of the experimental group and Δ the mean difference between groups (Cohen, 1988):

$$n = \frac{2(Z\alpha/2 - Z\beta)^2\sigma^2}{\Delta^2}$$

In our study, a sample size of 96 was obtained for α= .05 and β= .20 (80% power), considering,

Z α/2= 1.96

Zβ= -.84

σ= 3.5

Δ= 2

Anticipating that some cases might be lost due to equipment failure or other reasons, an extra 30% was added to the sample.

The sample was stratified in two age groups according to gestational age given that, at the time, evidence of the efficacy of skin-to-skin contact existed only for infants 32 to 36 weeks (Johnston *et al*., 2003) and published studies below this age are more recent (Johnston *et al*., 2008b). It was therefore not certain that the younger infants would respond to the intervention in the same way. The age groups considered were from 28 weeks until 31 weeks and 6 days; and from 32 weeks until 36 weeks and 6 days.

The inclusion criteria were:

- Gestational age between 28 and 36 weeks, 6 days;
- Postnatal age less than 28 days.

Infants eligible under the criteria above were definitely excluded if they had:

- Apgar score ≤ 6 at 5 minutes;
- Surgery;
- Major congenital anomalies;
- Genetic anomaly;
- Intra-ventricular hemorrhage (IVH) greater than Grade 2 or subsequent periventricular leucomalacia;
- Diabetic mother;
- Mother with history of drug abuse;
- Severe illness as defined by hypo or hyperthermia, need for respiratory support such as ventilation or nasal continuous positive airway pressure (CPAP), and inotropic therapy;
- Skin-breaking procedure in the previous 12 hours;
- Opioid or non-opioid sedation on the 48 hours prior to data collection;
- Mother absent or unable to do kangaroo care for clinical reasons.

These exclusion criteria were defined because the conditions described might interfere with the pain response, or for practical or ethical reasons.

6.4 Variables and outcome measures

The experimental variable, the outcome measures, the demographic and clinical variables related to the neonate and mother as well as the variables related to the procedure are operationalized next.

Experimental variable: interventions to relieve pain

The type of intervention was the experimental variable in this study.

As reported above, infants were randomly assigned to one of two interventions for pain relief before venepuncture for blood-draw (often referred in the text as the procedure):

- Oral sucrose with pacifier, or
- Oral sucrose with pacifier plus kangaroo mother care (S+KMC).

Venepuncture was chosen because it is the painful procedure that is performed more often in the units where the study took place.

Oral sweet solutions being the standard of care in both data collection sites this intervention group plays the role of the control group against which the combined intervention is compared. In the oral sucrose condition, the baby was positioned in the incubator or in the cot in prone position, head elevated, was nested and had minimal handling for 30 minutes prior to the procedure.

In the S+KMC condition, mothers were installed on a rest chair reclined at 45º. The baby was put on the mother's bare chest covered with her gown and a blanket and was not handled for 30 minutes. Mothers were instructed to clasp their hand to hold the baby and not to talk or stimulate the baby in any way during the procedure.

In both conditions, the nurse was asked to inspect the site of the before the baby was positioned so that the baby's head would face the video recording and no change in position would be needed before the procedure. Electrodes and sensors were placed at the time the baby was positioned.

Two minutes before the procedure, the infant was given 0,1 ml of a 24% sucrose solution via a syringe and offered a pacifier.

Given that standard pain relief for minor procedures is performed in one of the settings using a solution of 24% sucrose and in the other setting by means of a 30% solution of glucose, for the purposes of the study, this difference had to be reconciled. Therefore, the sucrose solution used in both settings was prepared under sterile conditions in 50 ml vials by the pharmacy of one of the hospitals. Every other day, according

to hospital routine procedure, two vials were delivered to the neonatal unit and kept in the refrigerator, while the previous ones were discarded. One intact vial was taken, as needed, to the other hospital, using a thermal bag to maintain low temperature during the ten-minute transportation. This vial was kept in the refrigerator for two weeks and was accessed only for the purposes of the study. The aim of this procedure was to ensure no risk of bacterial contamination. Harrison and colleagues (2007) have studied bacterial growth in samples of refrigerated and unrefrigerated solutions of 33% sucrose in use in a neonatal intensive care unit during one month. They found minimal, non-significant bacterial growth on two of the four refrigerated bottles on day 14. The bacteria found were common skin microorganisms, not consistently isolated in subsequent samples of the same bottles, suggesting contamination on the moment of the sample collection or inability of the solution to support the growth of these bacteria. No Gram-negatives were isolated. In addition, their results suggest a relation with the number of times the bottles were accessed, the contaminated ones having been used over 30 times in the 14 days. In our study, the vial that was kept for a longer time was accessed only for the purposes of the study and therefore a number of times significantly lower. Therefore, our procedure can be considered safe.

Outcome measures

Pain response was the dependent variable in this study and was measured through the following outcomes:

- The Premature Infant Pain Profile (PIPP) score and its components;
- Heart rate variability;
- Recovery time.

Premature Infant Pain Profile. The Premature Infant Pain Profile (PIPP) is a composite measure of pain that consists of:

- Three behavioral indicators (brow bulge, eye squeeze, nasolabial furrow);
- Two physiological indicators (heart rate and oxygen saturation), and
- Two contextual indicators (gestational age and behavioral state).

All the indicators are assessed at baseline, through observing the infant for 30 seconds. During the procedure, the PIPP score is computed by blocks of 30 seconds. In each block, heart rate is scored for the increase of maximum heart rate from baseline in beats per minute, oxygen saturation is scored for the decrease from baseline, and facial actions are scored for the percentage of time they are present during each block. Each indicator is scored on a 4-point scale (0-3), to obtain a total pain score between 0 and 21 (see Appendix A). Scores of 6 or less indicate minimal or no pain, scores from 7 to

12 indicate mild pain and scores over 12 indicate moderate to severe pain (Ballantyne, Stevens, McAllister, Dionne, & Jack, 1999; Stevens *et al.*, 1996).

A major strength of this scale is that it is a multidimensional measure and takes into consideration physiological and behavioral indicators of pain as well as contextual variables. These contextual variables, namely gestational age and behavioral state are known to influence the pain response of preterm infants. Preterm infants with low gestational ages compared to fullterm infants have behavioral responses that are less robust and shorter in duration (Johnston, Stevens, Craig, & Grunau, 1993; Gibbins *et al.*, 2008b). Behavioral state is also known to influence the intensity of the facial action response to pain which is why it is of major importance to include these contextual variables when measuring the response to pain.

In order to compute the PIPP score, the three facial actions of the baby were recorded on a Samsung DC165W digital camcorder.

Heart rate was recorded using three Neonatal ECG electrodes (Kendall Arbo, Tyco/Healthcare) placed on the babies' chest or back, that fed into a polysomnography device, Somte Compumedics- Series™ with a sampling rate of 100 Hz averaged beat-to-beat.

A pulse oximetry probe (Nellcor™ OxiMax™ sensor) was placed for the purpose of the study on the baby's hand or foot and was also connected to the Somte™.

Behavioral state was assessed using Prechtl's categories of quiet sleep, active sleep, quiet awake, and active awake (Prechtl & Beintema, 1977) .

Gestational age was taken from the infant's chart, based on early ultra-sound (12 weeks).

Since the different components of the PIPP do not contribute evenly to the PIPP score, namely the behavioral indicators (facial actions) contribute strongly while the physiological indicators may have low correlations with the total PIPP score, the different components were also analyzed as separate outcome measures.

Heart rate variability. Heart rate is under the influence of various peripheral and central control systems. It varies with blood pressure, temperature, respiration, oxygenation, but the main control of heart rate is exerted by the brain stem through the activity of the autonomic nervous system. Sympathetic activity increases heart rate while parasympathetic influence decreases heart rate. During inspiration, parasympathetic outflow to the sinoatrial node is temporarily reduced, causing a transient and subtle acceleration of heart rate. With expiration, heart rate falls as parasympathetic influence returns. This rhythmic change in heart rate coupled with the respiratory cycle is termed respiratory sinus arrhythmia.

Fluctuations of heart rate are most often examined in two peak ranges: low-frequency (LF) (0.04–0.15 Hz), indicating both sympathetic and parasympathetic activity; and high-frequency (HF) (>.015 Hz), influenced by parasympathetic (vagal) activity. The LF/HF ratio is also examined, increased ratio suggesting increased sympathetic cardiac modulation, decreased parasympathetic modulation, or both.

At rest, both the parasympathetic and sympathetic influences are active, although parasympathetic effects are dominant. Under stress conditions, heart rate increases and heart rate variability decreases as a result of decreased vagal activity. For this reason, like heart rate, heart rate variability has been used as a measure of neonatal stress (Porges, 1992; Porges, 1995) and more specifically as a biomarker of pain (Lindh, Wiklund, Sandman, & Hakansson, 1997; Lindh, Wiklund, & Hakansson, 1999). It is an index of sympathovagal balance that has been suggested to be influenced by kangaroo care (McCain, Ludington-Hoe, Swinth, & Hadeed, 2005).

Recovery time. After the disruption caused by a painful stimulus, physiological indicators such as heart rate, tend to return to baseline. The time needed for this recovery can be seen as an indicator of the infant's capacity for self- regulation (Stevens, Pillai Riddell, Oberlander & Gibbins, 2007).

In this study, recovery time was defined as the interval of time, in seconds, from the end of the procedure until heart rate returned to the value recorded at the bedside before sucrose was administered.

The mothers' perceptions. To explore mothers' perceptions of doing Kangaroo Care a semi-structured interview was used. People's feelings and emotions regarding their experiences, in this case, the experience of holding the baby skin-to-skin during a painful event are best captured by means of an interview that will allow them to express themselves without a strict set of questions.

The interviews were audio-taped using a Digital Voice Player version 2.1 from Sony Corp. A broad question was asked, "Please tell me, how was it for you?" followed by clarification questions to encourage the mothers to elaborate on their experience. Examples are: "Do you remember what crossed your mind at that moment?"; "How did you feel?"; "What makes you say that?"

After the mothers had expressed their feelings and emotions freely, two questions were asked to all the mothers: "Would you repeat this, if you had the choice?" and "Would you recommend it? or, "If another mother was hesitating to do it, what would you tell her, and why?"

These questions were aimed at identifying the diversity of feelings and emotions, and not only at looking for regularities in mothers' discourses.

Demographic and clinical variables

Demographic and clinical variables relate to the neonate and to the mother. Most were retrieved from the patients' charts and recorded on the data collection form.

Variables related to the neonate were: sex, gestational age in weeks as estimated by early ultra-sound, Apgar score at 1, 5 and 10 minutes, birth weight (Kg), primary diagnosis. Variables at the time of procedure included: postnatal age in days; vascular access (yes/no); gastric tube (no/orogastric/nasogastric); need for supplemental oxygen (yes/no); number of previous invasive procedures; time elapsed since last food intake.

Variables related to the mother were: age; gravidity; parity; type of delivery (vaginal, instrumental, caesarean section); previous experience of KC with present child or other (number of times); level of anxiety prior to blood draw as measured by the State Form of the State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1983). The State-Trait Anxiety Inventory (STAI) is a self-report inventory that consists of two 20 item scales: the State Anxiety Scale and the Trait Anxiety Scale (Spielberger et al., 1983). The Trait Anxiety Scale is intended to measure a person's disposition to respond to a stressful situation with anxiety. The State Anxiety Scale (S-STAI) is designed to assess the level of relatively transient situation-related stress perceived in a particular situation. Examples of these are stressful experimental procedures and real-life stressors such as imminent surgery or dental treatment. The S-STAI has been extensively used to measure the anxiety of mothers of preterm infants in the Neonatal Intensive Care Unit (Pinelli, 2000; Sisk, Lovelady, Dillard, & Gruber, 2006; Allen et al., 2004; Shields-Poe & Pinelli, 1997; Catlett, Miles, & Holditch-Davis, 1994).

The S-STAI consists of 20 statements that evaluate how respondents feel "right now, at this moment", on a four-point Likert scale, scored 1 to 4, from "Not at all", Somewhat", "Moderately so", "Very much so". Examples of these statements are: "I feel at ease" and "I feel upset".

Total scores may vary between 20 and 80, a minimum total score of 20 indicating a low level of anxiety and a maximum total score of 80 indicating a high level of anxiety. Items 1; 2; 5; 7; 9; 11; 12; 15; 19; 20 are scored in reverse. The scale has good construct validity, discriminating adults with generalized anxiety disorder (Silva, 2003).

The State Anxiety Scale (S-STAI) of the State-Trait Anxiety Inventory, (Spielberger et al., 1983), was used in its Portuguese version (Silva, 2003) to measure maternal anxiety before the baby had the blood test (see Appendix B).

Variables related to the procedure

The following variables were considered regarding the painful procedure:

- Duration of the phases of the procedure;
- Purpose of blood draw (biochemical or hematology tests/newborn screening/ both;
- Adverse events. The possibility of adverse events was considered and therefore monitored. Adverse events looked at were: sustained tachycardia (heart rate over 200 for more than 15 seconds); desaturation during the needle stick, defined by sustained oxygen saturation level below 85% for more than 15 seconds; and the need to repeat the venepuncture.

6.5 Research procedure

Pilot phase

After obtaining authorization from the Administration Boards of the two hospitals (see Appendix C), the pilot phase started. The aim was to test the equipment, gain training in using it, organize the data collection procedure on the wards and get the staff familiar with the researcher's presence. The data collected were not included in the study.

During this phase of the study, the information sheet for parents (Appendix D), the parents' written consent form (Appendix E), the protocol for recruitment and data collection procedure (Appendix F) and the data collection form were refined (Appendix G).

Data collection

Data were collected from March 25, 2007 to May 2, 2007 and from January 20, 2008 to September 1, 2008. All infants admitted to the Neonatal Intensive Care Units during these periods were assessed for eligibility according to the inclusion and exclusion criteria that follow.

Experimental protocol

Every day the researcher assessed newly admitted infants for eligibility criteria. The data collection form was inserted into the clinical file and the mother, father or both were approached for consent as soon as possible either when they came to the ward or by visiting the mother at the post natal unit. The purpose of the study was explained and the written information sheet was given to them with the request to inform the baby's nurse about their decision after they could read the paper. The parents' decision was noted on the data collection form.

When the physician ordered a test that was to involve venepuncture for a recruited baby, the baby was assessed again by the researcher to confirm that none of the exclusion criteria was present and was then randomly allocated to treatment group using a computerized randomization list by age group generated from GraphPad Software http://www.graphpad.com/quickcalcs/randomn2.cfm.

If the infant was allocated to receive S+KMC, the mother was asked to come to the ward if not already there.

The experimental protocol from assessment of eligibility to the end of data collection is illustrated on diagram 1.

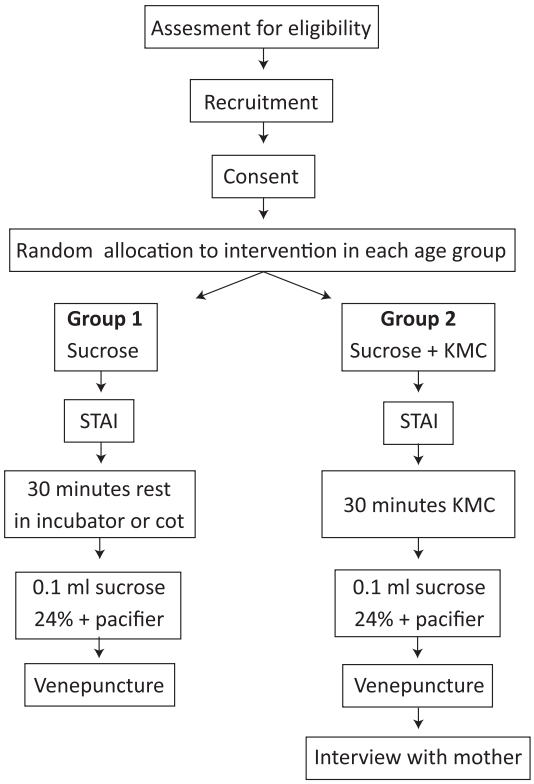

Diagram 1. Experimental protocol

Electrodes and pulse oxymeter probe were placed on the infant and recording equipment was tested.

Before the procedure started, mothers were asked by the researcher to respond to the STAI. Infants in the S+KMC group were then placed on the mother's chest and left quiet for 30 minutes. Infants in the Sucrose group remained in the incubator with minimal handling for 30 minutes.

Heart rate and oxygen saturation as displayed on the bedside monitor and behav-

ioral state observed by the researcher were noted on paper before the administration of sucrose. Sucrose was administered by the nurse, the recordings started 1 minute later and 2 minutes after sucrose administration the baby's nurse performed the blood draw following these steps: take the baby's hand, clean the insertion site, insert the needle, draw the blood, extract the needle and compress the site until no bleeding would occur, reposition baby's hand on mattress or on mother's chest. The duration of these steps varied according to the amount of blood needed and the nurse's judgment.

Records of facial action, heart rate and oxygen saturation were continuous throughout the 5 phases: 1) Sixty seconds before the procedure, 2) Skin preparation, 3) Needle Stick, 4) Compression after needle removal, and 4) Rest, after the end of the compression until heart rate returned to baseline or until five minutes had passed. This period of rest was not always as long, when the recording had to be ended to attend the mother's or the baby's needs.

The interviews to the mothers were performed by the researcher 2 to 72 hours after the procedure, in private, using a semi-structured interview.

The timeline for the experimental protocol considering the different phases of the procedure and the data recorded are illustrated on diagram 2.

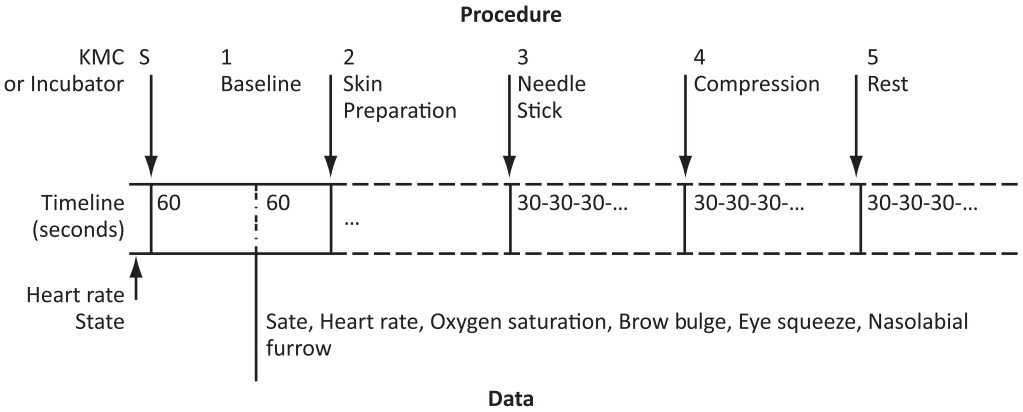

Diagram 2. Timeline of the procedure in the experimental protocol.

Data extraction

The analysis of the data comprised the facial coding and the extraction of the outcomes from physiological data. Both were done at McGill University.

Coding of Facial Actions. In order to compute the PIPP score, facial actions of infants were recorded continuously with a digital video camera for later coding and scoring. To keep coders blinded to the type of intervention the baby's face was filmed in

close-up with minimal surrounding and coders' copies of the films had no sound. Filming in close-up also avoided mothers' identification.

Three trained coders, two experienced and one recently trained, blinded to the purpose the study, performed the facial coding. Inter-rater reliability of coders was established when the correlation of the percentages for each facial action between the newly trained coder and the experienced coder was higher than 85%. Intra-rater reliability was reassessed by each coder every 15 sessions and was higher than 90%. In case reliability fell below this level, they were retrained. Each coder coded only one type of intervention. At the end of the coding, 10% of the sessions were coded by alternate coders to ensure that the the different conditions were coded similarly.

Each session was viewed three times in real time using Windows Media Payer. The upper facial actions - brow bulge, eye squeeze and naso-labial furrow – were identified using the Neonatal Facial Coding System Training Manual, which provides anatomically based objective descriptions of the facial actions displayed by newborns (see Appendix H).

The coders identified the facial actions second by second and the software developed for this purpose at McGill University, allows the conversion into the percentage of time that the baby displays each of the facial actions in a certain period of time.

Heart rate and oxygen saturation. Data from the electro-cardiogram were sampled at an effective accuracy of 1 millisecond and analyzed using Somté™ ECG analysis software. Artifacts, ectopic beats and noisy data on ECG traces were manually identified by visual inspection and not used for further analysis. Maximum, minimum, mean and standard deviation of heart rate in each of the previously defined blocks was extracted.

Oxygen saturation was extracted from second to second recordings of the Somté using the software Compumedics E-series Profusion PSG II and minimum, maximum and average values were calculated. Variations in heart rate and oxygen saturation from baseline across the different stages of the procedure were examined.

The patterns of response to the painful procedure were examined in terms of reactivity (change from baseline), intensity (magnitude of the change), direction (increase or decrease), regulation (change from pain to recovery) and slope (regression coefficient)

Heart Rate variability. Heart rate variability was analyzed using a frequency-domain approach.

On the electrocardiogram signal recorded with the Somté, and using the Somté software, normal beats, ectopic beats and artifacts were visually identified. Intervals with non-normal beats were interpolated to obtain an analyzable beat series and artifacts were excluded from the analysis.

Power for low frequency (LF), high frequency (HF) and the LF/HF ratio were analyzed for 3 intervals: 1) Baseline; 2) Needle stick and Compression phases together; 3) Rest.

Recovery time. Recovery time was calculated from the analysis of the output of second to second recordings on Somté™, obtained through the software Compumedics E-series Profusion PSG II. The number of seconds of recovery time was counted from the end of the procedure until heart rate returned to the baseline value and remained there, or below, for 5 or more consecutive beats.

Mothers' perceptions. Audio-taped interviews were transcribed verbatim by a person unrelated to the research, using the Digital Voice Editor from Sony Corp. In order to be corrected and validated, transcriptions were all read by the researcher while listening to the interviews. The written transcriptions were imported to QSR NVivo Version 8.0.180.0 SP1 from QSR International Pty Ltd. This software is designed to facilitate storing, sorting and organizing the data.

Interviews were read one by one to identify the main themes that were brought up by the mothers and to capture the dominant feeling of the interviewee regarding the research question. Following preliminary examination of the data, the text was broken down into units of analysis. In this study, the unit of analysis, i.e., the smallest bit of information used, was a word, sentence or part of a sentence conveying a meaningful feeling or emotion. The themes that responded to our research question were retained for analysis. Other issues that emerged during the interviews were ignored. Within a theme, units of analysis with the same meaning were grouped into a category that was named to best describe the dominant feeling or emotion. In this interactive way, a coding scheme was progressively built in which categories followed the rules indicated by Amado (2000): exhaustiveness, all units fitting under the category being coded there; exclusiveness, one unit belonging to one category only; homogeneity, all categories fitting under the same type of analysis; pertinence, the system being adjusted to the aims and the material; objectivity, the coding criteria being clear in a way that the coding could be replicated; productivity, the category system offering the possibility of a fruitful analysis.

Reliability of the coding scheme was tested by submitting a sample of units of analysis to an independent coder who was familiar with the research question and with the methods employed in content analysis. The reliability was 87%, calculated according to Polit and Hungler (1991), using the following equation:

$$\frac{\text{number of agreements}}{\text{number of agreements + number of disagreements}}$$

Frequencies of concepts (categories) were calculated in order to identify the number of mothers that shared the concept, so in this study, the frequencies represent the existence of that category in the interview, not the number of times that the concept appeared during the interview. Therefore, it indicates the number of mothers that had at least one unit of analysis coded into that category or indicator independently of the number of times the concept was brought up during the interview.

The recording units were translated from Portuguese to English trying to be faithful to the original meaning. Yet, we are aware that part of the richness of the idiomatic expressions is lost in the translation.

Statistical analysis

The statistical analysis of the data was conducted using the PASW 18 Statistics from SPSS Inc.

All tests were conducted considering significance for $\alpha < .05$.

For the sample characteristics, parametric tests were used when the assumptions were met namely, the normal distribution of the variables tested using the Kolmogorov-Smirnov test.

For the main outcomes, a mixed design ANOVA (Oberlander & Saul, 2002) was conducted to test the main effect of intervention, as an independent variable, on the dependent variables: PIPP scores, minimum, average and maximum heart rate, minimum oxygen saturation and percentage of each facial action at different epochs. Since our sample was stratified in two gestational age groups, gestational age was also introduced in the model as a between-subjects factor. Phase of procedure was considered a within-subjects independent variable. The mixed between-within ANOVA was preferred to a two-way ANOVA because each dependent variable was measured repeatedly and therefore this model allows introducing phase of the procedure as a repeated-measures independent variable along with the two other between-subjects independent variables: intervention and gestational age. It allows testing the main effect of the independent variables as well as the interaction effect between them. When Mauchly's test indicated that the assumption of sphericity was not met ($p < .05$), the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity. For post hoc pairwise comparisons, Bonferroni adjustment for multiple comparisons was used to control overall Type I error: for each test, the level of significance considered is α divided by the number of tests conducted.

The effect size for main effects (r) was calculated using the following formula (Field, 2005:514), where F is the value of the test and dfR is the residual degrees of freedom:

$$r = \sqrt{\frac{F(1, dfR)}{F(1, dfR) + dfR}}$$

According to (Cohen, 1988), $r = .10$ is considered a small effect, $r = .30$ a medium effect and $r = .50$ a large effect.

The Independent Student t-test was performed to test the difference between intervention groups for demographic data and for outcome variables at baseline.

Pearson correlation was used to test the relationship between the outcomes and the sample's characteristics, when the variables were continuous. The coefficient of determination (r^2), representing the proportion of shared variance between the variables, or the amount of variability in one variable that can be explained by the other variable, was calculated as a measure of the effect size in correlation tests (Field, 2005).

To test the association between categorical data such as behavioral state, Pearson Chi-Square statistics was used along with calculations for the Odds-ratio.

6.6 Ethical considerations

The ethics boards of the two Hospitals were consulted by the Administration before the study was authorized.

The study was conducted with full respect for the rights of human subjects as stated on the current version of the Declaration of Helsinki (World Medical Association, 2004) the Guidelines for the Ethical Conduct of Studies to Evaluate Drugs in Pediatric Populations (Committee on Drugs, 1995) and the Ethical Guidelines for Pain Research in Humans (International Association for the Study of Pain, 1995).

Expected health benefit and risk for the participants

Children in the study had the benefit from KC and from sucrose during a painful procedure which are know to reduce pain responses. Mothers also had the benefit from skin-to-skin contact with their infant, which is considered a very pleasant experience.

Blood draws were performed only when ordered to monitor the infants' condition so no extra burden was caused.

Concerning risks, no adverse events during Kangaroo Care have been described in the literature when infants are physiologically stabilized. As for sucrose, no adverse events have been reported either in infants older than 28 weeks, with a 24% concentration and with such small volume of solution.

Informed consent

Parents were given written and oral information about the study and asked for written consent. It was explained to the parents that they had the right to withdraw at any moment of the research with no need to give an explanation. Parents agreed that the images could be used for teaching and scientific purposes only.

Anonymity

No names were recorded on the data collection forms and the signed parental consent forms are kept in a separate file, so that they cannot be connected to the actual data. The video recordings did not include the mothers' face, to avoid identification. The videos will be kept in a file with security features (access limited by password). Paper and digital records will be kept in the archives of the Health Sciences Research Unit: Nursing Domain [HESC-Center-Coimbra-742] (UICISA-dE), hosted by the Nursing School of Coimbra.

Confidentiality

The identity of the participants will remain confidential and no individual recognizable identities will be used when presenting the data.

Reward

Mothers were offered the possibility of having a copy of the video recordings of their infant, and many accepted the offer. The copies were delivered with no sound so that conversations between the people involved in the procedure would remain confidential.

CHAPTER 7.
Results

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This chapter begins with a presentation of the sample flow and participants' characteristics in terms of demographic and clinical variables. Pain responses are examined next, followed by recovery time. Last, mothers' perceptions of doing Kangaroo Care during venepuncture on the baby are described.

Since a stratified sample by gestational age was used in this study, the results will be reported for the sample as a whole and for each gestational age group only when considered of interest for the analysis.

7.1 Participants' characteristics

The participants were recruited in two level II-III Neonatal Intensive Care Units in Coimbra, Portugal. Four-hundred and thirty-four (434) infants were assessed for eligibility. Two-hundred and twenty-three (223) were excluded based on the exclusion criteria previously defined. The parents (father, mother or both) of two-hundred and eleven (211) infants were approached for consent. Only six mothers (2.84%) refused to participate: two did not feel enough courage to hold the baby; one father did not accept his wife to participate; three mothers gave no reason for refusal. One of these mothers had five children at home, two of which had been admitted to the Unit at birth and was very anxious to go home.

Out of the two-hundred and five (205) neonates for whom consent was obtained, seventy-five (75) were lost to randomization: it was not possible to perform the data collection on eleven (11) neonates because the blood draw occurred during the night or staff failed to call the researcher, and for sixty-four (64) neonates because between the time that they were considered stable enough for study participation and discharge, there was no prescription for blood draw.

One-hundred and thirty neonates were randomized to receive sucrose or sucrose plus kangaroo mother care: forty-seven (47) were below 32 weeks gestational age and

eighty-three (83) were 32 weeks gestational age or more. Data collection was performed for these hundred and thirty neonates (130). Twenty cases (20) were lost for analysis, because of insufficient data, which occurred when the infant exhibited vigorous movements and the equipment failed to capture reliable data (twelve in the sucrose group and six in the sucrose plus kangaroo mother care or, in two cases, because the researcher failed to notice that the equipment was off (one infant in each intervention group).

The final sample was composed of one hundred and ten (110) neonates, thirty-five (35) below 32 weeks and seventy-five (75) with 32 or more weeks gestational age: forty-nine (49) infants were randomized to the sucrose group (S) and 61 to the sucrose plus kangaroo mother care (S+KMC) group (see Figure 5).

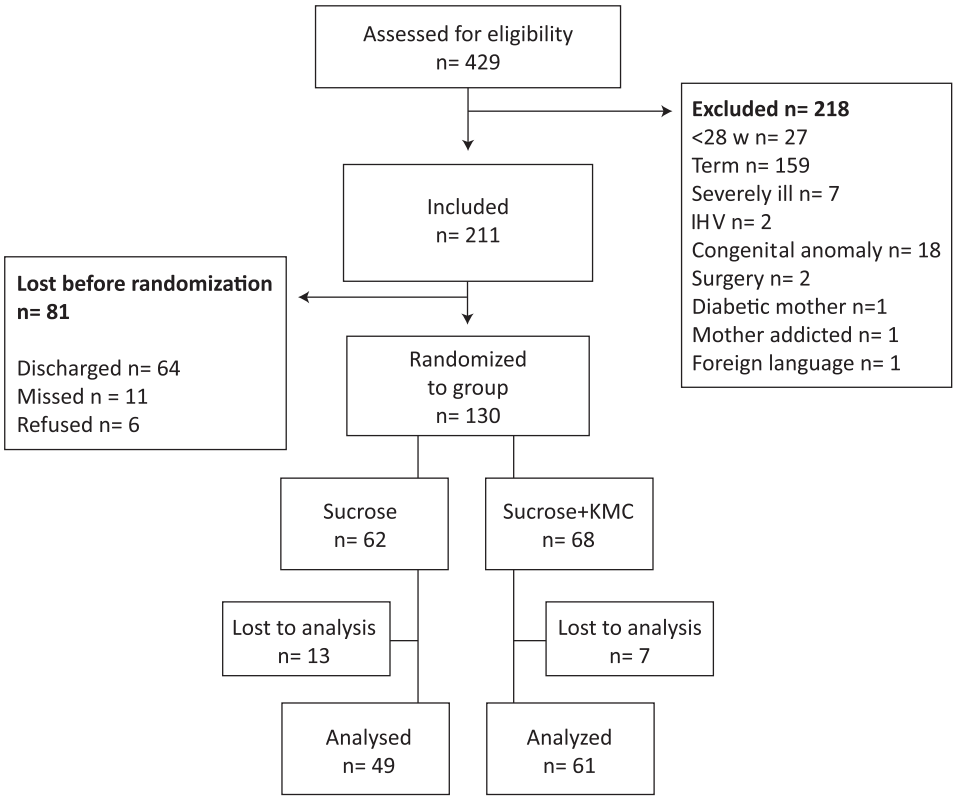

Figure 5. Sample flow. Note: IVH - Intra-ventricular hemorrage.

The sample characteristics regarding demographic and clinical variables related to infants and mothers and variables related to the procedure are presented below. Most of the neonates (65.55%) in this study were admitted to Unit 1, which is affiliated to the University Hospital. The group below 32 weeks gestational age was nearly half the size of the group 32 weeks or more. The percentage of male infants in the total sample is slightly higher (54.55%) than the percentage of female infants (45.45%). The most fre-

quent type of delivery was the caesarean section (61.82%), followed by vaginal delivery (33.64%) and instrumented vaginal delivery (4.55%). About half the infants were born from a first gestation and first delivery, and therefore were the first child in the family. One fourth of the neonates were twins (25.45%). The condition of these infants at birth was good, more than 75% scoring an Apgar of 6 or higher on the 1st minute of life. As this was a requirement to be included in the study, all the infants had an Apgar of 6 or higher on the 5th minute. Even though all the infants were preterm, one third of the cases had no other diagnosis than prematurity. Other main diagnoses were intra-uterine growth restriction, twin birth, respiratory distress, hyperbilirubinemia and infection. Three quarters of the neonates had a feeding tube either oral (68.8) or nasal (8%) and 31.82% had an intra-venous line. Only two infants were under supplemental oxygen, in a low fraction (FiO2= 25-28%). There were two purposes for the blood draw: 1) to obtain a blood sample to monitor biochemical or hematological values (monitoring); 2) to obtain a blood sample for neonatal screening of metabolic diseases (screening). In twenty infants (18.18), the blood draw was performed for both reasons (monitoring and screening). As for mothers' previous experience, only 23.64% of mothers had experienced skin-to-skin care before, either with this or another child. In the sucrose plus kangaroo mother care, only 18 (29.51%) mothers had previous experience, which leaves 43 mothers (70.49%) for whom this was their first experience of holding the infant skin-to-skin.

In order to see whether the distribution of the infants in each intervention group was similar regarding the characteristics described above, the Pearson Chi-Square test was performed. No significant differences were found between the two groups except in the purpose of blood draw for which the sucrose group had a lower percentage of blood draws for screening compared to the sucrose plus kangaroo mother care group.

Table 5 displays the distribution of neonates (total sample and each intervention group) by these characteristics and presents the p value for the Pearson Chi-Square test.

The mean, standard deviation, median and range values for the numerical variables that characterize the sample can be seen on Table 6, as well as the results of the comparison between the two intervention groups. An Independent Students t-test was used to compare the groups regarding variables with a normal distribution (Kolmogorov-Smirnov test $p>.05$). Since the distribution of gestational age, post natal age and number of previous painful procedures could not be assumed as normal, the Mann-Whitney U test was used to compare the two groups. Median gestational age was 32 weeks in both groups and post natal age was 6 days. Infants weighed on average 1657.15 grams with a wide range from 920 to 2860 grams. The time interval since the last meal was in average 108 seconds, ranging from 0 seconds, in the case of an infant who was fed by gavage dur-

ing the skin-to-skin care and to whom the blood draw occurred immediately after the end of feeding, to 360 seconds (6 hours) in a 34 weeks gestational age neonate who was breastfed. The number of previous painful procedures such as heel lances, venepuncture for blood draw of intra-venous lines, gastric tubing, tracheal tubing, suctioning, pose of chest drain was examined. The median number of previous painful procedures endured by these neonates was 9.5. One infant 28 weeks gestational age and 10 days old had 99 procedures before the one that was observed. Regarding maternal variables, mean maternal age was 30 years-old.

Table 5 - Sample characteristics (categorical variables) by intervention group

Characteristics		Total sample N= 110		Intervention group S n= 49		S+KMC n= 61		Chi-Square
		n	%	n	%	n	%	
Site	Unit 1	71	64.55	31	63.27	40	65.57	$\chi^2(1)= .063$ $p= .801$
	Unit 2	39	35.45	18	36.73	21	34.43	
Gestational age group	< 32 weeks	35	31.82	18	36.73	17	27.87	$\chi^2(1)= .985.$ $p= .321$
	≥32 weeks	75	68.18	31	63.27	44	72.13	
Sex	Male	60	54.55	24	48.98	36	59.02	$\chi^2(1)= 1.104$ $p= .293$
Type of delivery	Cesarean	68	61.82	32	65.31	36	59.02	$\chi^2(2)= 1.331^a$ $p= .514$
	Vaginal	37	33.64	14	28.57	23	37.70	
	Instrumented	5	4.55	3	6.12	2	3.28	
Order of gestation	1	59	53.64	29	59.18	30	49.18	$\chi^2(2)= 1.094$ $p= .579$
	2	28	25.45	11	22.45	17	27.87	
	≥ 3	23	20.91	9	18.37	14	22.95	
Order of parity	1	57	51.82	29	59.19	28	45.90	$\chi^2(2)= 1.924$ $p= .382$
	2	40	36.36	15	30.61	25	40.98	
	≥ 3	13	11.82	5	10.20	8	13.12	
Twin birth	Yes	28	25.45	12	24.49	16	26.23	$\chi^2(1)= .043$ $p= .835$
Apgar>6	1st minute	88	80.00	42	85.71	46	75.41	$\chi^2(1)= 1.803$ $p= .179$
	5th minute	110	100.00	49	100.00	61	100.00	b
Main diagnosis	Prematurity	38	34.55	16	32.65	22	36.07	$\chi^2(5)= 1.522^a$ $p= .910$
	IUGR	25	22.73	13	26.53	12	19.67	
	Twin birth	19	17.27	8	16.33	11	18.03	
	Respiratory distress	15	13.64	7	14.29	8	13.11	
	Hyperbilirubinemia	7	6.36	2	4.08	5	8.20	
	Infection	6	5.45	3	6.12	3	4.92	
Gastric tube	No	26	23.64	14	28.57	12	19.67	$\chi^2(2)= 5.002^a$ $p= .082$
	Oral	75	68.18	34	69.39	41	67.22	
	Nasal	9	8.18	1	2.04	8	13.11	
IV line	Yes	35	31.82	19	38.78	16	26.23	$\chi^2(1)= 1.972$ $p= .160$
Oxygen therapy	Yes	2	1.82	1	2.04	1	1.64	$p= 1.000^c$
Purpose of blood draw	Monitoring	45	40.91	26	53.06	19	31.15	$\chi^2(2)= 9.898$ $p= .007$**
	Screening	45	40.91	12	24.49	33	54.10	
	Monitoring+Screening	20	18.18	11	22.45	9	14.75	
Mothers previous experience of KMC	Yes	26	23.64	8	16.33	18	29.51	$\chi^2(1)= 2.616$ $p= .106$
	With this child	24	21.84	8	16.33	16	26.23	$\chi^2(1)= 1.562$ $p= .211$
	With another child	6	5.45	2	4.08	4	6.56	$p= .690^c$

Notes: IUGR, Intra-Uterine Growth Restriction; IV, Intra-Venous line. [a] Expected count less than 5 in 33.33% of cells; [b] No statistics computed because variable is constant; [c] p for the Fisher's Exact test because 50% of cells have expected counts less than 5. **Significant for α< .01.

Table 6 - Sample characteristics (numerical variables) by intervention group

		All n= 110	S n= 49	S+KMC n= 61	Test
Gestational age (weeks) n= 110	Mean	32.12	31.96	32.25	U= 1379.5
	SD	1.97	1.88	2.05	*p*= .483
	Median	32. 0	32	32	
	Range	28-36	28-35	28-36	
Postnatal age (days) n= 110	Mean	6.51	6.20	6.75	U= 1328.5
	SD	3.99	4.21	3.83	*p*= .315
	Median	6	6	6	
	Range	0-24	0-24	1-21	
Birth weight (grams) n= 110	Mean	1657.15	1586.20	1714.13	t(108)= 1.534
	SD	437.32	409.89	453.45	*p*= .128
	Median	1663	1550	1690	
	Range	920-2860	920-2410	935-2860	
Interval since last meal (minutes) n= 99	Mean	108.31	107.87	108.69	t(96)= 0.060a
	SD	69.02	58.21	77.43	*p*= .952
	Median	120	120	117.5	
	Range	0-360	0-240	0-360	
Number of previous painful procedures n= 106	Mean	16.65	16.80	16.53	U= 1377.5
	SD	19.38	19.39	19.54	*p*= .987
	Median	9.5	10.00	9.5	
	Range	1-99	1-99	2-91	
Maternal age (years) n= 110	Mean	30.29	30.08	30.46	t(108)= .420
	SD	4.67	4.20	5.04	*p*= .675
	Median	30	30	30	
	Range	15-42	15-39	17-42	

Abbreviations: MD, Mean Difference; STAI, State-Trait Anxiety Inventory. [a] Equal variances not assumed by the Levene test. **Significant for α< 0,01.

The characteristics presented on Table 6, pertaining to the infants in our final sample, were compared to the characteristics of the 20 infants that were lost for analysis because of insufficient data. No significant differences were found between the final sample and the group of lost cases regarding gestational age, postnatal age, birth weight, number of previous painful procedures, maternal age and maternal anxiety. The results of the tests performed can be seen on Appendix I.

Duration of the procedure

Duration of the procedure was highly variable, due to uncontrollable factors such as: volume of blood needed, speed of blood flow and nurses judgement about the time needed for compression.

The standard procedure for blood harvesting by venepuncture consisted of three phases: 1) Skin preparation (P), when the nurse took the neonates' hand, inspected the vein and used a swab to cleanse the dorsum of the hand; 2) Needle Stick (S), when the nurse pierced the skin with the needle and harvested the required amount of blood; and 3) Compression (C), when the nurse withdrew the needle compressing the site of puncture with a cotton ball or gauze swab. The duration of each phase was counted in seconds from the beginning of that phase until the beginning of the next phase. Total duration of procedure was counted from the moment the nurse started the preparation of

the skin until the end of the compression phase (P+S+C). The duration of the painful phases was counted from the beginning of the needle stick phase until the end of the compression phase (S+C).

In one case, there was no compression phase because the puncture was unsuccessful and a second puncture followed without compression of the hand dorsum which is why the duration of this phase could only be counted for 109 infants.

A significant difference in the duration was found between the groups for the skin preparation phase (P), which was longer in the S+KMC group and in the needle stick phase (S) which was shorter in the S+KMC group (see Table 7).

There was no correlation between duration of preparation, stick and compression phases and birth weight, gestational age and postnatal age (See Appendix J).

Table 7 - Duration of the different phases of the procedure (seconds)

Phases of the procedure		All	Sucrose	S+KMC	Mann-Whitney test
Skin preparation (P)	Mean	33.95	27.08	39.48	*U*= 1043.0
n= 110	SD	21.66	15.22	24.44	*p*= .007**
	Median	27.5	24	31	
	Range	9-114	10-83	9-114	
Needle stick	Mean	149.30	170.05	132.63	*U*= 1052.0
(S)	SD	93.25	88.45	94.37	*p*= .008**
n= 110	Median	123	153	112	
	Range	9-561	9-425	23-561	
Compression	Mean	89.01	91.42	87.03	*U*= 1402.0
(C)	SD	47.01	49.00	45.65	*p*= .679
n= 109	Median	82	83	81.50	
	Range	10-259	22-259	10-248	
Total duration of	Mean	272.23	288.55	258.92	*U*= 1115.0
procedure (P+S+C)	SD	112.44	97.74	122.36	*p*= .031*
n= 109	Median	251	265	220.5	
	Range	100-715	149-526	100-715	
Duration of painful	Mean	238.42	261.47	219.59	*U*= 101.0
phases (S+C)	SD	106.47	94.67	112.51	*p*= .005**
n= 109	Median	218	240	192.5	
	Range	69-668	137-507	69-668	

Abbreviations: MD, Mean Difference; CI, 95% Confidence Interval. *Significant for α< .05; **Significant for α< .01;

Adverse events

Regarding heart rate and oxygen levels during needle stick, there were no records of prolonged tachycardia or desaturation that needed intervention. As for the need to repeat the puncture, it occurred in 8 neonates (7.30%), 3 in the Sucrose group and 5 in the S+KMC group. The relation between the intervention and the need to repeat the puncture was examined using Pearson Chi-square test. The proportion of infants who needed a second venepuncture was similar in the two intervention groups (see Table 8).

Table 8 - Need to repeat the procedure by intervention group

	Sucrose		S+KMC		
Need to repeat	n	%	n	%	Chi-square test
No	46	93,9%	56	91,8%	*p*= .73[a]
Yes	3	6,1%	5	8,2%	
Total	49	100%	61	100	

[a] Fisher's Exact Test was used because 2 cells have expected count less than five.

In conclusion, we may say that considering the demographic and clinical characteristics of infants and mothers presented above, the two intervention groups did not differ significantly. Regarding the characteristics of the procedure, there was a difference regarding the purpose of the blood draw that may account for differences found in the duration of phases of the procedure. The only adverse event found was the need to repeat the venepuncture, which does not seem to be related to the intervention group the infant belonged to.

7.2 Responses to the interventions

The results of the Premature Infant Pain Profile (PIPP) will be analysed in this section as well as the PIPP components individually, namely heart rate, oxygen saturation, facial actions and state. The analysis of heart rate variability will also be presented in this section.

Given that the duration of the phases was not the same for all infants, only preparation phase and the first 30 seconds of needle stick (S30), of compression (C30) and of rest (R30) were retained for analysis.

Tables with results of two-way repeated-measures ANOVA display for each factor the degrees of freedom (df), the value of the test (F) and the p value. When a significant effect is found, the effect size (r) and the observed power of the test (OP) are also displayed.

7.2.1 Premature Infant Pain Profile

The PIPP score was obtained by adding the scores of its components as explained earlier. Behavioral state at baseline and postconceptional age at time of procedure were used for PIPP calculations. Heart rate, oxygen saturation and facial actions were recorded for 30 seconds before the procedure started (baseline). During the procedure, the PIPP was computed for the preparation phase, and in 30 second epochs during the needle stick phase, compression phase and after the end of the procedure (rest phase).

PIPP scores across the procedure

The hypothesis was that there would be a main effect of intervention on PIPP scores. To test this hypothesis, a two-factor repeated-measures ANOVA was conducted with intervention and age group as between-subjects factors and phase as the within-subjects factor. Gestational age was introduced in the model as a factor, because the sample was stratified in two age groups.

The overall effect was not significant, Pillai's Trace= .013, F(3, 94)= .43, *p*= .74.

The mean, standard error and 95% confidence interval for each intervention group can be found on Table 9. The mean difference (MD) between the two intervention groups was very small and not significant (*MD*= -.15, 95% CI [-.97, .65]).

Table 9 - PIPP scores by intervention and gestational age group

				95% CI	
Intervention		M	SE	Lower limit	Upper limit
Sucrose	All n= 44	4.85	0.29	4.26	5.43
	< 32 weeks n= 16	4.8	0.47	3.87	5.73
	≥ 32 weeks n= 28	4.89	0.35	4.19	.5.60
S+KMC	All n= 56	5	0.28	4.44	5.56
	< 32 weeks n= 15	5.25	0.48	4.29	6.21
	≥ 32 weeks n= 41	4.75	0.29	4.17	5.33

The results of the repeated-measures ANOVA are displayed on Table 10. There was no significant overall effect, Pillai's Trace= .01, F(3.94)= .43, *p*= .74. They do not support the hypothesis of an effect of intervention, F(1,96)= .15. No effect of gestational age group was found, F(1,96)= .25. There was, however, a highly significant main effect of phase on PIPP scores, F(2.12, 203.47)= 26.94, p= .000.

Table 10 - Results of repeated-measures ANOVA for PIPP scores

Factors	df	F	p	r	OP
	Between subjects				
Intervention	1, 96	.15	.705		
Gestational age	1, 96	.25	.622		
Intervention x Gestational age	1, 96	.53	.467		
	Within subjects[a]				
Phase	2.12, 203.47	26.94	.000***	.32	.99
Phase x Intervention	2.12, 203.47	2.45	.085		
Phase x Gestational age	2.12, 203.47	2.61	.073		
Phase x Intervention x Gestational age	2.12, 203.47	.53	.600		

[a] The Greenhouse-Geisser correction was used. *** Significant for α< .001. OP, observed power.

PIPP scores increased from preparation to needle stick, decreasing at compression and rest. Tests of within-subjects contrasts show a significant difference between preparation and S30 $F(1, 96)= 30.85$, $p< .000$; between S30 and C30 $F(1, 96)= 27.41$, $p< .000$; and between C30 and R30 $F(1, 96)= 13.99$, $p< .000$. The interaction between phase and gestational age group was significant only for the difference between S30 and C30, $F(1, 96)= 49.94$, $p= .042$. At needle stick, mean PIPP score was higher in the Sucrose group ($M= 7.18$, $SD= 4.27$) than in the S+KMC group ($M= 6.21$, $SD= 3.50$) (See Figure 6).

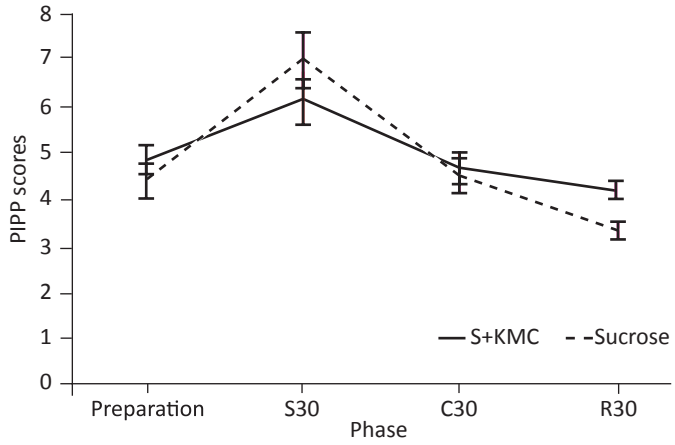

Figure 6. Evolution of PIPP scores across phases of the procedure, by intervention group (N= 110). Bars represent standard error.

Pairwise comparisons using the Bonferroni adjustment show a significant mean difference between all pairs of phases ($p< .001$), with an exception between preparation and compression (C30) ($p> .05$). Means and standard deviation of PIPP scores at every phase of the procedure as well as tables of pairwise comparisons can be found in APPENDIX J.

PIPP scores and infants' characteristics

The relation between PIPP scores and other variables such as site, infants' characteristics and duration of the procedure was explored. The differences in mean PIPP scores related to site and sex were not significant at any phase of the procedure using the t-test. The difference related to the infant having a vascular line was significant at S30, PIPP scores being higher in infants who did not have a vascular line ($M= 7.40$, $SD= 4.04$), compared to those with a vascular line ($M= 5.17$, $SD= 3.38$), $t(78)= -3.021$, $p< .01$. PIPP scores at S30 in the Sucrose groups were moderate and positively correlated with postnatal age ($r= .35$, $p= .01$, $r^2= .12$) and with birth weight ($r= .30$, $p= .04$, $r^2=$

.09) . In the S+KMC group, PIPP scores at S30 were correlated with postnatal age (r= .27, p= .04, r^2= .07). Correlations between the PIPP and time elapsed since last meal, number of previous painful procedures and duration of the Preparation phase were not significant (Appendix K).

In summary, PIPP scores changed significantly across phases of the procedure, increasing from preparation to needle stick and descending from needle stick to rest. These changes occurred in both intervention groups with no significant differences.

7.2.2 Heart rate

The results for maximum, average and minimum heart rate will be presented at baseline and across the procedure. Correlations with maternal anxiety and other infant characteristics will be examined at end of the section.

Data for heart rate were missing for a maximum of 11/110 infants in the epochs considered, which was attributable to the loss of signal as a result of infants' movements.

Heart rate at baseline

In order to confirm that no differences existed at baseline between infants in the S+KMC group and infants in the Sucrose group regarding minimum, average and maximum heart rate, the Independent Student t-test was performed. The results indicated that there was no significant difference in minimum, average and maximum heart rate at baseline (see Table 11)

Table 11 - Results of the t-test for maximum, average and minimum heart rate at Baseline

	Sucrose n= 48		S+KMC n= 58			
Heart Rate	M	SD	M	SD	Student t-test	
Maximum	167.10	13.77	167.81	14.15	t(104)= - .259	p= .796
Average	156.31	13.11	155.19	13.51	t(104)= .432	p= .667
Minimum	144.48	15.25	143.86	14.59	t(104)= .212	p= .832

Maximum heart rate across the procedure

It was hypothesized that there would be a main effect of intervention on maximum, average and minimum heart rate. To test this hypothesis, a two-factor repeated-measures ANOVA was conducted with intervention group and gestational age group as between-subjects factors, and phase as within-subjects factor. Taking into consideration that the duration of the phases was not the same in the two intervention groups, as reported in the first section of this chapter, the analysis was also conducted introducing the duration of the preparation phase as covariate (repeated-measures ANCOVA). However, it did not change the results of the repeated-measures ANOVA, which will be presented.

Regarding maximum heart rate, the overall effect was not significant, Pillai's Trace= .054, F(4, 90)= 1.29, *p*= .280.

The means for each intervention group by gestational age are displayed in Table 12. The mean difference (MD) between the two intervention groups was very small and not significant (*MD*= -.60, *SE*= .85, 95% CI [-6.89, 5.70])

Table 12 - Maximum heart rate for each intervention group and gestational age

				95% CI	
Intervention		M	SE	Lower limit	Upper limit
Sucrose	All n= 43	172.12	2.28	167.59	176.66
	< 32 weeks n= 16	172.18	3.62	165	179.36
	≥ 32 weeks n= 27	172.07	2.79	166.54	177.6
S+KMC	All n= 54	172.72	2.2	168.35	177.09
	< 32 weeks n= 15	177.45	3.74	170.03	184.87
	≥ 32 weeks n= 39	167.98	2.32	163.38	172.59

From the results of the repeated-measures ANOVA for maximum heart rate displayed on Table 13, it is possible to see there was no significant main effect of intervention F(1,93)= .04 or gestational age F(1,93)= 2.28, but there was a significant main effect of phase, F(3.09, 286.96)= 24.67, with a medium effect size (*r*= .28).

The interaction effect between gestational age and phase was also highly significant, F(3.09, 286.96)= 3.97, indicating that gestational age played an important role in maximum heart rate values across phases of the procedure. The effect size was small (*r*= .12).

Table 13 - Results of repeated-measures ANOVA for maximum heart rate

Factors	df	F	p	r	OP
	Between subjects				
Intervention	1, 93	.04	.851		
Gestational age	1, 93	2.28	.134		
Intervention x Gestational age	1, 93	2.18	.143		
	Within subjects[a]				
Phase	3.09, 286.96	24.67	.000***	.28	.99
Phase x Intervention	3.09, 286.96	.36	.784		
Phase x Gestational age	3.09, 286.96	3.97	.008**	.12	.84
Phase x Intervention x Gestational age	3.09, 286.96	1.53	.205		

Note: [a] The Greenhouse-Geisser correction was used. ** Significant for α< .01; *** Significant for α< .001. OP, observed power.

Variations in maximum heart rate across phases of the procedure are displayed in Figure 7. Maximum heart rate increased from baseline to preparation and again at needle stick, and then started to decrease. At the rest phase, heart rate values were back to or lower than baseline.

Test of within-subjects contrasts showed a significant difference from phase to phase.

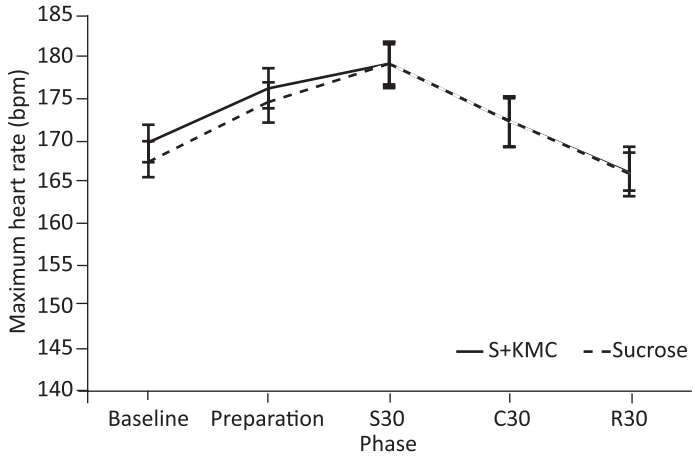

Figure 7. Maximum heart rate in beats per minute (bpm) across phases of the procedure, by intervention group. Bars represent standard error.

Pairwise comparisons using the Bonferroni adjustment showed a significant increase from baseline to preparation (*MD*= -6.60, *SE*= .89) and to needle stick (*MD*= -10.27, *SE*= 1.40 but not to compression and rest. Maximum heart rate at preparation was significantly lower than needle stick (*MD*= -3.67, *SE*= 1.03 and higher than rest (*MD*= 9.15, *SE*= 1.26 but not different from compression. At needle stick, maximum heart rate was significantly higher than at all the other phases.

Means and standard deviation of maximum heart rate at every phase of the procedure as well as tables of within-subjects contrasts and pairwise comparisons can be found in Appendix L, tables 1, 2 and 3.

Average heart rate across the procedure

Regarding average heart rate, the overall effect was not significant, Pillai's Trace= .024, F(4, 90)= .54, p= .705, η^2p= .02, O*P*= .18.

The mean, standard error and 95% confidence interval for each intervention group can be found on Table 14. The mean difference (MD) between the two intervention groups was very small and not significant (*MD*= -.06, *SE*= 2.64, 95% CI [-5.17, 5.30]).

Repeated-measures ANOVA yielded no significant effect of intervention on average heart rate, F(1,93)= .001.

A significant main effect of gestational age was found, F(1,93)= 7.88, *p*= . indicating that average heart rate was significantly different according to gestational age: the mean difference (MD) in heart rate between neonates below 32 weeks and those 32 weeks and more was *MD*= 7.40, *SE*= 2.64, 95% CI [2.16, 12.63].

A significant main effect of phase was also found, F(2.90, 270.15)= 48.25, indicating that average heart rate was different across phases of the procedure, the effect size being medium to large.

Table 14 - Average heart rate for each intervention group and gestational age

				95% CI	
Intervention		M	SE	Lower limit	Upper limit
Sucrose	All n= 43	161.77	1.9	158	165.54
	< 32 weeks n= 16	163.15	3.01	157.18	169.13
	≥ 32 weeks n= 27	160.39	2.32	155.79	164.99
S+KMC	All n= 54	161.7	1.83	158.07	165.33
	< 32 weeks n= 15	167.72	3.11	161.55	173.89
	≥ 32 weeks n= 39	155.69	1.93	151.86	159.51

There was a significant interaction effect between gestational age and phase on average heart rate, with a small effect size. This indicates that average heart rate across the phases of the procedure differed according to gestational age (see Table 15).

Table 15 - Results of repeated-measures ANOVA for average heart rate

Factors	df	F	p	r	OP
	Between subjects				
Intervention	1, 93	.001	.981		
Gestational age	1, 93	7.88	.006**	.28	.79
Intervention x Gestational age	1, 93	3.09	.082		
	Within subjects[a]				
Phase	2.90, 270.15	48.25	.000***	.39	.99
Phase x Intervention	2.90, 270.15	.067	.975		
Phase x Gestational age	2.90, 270.15	3.57	.016*	.11	.78
Phase x Intervention x Gestational age	2.90, 270.15	.76	.512		

Note: [a] The Greenhouse-Geisser correction was used. * Significant for α< .05; ** Significant for α< .01; *** Significant for α< .001. OP, observed power.

Variations in average heart rate across phases of the procedure are displayed in Figure 8. Changes in average heart rate followed the same trend as maximum heart rate.

Tests of within-subjects contrasts showed a significant change in average heart rate from phase to phase, with no interaction effect of intervention or gestational age.

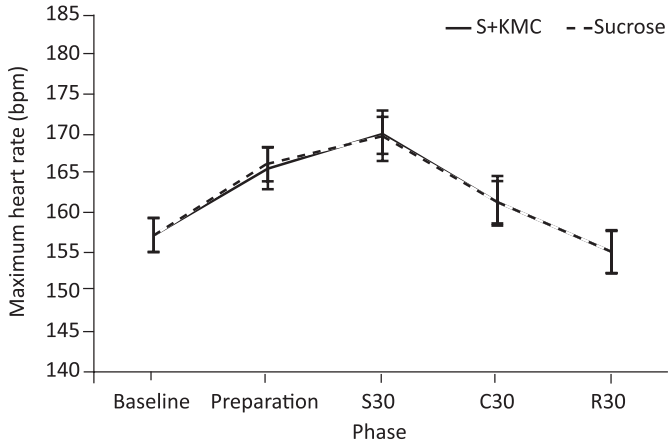

Figure 8. Average heart rate in beats per minute (bpm) across phases of the procedure, by intervention group. Bars represent standard error.

Pairwise comparisons using the Bonferroni adjustment showed a significant difference between all pairs of phases, except between baseline and rest.

Means and standard deviation of average heart rate at every phase of the procedure as well as tables of within-subjects contrasts and pairwise comparisons can be found in APPENDIX L, tables 4, 5 and 6.

Minimum heart rate across the procedure

Regarding minimum heart rate, the overall effect was not significant, Pillai's Trace= .023, F(4, 90)= .53, p= .711.

The mean minimum heart rate, standard error and 95% confidence interval for each intervention group can be found on Table 16. The mean difference (MD) between the two intervention groups was very small and not significant (*MD*= -.11, *SE*= 2.66, 95% CI [-5.38, 5.17]).

Table 16 - Minimum heart rate for each intervention group and gestational age

				95% CI	
Intervention		M	SE	Lower limit	Upper limit
Sucrose	All n= 43	149.68	1.91	145.88	153.48
	< 32 weeks n= 16	153.35	3.03	147.33	159.37
	≥ 32 weeks n= 27	146.01	2.33	141.73	150.64
S+KMC	All n= 54	149.79	1.8	146.13	153.45
	< 32 weeks n= 15	158.64	3.13	152.42	164.86
	≥ 32 weeks n= 39	140.93	1.94	137.08	144.79

Regarding minimum heart rate, no effect of intervention was found, F(1, 93)= .00 (see Table 17).

Gestational age had a significant main effect on minimum heart rate F(1, 93)= 22.24, the mean difference between younger infants and older infants being significant (*MD*= 12.53, *SE*= 2.66, 95% CI [7.25, 17.8]). The effect size was large.

Phase had a significant main effect on minimum heart rate F(3.27, 303.92)= 25.16, with a medium effect size, indicating that minimum heart rate significantly differed across phases of the procedure.

Table 17 - Results of repeated-measures ANOVA for minimum heart rate

Factors	df	F	p	r	OP
	Between subjects				
Intervention	1,93	.00	.968		
Gestational age	1,93	22.24	.000***	.44	.99
Intervention x Gestational age	1,93	3.81	.054		
	Within subjects[a]				
Phase	3.27, 303.92	25.16	.000***	.28	.99
Phase x Intervention	3.27, 303.92	.304	.839		
Phase x Gestational age	3.27, 303.92	.704	.562		
Phase x Intervention x Gestational age	3.27, 303.92	.532	.676		

Note: [a] The Greenhouse-Geisser correction was used. *** Significant for α< .001. OP, observed power.

Paiwise comparisons with Bonferroni correction showed that minimum heart rate changed significantly from baseline to preparation (*MD*= -8.34, *SE*= 1.47, 95% CI [-12.57, -4.11]), needle stick (*MD*= -10.76, *SE*= 1.98, 95% CI [-16.47, -5.06]), and rest (*MD*= 3.74, *SE*= 1.29, 95% CI [.03, 7.45]). The difference between baseline and compression was small and not significant (*MD*= -1.10, *SE*= 1.87, 95% CI [-6.48, 4.28]). Minimum heart rate at needle stick was significantly different from all the other phases (*p*> .00) except preparation. Minimum heart rate values across the procedure are represented in Figure 9.

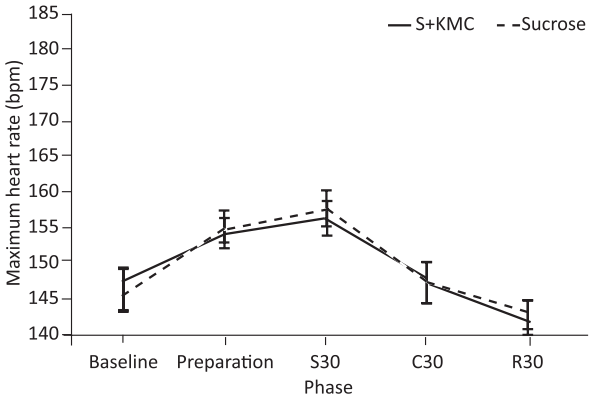

Figure 9. Minimum heart rate in beats per minute (bpm) across phases of the procedure, by intervention group. Bars represent standard error.

Means and standard deviation of minimum hearth rate at every phase of the procedure and tables of within-subjects constrasts and pairwise comparisons can be found in APPENDIX L, tables 7, 8 and 9.

The relationship between maximum heart rate and infants' background characteristics, namely birth weight, postnatal age, number of previous painful procedures and time elapsed since the last meal, was also examined using Pearson product-moment correlations. No significant correlation was found between maximum heart rate and the time elapsed since the last meal, $p > .05$. There was a significant moderate to strong positive correlation between maximum heart rate and postnatal age across all phases of the procedure (see Table 18). These correlations were strong in the Sucrose group, and according to the coefficients of determination, 25% to 47% of the variance of maximum heart rate could be accounted for by postnatal age. A moderate negative correlation was found at rest between maximum heart rate and birth weight ($r = -.34$) although with a low coefficient of determination. A moderate positive correlation between maximum heart rate and number of previous painful procedures was found only at compression, $r = .44$, again with a low coefficient of determination.

Table 18 - Pearson product-moment correlation coefficients (*r*) between maximum heart rate values in different phases and infants' characteristics by intervention, and corresponding coefficients of determination (r^2) (only significant correlations are displayed)

		Birth weight		Postnatal age		Number of previous painful procedures	
	Phase	Sucrose	S+KMC	Sucrose	S+KMC	Sucrose	S+KMC
Maximum heart hate	Baseline	ns	ns	$r = .50$ $p = .000$*** n= 48 $r^2 = .25$	$r = .34$ $p = .009$** n= 58 $r^2 = .12$	ns	ns
	Preparation	ns	ns	$r = .63$ $p = .000$*** n= 48 $r^2 = .40$	$r = .34$ $p = .009$** n= 58 $r^2 = .12$	ns	ns
	S30	ns	ns	$r = .56$ $p = .000$*** n= 48 $r^2 = .31$	$r = .41$ $p = .001$** n= 58 $r^2 = .17$	ns	ns
	C30	ns	ns	$r = .69$ $p = .000$*** n= 48 $r^2 = .47$	ns	$r = .44$ $p = .003$** n= 44 $r^2 = .19$	ns
	R30	$r = -.34$ $p = .022$* n= 44 $r^2 = .12$	ns	$r = .51$ $p = .000$*** n= 44 $r^2 = .26$	$r = .43$ $p = .001$** n= 54 $r^2 = .19$	ns	ns

Note: *Significant for $\alpha < .05$; **Significant for $\alpha < .01$; *** Significant for $\alpha < .001$.

In summary, phase had a significant main effect on maximum, average and minimum heart rate values, which changed significantly across phases of the procedure, in-

creasing from baseline until needle stick and descending from needle stick to rest, following a quadratic trend. These changes occurred in both intervention groups with no significant effect of intervention. A main effect of gestational age was found for minimum and average heart rate and an interaction effect between gestational age and phase was significant for maximum and average heart rate.

Postnatal age correlated to maximum heart rate at all phases of the procedure, with stronger coefficients of determination in the Sucrose group.

7.2.3 Oxygen saturation

Minimum, average and maximum oxygen saturation (SpO2) at baseline and across phases of the procedure will be presented, followed by the correlation between oxygen saturation and maternal anxiety and the correlation between minimum oxygen saturation and maximum heart rate.

Maximum missing data on oxygen saturation was 18/110 infants in only one phase: rest.

Oxygen saturation at Baseline

The Independent Student t-test was performed to identify differences between the two intervention groups at baseline. The results show that the intervention groups did not differ at baseline in minimum, average and maximum oxygen saturation values (p> .05). The exact statistics at baseline are displayed on Table 19.

Table 19 - Baseline values for minimum, average and maximum oxygen saturation by intervention, and results of t-test

	Sucrose n= 49		S+KMC n= 59			
SpO2	M	SD	M	SD	Independent Student t-test	
Minimum	96.51	2.47	96.8	2.33	t (106)= -.62	p= .537
Average	97.31	2.16	97.62	1.93	t (106)= -.78	p= .439
Maximum	98.12	2.09	98.32	1.42	t (106)= -.59	p= .537

Minimum oxygen saturation across phases of the procedure

The hypothesis of an effect of intervention on oxygen saturation levels was tested using repeated-measures ANOVA with intervention and gestational age group as between-subjects factors and phase of procedure as within-subjects factor. The test was done for minimum, average and maximum oxygen saturation.

The results for minimum oxygen saturation levels did not support this hypothesis. The overall effect was not significant, Pillai's Trace= .50, F(4, 80)= 1.06, p= .384.

The mean, standard error and 95% confidence interval for each intervention group can be found on Table 20. The mean difference (MD) between the two interven-

tion groups regarding minimum oxygen saturation was very small and not significant (*MD*= -.12, *SE*= .53, 95% CI [-1.17, .93]).

Table 20 - Minimum oxygen saturation (%) for each intervention group and gestational age

				95% CI	
Intervention		M	SE	Lower limit	Upper limit
Sucrose	All n= 36	96.23	0.39	95.47	97
	< 32 weeks n= 16	95.71	0.57	94.57	96.85
	≥ 32 weeks n= 20	96.75	0.51	95.73	97.77
S+KMC	All n= 51	96.36	0.36	95.64	97.07
	< 32 weeks n= 14	96.59	0.61	95.37	97.81
	≥ 32 weeks n= 37	96.12	0.38	95.37	96.88

There was no significant main effect of intervention F(1, 83)= .05, or gestational age F(1, 83)= .30 on minimum oxygen saturation levels. The effect of phase was also not significant F(2.41, 199.96)= 2.01, indicating that there were no significant variations of minimum oxygen saturation levels across phases of the procedure. There were also no significant interaction effects on minimum oxygen saturation levels (see Table 21).

Table 21 - Results of repeated-measures ANOVA for minimum oxygen saturation

Factors	df	F	p
	Between subjects		
Intervention	1, 83	.06	.815
Gestational age	1, 83	.30	.586
Intervention x GA	1, 83	2.02	.159
	Within subjects[a]		
Phase	2.41, 199.96	2.01	.128
Phase x Intervention	2.41, 199.96	.49	.646
Phase x GA	2.41, 199.96	.64	.558
Phase x Intervention x GA	2.41, 199.96	.91	.421

Note: [a] The Greenhouse-Geisser correction was used

During the procedure, minimum oxygen saturation levels ranged within a very narrow window. There was a decrease from baseline to needle stick and the values increased from needle stick to rest (Figure 10).

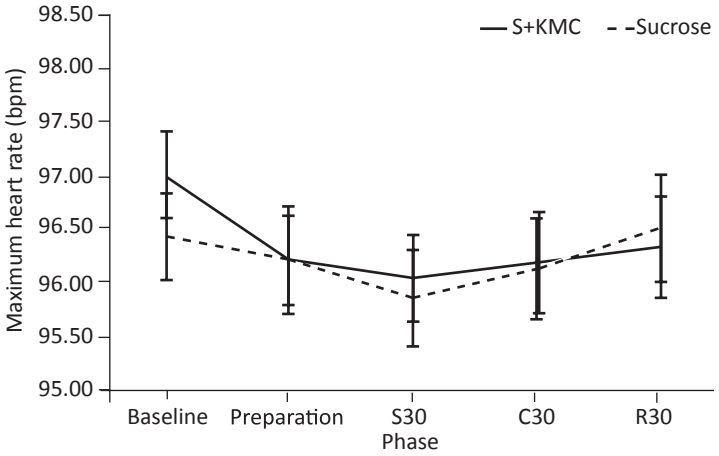

Figure 10. Minimum oxygen saturation levels (%) across phases of the procedure, by intervention group. Bars represent standard error.

Pairwise comparisons showed a significant mean difference in minimum oxygen saturation between baseline and needle stick (*MD*= .77, *SE*= .26, 95% CI [0.01, 1.52]).

Full tables with means and standard deviation of minimum oxygen saturation by intervention and gestational age groups as well as results of within-subjects contrasts and pairwise comparisons are presented in APPENDIX M, table 1.

Average oxygen saturation across phases of the procedure

Using a repeated-measures ANOVA with the same model described for minimum oxygen saturation, there was no overall effect of intervention and gestational age group, Pillai's Trace= .02, F(4, 80)= .44, *p*= .780.

The mean, standard error and 95% confidence interval for each intervention group is displayed on Table 22. The mean difference (MD) between the two intervention groups was very small and not significant (*MD*= -.29, *SE*= .46, 95% CI [-1.20, 0.62]).

Table 22 - Average oxygen saturation (%) for each intervention group and gestational age

				95% CI	
Intervention		M	SE	Lower limit	Upper limit
Sucrose	All n= 36	97.05	0.33	96.39	97.71
	< 32 weeks n= 16	96.62	0.5	95.64	97.61
	≥ 32 weeks n= 20	97.47	0.44	96.59	98.35
S+KMC	All n= 51	97.33	0.31	96.72	97.95
	< 32 weeks n= 14	97.43	0.53	96.38	98.42
	≥ 32 weeks n= 37	97.24	0.33	96.59	97.89

No main effect of intervention, F(1, 83)= .40, gestational age F(1, 83)= .518, or phase F(2.21, 183.49)= 2.34 was detected, indicating that none of these variables played a significant role in average saturation levels during the procedure (Table 23).

Table 23 - Results of repeated-measures ANOVA for average oxygen saturation

Factors	df	F	p
	Between subjects		
Intervention	1,83	.40	.528
Gestational age	1,83	.52	.474
Intervention x GA	1,83	1.29	.260
	Within subjects[a]		
Phase	2.21,183.49	2.34	.094
Phase x Intervention	2.21,183.49	.50	.629
Phase x GA	2.21,183.49	1.67	.189
Phase x Intervention x GA	2.21,183.49	.48	.639

Note: [a] The Greenhouse-Geisser correction was used

Average oxygen saturation levels variation was very small across the procedure, with a slight decrease from Baseline until Needle stick and a recover after that (see Figure 11).

Tests of within subjects contrasts showed a significant effect of phase on the change from Baseline to Preparation, F(1, 83)= 4.72, *p*= .033. However, pairwise comparisons showed the difference between these two phases was not significant.

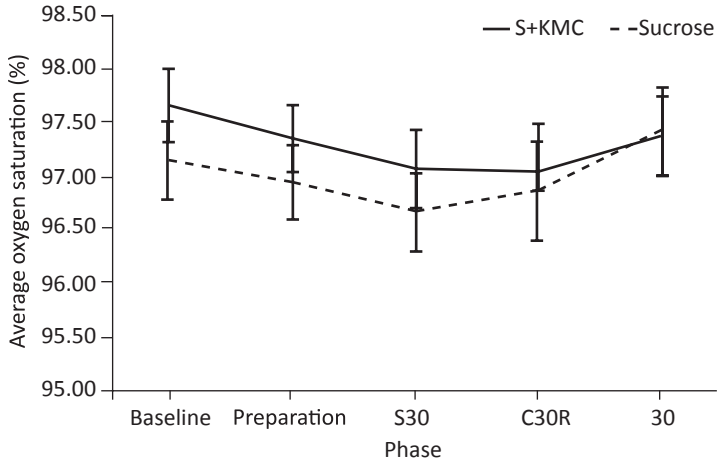

Figure 11. Average oxygen saturation levels (%) across phases of the procedure, by intervention group. Bars represent standard error.

Tables with means and standard deviation of average oxygen saturation by intervention and gestational age groups as well as results of within-subjects contrasts and pairwise comparisons are presented in APPENDIX M, table 2.

Maximum oxygen saturation across phases of the procedure

Results of the two-factor repeated-measures ANOVA yielded no overall effect on maximum oxygen saturation levels, Pillai's Trace= .04, F(4, 80)= .85, *p*= .496.

Means for each group are displayed in Table 24. The mean difference between the two intervention groups regarding maximum oxygen saturation levels was not significant, (*MD*= -.34, *SE*= .39, 95% CI [-1.12, 0.45]).

Table 24 - Average oxygen saturation (%) for each intervention group and gestational age

				95% CI	
Intervention		M	SE	Lower limit	Upper limit
Sucrose	All n= 36	97.05	0.33	96.39	97.71
	< 32 weeks n= 16	96.62	0.5	95.64	97.61
	≥ 32 weeks n= 20	97.47	0.44	96.59	98.35
S+KMC	All n= 51	97.33	0.31	96.72	97.95
	< 32 weeks n= 14	97.43	0.53	96.38	98.42
	≥ 32 weeks n= 37	97.24	0.33	96.59	97.89

There was no main effect of intervention, F(1, 83)= .73, or gestational age, F(1, 83)= .48, but the main effect of phase was significant, F(2.37, 196.85)= 3.69. This indicates that maximum oxygen saturation levels varied significantly across phases of the procedure. A significant interaction effect between gestational age group and time F(2.37, 196.85)= 3.71 indicates that the variation of maximum oxygen levels across phases of the procedure was different according to the gestational age of the infant (see Table 25).

Table 25 - Results of repeated-measures ANOVA for maximum oxygen saturation

Factors	df	F	p	r	OP
	Between subjects				
Intervention	1,83	.73	.397		
Gestational age	1,83	.48	.491		
Intervention x Gestational age	1,83	1.02	.317		
	Within subjects[a]				
Phase	2.37, 196.85	3.69	.020*	.14	.73
Phase x Intervention	2.37, 196.85	.53	.618		
Phase x Gestational age	2.37, 196.85	3.71	.020*	.14	.73
Phase x Intervention x Gestational age	2.37, 196.85	.414	.696		

Note: a The Greenhouse-Geisser correction was used. * Significant for α< .05. OP, observed power.

The variation range of maximum oxygen saturation levels across the procedure was within one percent (Figure 12). Still, although there were no significant differences in the contrast tests, pairwise comparisons with Bonferroni correction showed a significant mean difference (MD) between needle stick and rest (*MD*= -.65, *SE*= .21, 95% CI [-1.26, -0.04]).

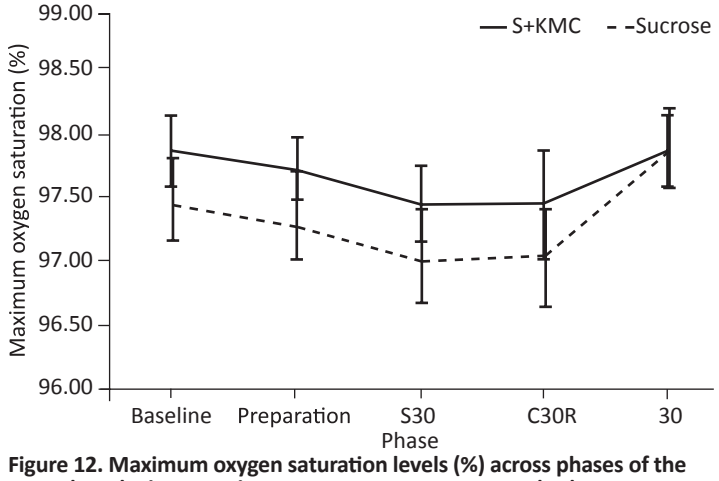

Figure 12. Maximum oxygen saturation levels (%) across phases of the procedure, by intervention group. Bars represent standard error.

Tables with means and standard deviation of average oxygen saturation by intervention and gestational age groups as well as results of within-subjects contrasts and pairwise comparisons are presented in APPENDIX M, table 3.

Summarizing, no significant differences between the intervention groups were found for minimum, average and maximum oxygen saturation levels. Levels of minimum and average oxygen saturation did not change significantly across phases of the procedure. For maximum oxygen saturation levels, however, there was a significant main effect of phase and a significant interaction between phase and gestational age group.

7.2.4 Facial Behavior

Each of the three facial actions recorded: brow bulge (BB), eye squeeze (ES) and nasolabial furrow (NLF) will be presented separately at baseline and across phases of the procedure. The correlation between facial actions and maternal anxiety, and infant characteristics will also be examined, as well as the correlation among the three facial actions and between facial actions and heart rate. Values express the percentage of time that infants' displayed the facial action in each phase of the procedure.

It was hypothesized that the percentage of time the infants displayed brow bulge, eye squeeze and nasolabial furrow during the painful phases of the procedure would be different in the Sucrose condition and in the S+KMC condition.

Brow bulge

Differences between the two intervention groups at baseline were examined using the Independent Student t-test. At baseline, the t-test showed there was no significant difference in the percentage of time in brow bulge between the Sucrose group (M= 1.20, SD= 4.20) and the S+KMC group (M= 1.24, SD= 4.54), t(108)= .046, p= .964.

A two-factor repeated-measures ANOVA was performed with intervention and gestational age group as between-subjects factors and phase as the within-subjects factor. The mean, standard error, and confidence interval for each group by gestational age are presented in Table 26.

Table 26 - Percentage of time in brow bulge by intervention group and gestational age

				95% CI	
Intervention		M	SE	Lower limit	Upper limit
Sucrose	All n= 45	9.68	1.64	6.43	12.94
	< 32 weeks n= 17	8.59	2.59	3.45	13.72
	≥ 32 weeks n= 28	10.78	2.02	6.78	14.78
S+KMC	All n= 57	4.49	1.61	1.31	7.68
	< 32 weeks n= 15	2.33	1.76	-3.14	7.8
	≥ 32 weeks n= 42	6.65	1.65	3.39	9.92

The overall effect was not significant, Pillai's Trace= .024, F(4, 95)= .58, p= .676. However, the mean difference (MD) between the two intervention groups was significant (MD= 5.19, SE= 2.30, 95% CI [0.64, 9.74]), infants in the S+KMC group spending significantly less time in brow bulge.

The results of repeated-measures ANOVA supported the hypothesis of a main effect of intervention, F(1, 98)= 5.12, p= .026, on brow bulge, with a small to medium effect size of r= .22.

A significant effect of phase was also found, F(2, 212)= 23.18, with an effect size of r= .31, indicating that the percentage of brow bulge varied significantly across the procedure.

The significant interaction effect between intervention and time F(3, 212)= 3.24,

with a small effect size, suggests that variations of brow bulge across the procedure were related to the intervention (see Table 27). Introducing the duration of preparation phase as covariate did not change the results.

Table 27 - Results of repeated-measures ANOVA for percentage of time in brow bulge

Factors	df	F	p	r	OP
	Between subjects				
Intervention	1, 98	5.12	.026*	.22	.61
Gestational age	1, 98	2.01	.159		
Intervention x Gestational age	1, 98	.22	.643		
	Within subjects[a]				
Phase	2.16, 211.97	23.18	.000***	.31	.99
Phase x Intervention	2.16, 211.97	3.24	.037*	.12	.64
Phase x Gestational age	2.16, 211.97	2.66	.068		
Phase x Intervention x Gestational age	2.16, 211.97	.61	.559		

Note: a The Greenhouse-Geisser correction was used. * Significant for α< .05; *** Significant for α< .001
OP, observed power.

Tests of within-subjects contrasts showed a significant difference from phase to phase. Brow bulge increased from Baseline to preparation, reaching a peak at needle stick and declining at compression, to reach baseline values at rest, as displayed on Figure 13.

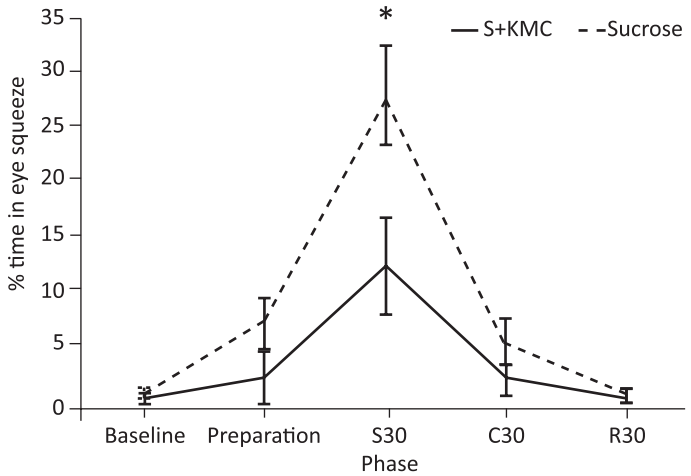

Figure 13. Percentage of time in brow bulge across phases of the procedure, by intervention group. Bars represent standard error. Note: * Significant for α< .05

Pairwise comparisons using Bonferroni adjustment show a significant mean difference (MD) between needle stick phase (S30) and baseline (*MD*= 20.41, *SE*= 3.24, 95% CI [11.10, 29.72]), preparation (*MD*= 16.11, *SE*= 2.89, 95 % CI [7.81, 24.40]), compression (*MD*= 14.89, *SE*= 3.03, 95% CI [6.18, 23.60]), and rest (*MD*= 19.90, *SE*=

3.38, 95% CI [10.20, 29.60]). Differences between the other phases were not significant.

Post-hoc analysis using a two-way ANOVA to examine differences between groups by phase showed that the significant difference takes place at needle stick, the Sucrose group displaying brow bulge in a much higher percentage of time, (*M*= 29.22, *SE*= 4.75) than the S+KMC group (*M*= 15.89, *SE*= 4.58), *p*= .046.

Means and standard deviation for the percentage of time in brow bulge by intervention group and gestational age group at each phase of the procedure as well as tables of within-subjects contrasts and pairwise comparisons can be found in APPENDIX N, tables 1, 2 and 3.

Eye squeeze

The Student t-test was used to compare the means of the two intervention groups at baseline and showed no difference, t(71)= 1.525, *p*> .05.

The means, standard error and confidence interval for eye squeeze in each intervention group and gestational age group are displayed in Table 28.

Table 28 - Percentage of time in eye squeeze by intervention group and gestational age

				95% CI	
Intervention		M	SE	Lower limit	Upper limit
Sucrose	All n= 45	8.94	1.47	6.03	11.86
	< 32 weeks n= 17	7.8	2.32	3.2	12.39
	≥ 32 weeks n= 28	10.09	1.8	6.51	13.67
S+KMC	All n= 57	3.87	1.44	1.02	6.72
	< 32 weeks n= 15	1.8	2.47	-3.09	6.69
	≥ 32 weeks n= 42	5.95	1.47	3.03	8.87

Using intervention and gestational age group as between-subjects factors and phase as the within-subjects factor, repeated-measures ANOVA was performed (see Table 29). The overall effect was not significant, Pillai's Trace= .025, F(4, 95)= .62, *p*= .65.

As for brow bulge, the results support the hypothesis of a main effect of intervention on the percentage of time in eye squeeze, F(1, 98)= 6.02, *p*= .015. The mean difference (MD) between the two intervention groups being significant (*MD*= 5.07, *SE*=

2.05, 95% CI [0.99, 9.15]). The calculated effect size was r= .24.

Within subjects, a significant main effect of phase, F(1.86, 182.37)= 27.06, p= .000, indicates that eye squeeze varied significantly across the procedure. The significant interaction effect found between phase and intervention, F(1.86, 182.37)= 4.02, p= .022, suggests that the variations of eye squeeze across the procedure were related to the intervention.

Table 29 - Results of repeated-measures ANOVA for percentage of time in eye squeeze

Factors	df	F	p	r	OP
	Between subjects				
Intervention	1, 98	6.11	.015*	.24	.69
Gestational age	1, 98	2.47	.119		
Intervention x Gestational age	1, 98	.20	.652		
	Within subjects[a]				
Phase	1.86, 182.37	27.06	.000***	.36	.99
Phase x Intervention	1.86, 182.37	4.02	.022*	.15	.69
Phase x GA	1.86, 182.37	2.69	.075		
Phase x Intervention x Gestational age	1.86, 182.37	.137	.858		

Note: [a] The Greenhouse-Geisser correction was used. * Significant for α< .05; *** Significant for α< .001. OP, observed power.

The display of eye squeeze across phases of the procedure was similar to that of brow bulge. An increase from baseline to needle stick was followed by a decrease from needle stick to rest (Figure 14). Tests of within-subjects contrasts show a significant difference from phase to phase. The interaction effect of phase and group was significant for the difference between S30 and C30, F(1, 98)= 5.26, p= .024.

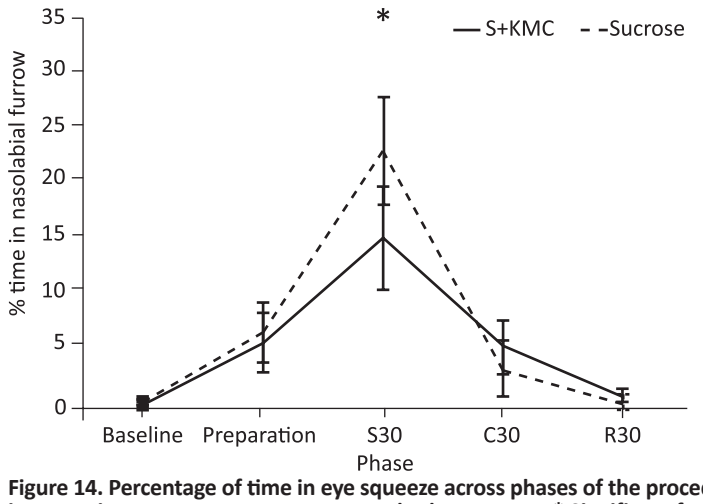

Figure 14. Percentage of time in eye squeeze across phases of the procedure by intervention group. Bars represent standard error.Note: * Significant for α< .05

Pairwise comparisons using Bonferroni adjustment for multiple comparisons

show a significant mean difference (MD) between S30 and baseline (*MD*= 20.18, 95% CI [10.94, 29.43]); S30 and preparation (*MD*= 15.89, 95% CI [7.54, 24.24]); S30 and C30 (*MD*= 16.54, 95% CI [8.30, 24.77]); and between S30 and R30 (*MD*= 20.17, 95% CI [10.71, 29.63]).

Means and standard deviation for the percentage of time in eye squeeze by intervention group and gestational age action the procedure, as well as tables of within-subjects contrasts and pairwise comparisons can be found in APPENDIX N, tables 4, 5, and 6.

To examine differences between groups by phase, a post-hoc analysis using a two-way ANOVA was conducted and showed that the significant difference takes place at needle stick, the Sucrose group displaying eye squeeze in a much higher percentage of time (*M*= 29.13, *SE*= 4.52) than the S+KMC group (*M*= 13.85, *SE*= 4.36) *p*= .017.

Nasolabial furrow

The *t* test was used to examine differences at baseline, and no significant difference was found in the percentage of time in nasolabial furrow *t*(108)= .854, *p*= .395.

Table 30 displays the means, standard error and confidence interval for nasolabial furrow in each intervention group and gestational age group.

Table 30 - Percentage of time in nasolabial furrow by intervention group and gestational age

				95% CI	
Intervention		M	SE	Lower limit	Upper limit
Sucrose	All n= 45	7.00	1.66	3.71	10.29
	< 32 weeks n= 17	5.80	2.61	.62	10.99
	≥ 32 weeks n= 28	8.20	2.04	4.16	12.24
S+KMC	All n= 57	5.69	1.62	2.47	8.91
	< 32 weeks n= 15	3.86	2.78	-1.67	9.38
	≥ 32 weeks n= 42	7.52	1.66	4.22	10.82

Using intervention and gestational age group as between-subjects factors and phase as within-subjects factor, repeated-measures ANOVA was performed. The overall effect was not significant, Pillai's Trace= .012, *F*(4, 95)= .30, *p*= .881.

The mean difference (MD) between the two intervention groups was also not significant (*MD*= 1.31, *SE*= 2.32, 95% CI [-3.29, 5.91]).

The results do not support the hypothesis of an effect of intervention on nasolabial furrow display, nor an effect of gestational age (see Table 31). These results are not modified by introducing the duration of preparation as covariate.

Differences across phases of the procedure were significant, as indicated by a significant main effect of phase $F(2, 212)= 21.04$, $p= .000$.

Table 31 - Results of repeated-measures ANOVA for percentage of time in nasolabial furrow

Factors	df	F	p	r	OP
	Between subjects				
Intervention	1, 98	.32	.573		
Gestational age	1, 98	1.71	.194		
Intervention x Gestational age	1, 98	.07	.786		
	Within subjects[a]				
Phase	2.02, 197.56	21.04	.000***	.31	.99
Phase x Intervention	2.02, 197.56	1.30	.275		
Phase x Gestational age	2.02, 197.56	1.11	.331		
Phase x Intervention x Gestational age	2.02, 197.56	.34	.716		

Note: a The Greenhouse-Geisser correction was used. *** Significant for α< .001. OP, observed power.
OP, observed power.

Tests of within-subjects contrasts show a significant difference in the percentage of nasolabial furrow from phase to phase. Like other facial actions, infants' display of nasolabial furrow increased from baseline to needle stick and decreased from there to rest (Figure 15).

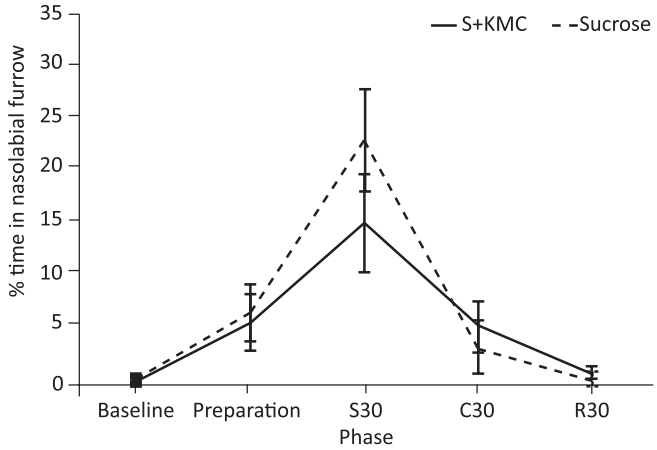

Figure 15. Percentage of time in nasolabial furrow across phases of the procedure by intervention group. Bars represent standard error.

Pairwise comparisons using Bonferroni adjustment for multiple comparisons showed a significant mean difference between baseline and preparation (*MD*= -5.98, 95% CI [-11.33, -.63]). Baseline, compression and rest did not differ significantly. Percentage of nasolabial furrow during needle stick was significantly different from

baseline (*MD*= 18.70, 95% CI 9.22, 28.17]), preparation (*MD*= 12.72, 95% CI [4.68, 20.76]), compression (*MD*= 14.56, 95% CI [6.26, 22.85]), and rest (*MD*= 18.20, 95% CI [8.70, 27.71]). Means and standard deviation for the percentage of time in nasolabial furrow by intervention group and gestational age action the procedure, as well as tables of within-subjects contrasts and pairwise comparisons can be found in APPENDIX N, tables 7, 8, and 9.

Facial actions and sex

To test the effect of sex on facial actions, a factorial ANOVA was conducted for each facial action at each point in time, adding sex to the between-subjects factors intervention and gestational age. The results indicate that during the first 30 seconds of compression (C30), boys displayed brow bulge (*M*= 10.21, *SE*= 2.73) significantly more than girls (*M*= 2.41, *SE*= 2.81), *F*(1, 101)= 3.97, *p*= .040. The effect size for both brow bulge was *r*= .19, which can be considered a small to moderate effect size of sex.

Correlation between facial actions

The correlation among facial actions was examined through a bivariate Pearson product-moment correlation for each phase and significant correlations are displayed in Table 32.

Table 32 - Significant Pearson product-moment correlation coefficients (r) between facial actions and corresponding coefficients of determination (r^2)

Phase	Facial actions	Eye squeeze	Nasolabial furrow
Baseline	Brow bulge	*r*= .47 *p*= .000*** n= 110 r^2= .22	ns
	Eye squeeze	--	*r*= .41 *p*= .000*** n= 110 r^2= .17
Preparation	Brow bulge	*r*= .91 *p*= .000*** n= 110 r^2= .83	*r*= .85 *p*= .000*** n= 110 r^2= .72
	Eye squeeze	--	*r*= .86 *p*= .000*** n= 110 r^2= .73
Needle Stick (S30)	Brow bulge	*r*= .95 *p*= .000*** n= 110 r^2= .89	*r*= .90 *p*= .000*** n= 110 r^2= .80
	Eye squeeze	--	*r*= .90 *p*= .000*** n= 110 r^2= .80
Compression (C30)	Brow bulge	*r*= .88 *p*= .000*** n= 109 r^2= .77	*r*= .79 *p*= .000*** n= 109 r^2= .62
	Eye squeeze	--	*r*= .77 *p*= .000*** n= 109 r^2= .59
Rest (R30)	Brow bulge	*r*= .56 *p*= .000*** n= 102 r^2= .31	*r*= .27 *p*= .006** n= 102 r^2= .07
	Eye squeeze	--	*r*= .26 *p*= .009** n= 102 r^2= .07

Note: **Significant for α< .01; *** Significant for α< .001

The two upper facial actions, brow bulge and eye squeeze, were correlated at all phases of the procedure. During preparation, needle stick and compression, there was a very strong positive correlation between the three facial actions, whereas at baseline and rest, the correlation between the facial actions was only moderate. In the case of nasolabial furrow, at baseline there was no significant correlation with brow bulge, and at rest, the correlation with brow bulge and eye squeeze was significant but weak. The shared variance between brow bulge and eye squeeze during preparation, needle stick and compression was much higher (77% to 89%) than at baseline and rest (22% to 31%).

Facial actions and heart rate

The relationship between each facial action and maximum heart rate was examined using a bivariate Pearson product-moment correlation. At preparation, needle stick (S30) and compression (C30), all three facial actions were significantly low to moderately correlated to maximum heart rate but coefficients of determination were low (see Table 33 for significant correlations).

Table 33 - Significant Pearson product-moment correlation (r) between brow 5bulge, eye squeeze, nasolabial furrow, and maximum heart rate during needle stick and compression phases and coefficients of determination (r^2)

Phase	Facial actions	Maximum heart rate
Preparation	Brow bulge	*r*= .28 *p*= .004** n= 106 r^2= .08
	Eye squeeze	*r*= .29 *p*= .002** n= 106 r^2= .09
	Nasolabial furrow	*r*= .27 *p*= .005** n= 106 r^2= .07
Needle Stick (S30)	Brow bulge	*r*= .38 *p*= .000*** n= 107 r^2= .15
	Eye squeeze	*r*= .41 *p*= .000*** n= 107 r^2= .17
	Nasolabial furrow	*r*= .40 *p*= .000*** n= 107 r^2= .16
Compression (C30)	Brow bulge	*r*= .35 *p*= .000*** n= 104 r^2= .12
	Eye squeeze	*r*= .39 *p*= .000*** n= 104 r^2= .15
	Nasolabial furrow	*r*= .44 *p*= .000*** n= 104 r^2= .20

Note: **Significant for α< .01; *** Significant for α< .001

In conclusion, the percentage of time displaying facial actions increased from

baseline to preparation and needle stick and decreased there after. A significant main effect of intervention was found on brow bulge and eye squeeze, and a significant effect of phase was found for all three facial actions. Boys and girls responded differently at C30, for brow bulge. Strong correlations were found between facial actions during the manipulative phases of the procedure: preparation, needle stick and compression, with high coefficients of determination. Weak to moderate correlations between facial actions and maximum heart rate were significant at preparation, needle stick and compression with low shared variability.

7.2.5 Behavioral state

Behavioral state was recorded and analyzed across the procedure. Given the ordinal nature of the variable, non-parametric tests were used to examine the relation between behavioral state and intervention.

Four behavioral states are considered in the PIPP: active/awake, quiet/awake, active/sleep and quiet/sleep. Figure 16 displays the percentage of infants in sleep states (active sleep and quiet sleep). At baseline, most infants in both groups were asleep with a higher percentage in the S+KMC group. The majority of infants in the S+KMC group remained asleep throughout the procedure. In the Sucrose group, the number of infants in sleep states decreased from baseline to preparation and needle stick, the percentage of infants asleep and awake being nearly the same at preparation (51.02% and 48.98%, respectively) and at needle stick phase (48.98% asleep and 51.02% awake). At compression and rest phases again, most infants in both groups were in sleep states.

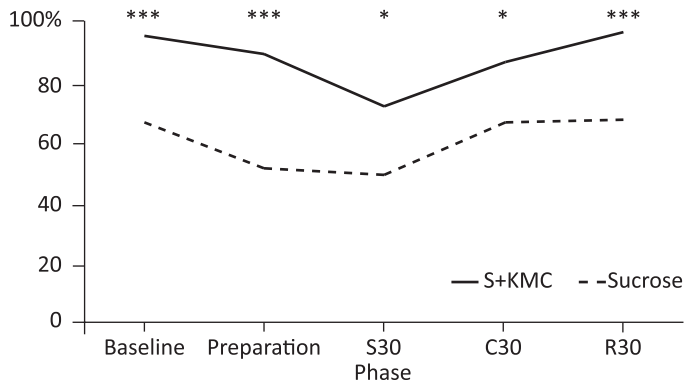

Figure 16. Percentage of neonates in sleep states across phases of the procedure, by intervention group. Note: * Significant for α< .05; * Significant for α< .001**

A Chi-Square test of independence was used to examine the relation between intervention and state at each phase of the procedure. In order to create 2x2 tables, active

sleep and quiet sleep were grouped together, and so were active awake and quiet awake. Results of the Chi-Square test are displayed in Table 34.

The proportion of infants in sleep states was significantly higher in the S+KMC group compared to the Sucrose group at every point in time. Odds Ratio for being asleep in the S+KMC condition was 2.49, which means infants in this condition were 2. 49 times more likely to be asleep during the needle stick than infants in the Sucrose condition.

Table 34 - Number and percentage (below) of infants in each behavioral state, by phase of procedure and intervention group, and results of the Chi-square test.

	Baseline		Preparation		S30		C30		R30	
State	S n= 49	S+KMC n= 61	S n= 49	S+KMC n= 61	S n= 49	S+KMC n= 61	S n= 49	S+KMC n= 60	S n= 45	S+KMC n= 57
Active awake	9 18.37	2 3.28	**18** 36.73	5 8.20	**22** 44.90	17 27.87	8 16.33	8 13.33	8 17.78	2 3.51
Quiet awake	8 16.33	2 3.28	6 12.24	2 3.28	3 6.12	1 1.64	9 18.37	1 1.67	7 15.56	1 1.75
Active sleep	**20** 40.82	**30** 49.18	17 34.69	**39** 63.93	20 40.82	**33** 54.10	**22** 44.9	**29** 48.33	**20** 44.44	**28** 49.12
Quiet sleep	12 24.49	27 44.26	8 16.33	15 25.59	4 8.16	10 16.39	10 20.41	22 36.67	10 22.22	26 45.61
Chi-Square test a)	$\chi^2_{(1)}$= 13.93 *p*= .000***		$\chi^2_{(1)}$= 18.88 *p*= .000***		$\chi^2_{(1)}$= 5.28 *p*= .022*		$\chi^2_{(1)}$= 5.76 *p*= .016*		$\chi^2_{(1)}$= 13.63 *p*= .000***	

Notes: a) Chi-square test was performed grouping the two wake states and the two sleep states, to create 2x2 tables. Numbers in bold represent the highest frequency in each column. * Significant for α< .05; *** Significant for α< .001

Behavioral state and facial actions

The relation between the infant's behavioral state in each phase of the procedure and the mean percentage of time displaying facial actions during the same phase was examined using Spearman correlation test (r_s) (Figure 17). State was categorized as for the PIPP score: 0, active awake; 1, quiet awake; 2, active sleep; and 3 quiet sleep.

The correlation at baseline was not significant (r_s= -.18, p= .062). A significant moderate negative correlation was found at preparation (r_s= -.42, p< .001, r^2= .18), needle stick (r_s= -.62, p< .001, r^2= .39) and compression (r_s= -.54, p< .001, r^2= .29). At rest, a negative correlation was significant but low (r_s= -.29, p< .001, r^2= .08). This indicates that during the manipulative phases of the procedure, the more asleep the infants were the less facial actions they displayed.

Behavioral state and other variables

The relation between behavioral state and site, gender and gestational age group was tested using the Chi-Square test but no significant relation was found.

To summarize, at baseline, behavioral state was related to the intervention group being Sucrose or S+KMC, more infants being asleep in the S+KMC group. This relation persisted across the procedure, a significantly higher percentage of infants being in sleep states during needle stick in the S+KMC group compared to the Sucrose group.

Behavioral state was negatively correlated to mean percentage of facial action.

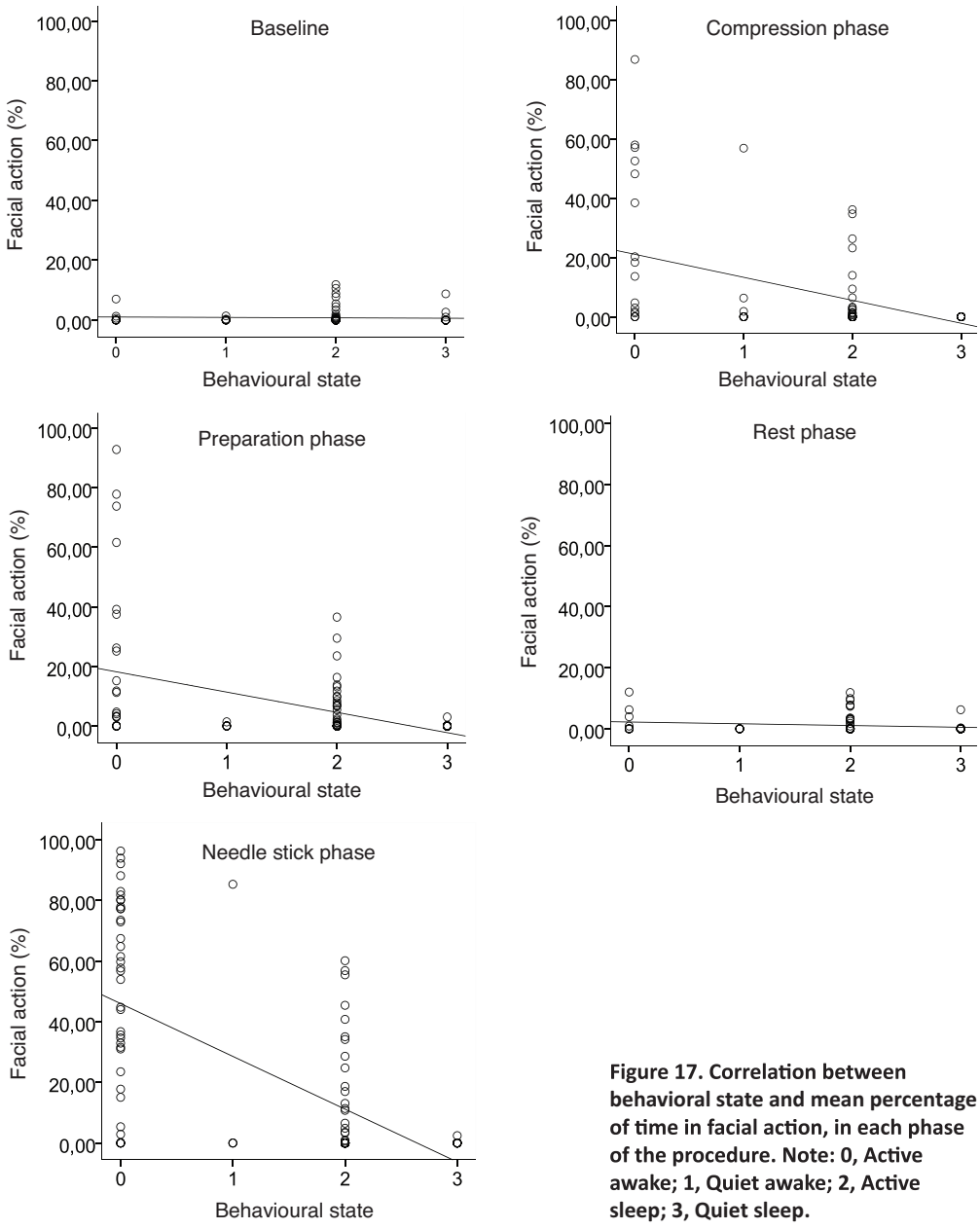

Figure 17. Correlation between behavioral state and mean percentage of time in facial action, in each phase of the procedure. Note: 0, Active awake; 1, Quiet awake; 2, Active sleep; 3, Quiet sleep.

7.2.6 Heart rate variability

Heart rate variability was examined in terms of frequency-domain analysis. Low-

frequency (LF), high-frequency (HF) and the ratio LF to HF were estimated for three phases: baseline, needle stick plus compression, and rest. At baseline, the 30 seconds segment analyzed was insufficient to provide any data, so only two time periods were included in the analysis: needle stick and compression phases together, which will be designated as manipulation phase, and rest. Due to the short duration of the blood and consequent small length of the recorded segments, data were missing for 13 neonates (11.82%). The percentage was similar in the Sucrose group (12.25%) and in the S+KMC group (11.48%).

The hypothesis was that heart rate variability measured through the LH to HF ratio would be different between infants under S+KMC and infants under Sucrose. To test this hypothesis, a two-factor repeated measures ANOVA with intervention and gestational age as between-subjects factors and phase with two levels as within-subjects factor was used.

The results will be presented for LF, HF and Ratio across the procedure.

LF across the procedure

Mean, standard error and confidence interval for low frequency are displayed in Table 35.

Table 35 - Mean low frequency peaks by intervention group and gestational age

				95% CI	
Intervention	Age group	M	SE	Lower limit	Upper limit
Sucrose	All n= 37	115.27	26.88	61.72	168.81
	< 32 weeks n= 16	65.13	40.51	-15,55	145.80
	≥ 32 weeks n= 21	165.41	35.36	94.99	235.82
S+KMC	All n= 43	101.65	26.36	49.14	154.15
	< 32 weeks n= 14	46.39	43.30	-39.850	132.64
	≥ 32 weeks n= 29	156.90	30.09	96.97	216.82

The results of the hypotheses testing showed the overall effect was not significant, Pillai's Trace= .012, $F(1, 76)= .92$, $p= .34$.

There was a main effect of gestational age on LF, $F(1, 76)= 7.84$, $p= .006$, younger infants having significant less low frequency peaks than infants in the older group ($MD= - 105.39$, $SE= 37.65$, 95% CI [-108.38, - 30.40]) (see Table 36).

There was also a main effect of phase $F(1, 76)= 5.91$, $p= .017$, infants displaying

less low-frequency peaks during the manipulation phase, *M*= 75.57, *SE*= 15.55, than during the rest phase, *M*= 141,34, *SE*= 28.87, *p*= .017.

Table 36 - Results of repeated-measures ANOVA for LF

Factors	df	F	p	r	OP
Between subjects					
Intervention	1, 76	.13	.719		
Gestational age	1, 76	7.84	.006**	.31	.79
Intervention x Gestational age	1, 76	.02	.89		
Within subjects [a]					
Phase	1, 76	5.91	.017*	.27	.67
Phase x Intervention	1, 76	.00	.98		
Phase x Gestational age	1, 76	.64	.425		
Phase x Intervention x Gestational age	1, 76	.92	.342		

Note: LF, low-frequency; a The Greenhouse-Geisser correction was used.
*Significant for α< .05; ***Significant for α< .001

HF across the procedure

Table 37 displays the mean, standard error and confidence intervals for high frequency peaks during the procedure.

Table 37 - High-frequency by intervention group and gestational age

				95% CI	
Intervention	Age group	*M*	*SE*	Lower limit	Upper limit
Sucrose	All n= 35	46.13	13.69	18.84	73.41
	< 32 weeks n= 16	22.25	20.17	-17.96	62.46
	≥ 32 weeks n= 19	70.00	18.51	33.11	106.90
S+KMC	All n= 41	17.33	13.85	-10.27	44.93
	< 32 weeks n= 12	9.04	23.29	-37.38	55.47
	≥ 32 weeks n= 29	25.62	14.98	-4.24	55.49

The results of the repeated-measures ANOVA showed the overall effect was not significant, Pillai's Trace= .00, $F(1, 72)$= .00, *p*= .970.

There were no significant main effects or interaction effects. HF did not change significantly across the procedure nor did it vary with intervention and gestational age (see Table 38). Introducing duration of the preparation phase as covariate did not change the results.

Table 38 - Results of repeated-measures ANOVA for HF

Factors	df	F	p
Between subjects			
Intervention	1 , 72	2.19	.144
Gestational age	1 , 72	2.73	.103
Intervention x Gestational age	1 , 72	.64	.426
Within subjects			
Phase	1 , 72	3.37	.071
Phase x Intervention	1 , 72	1.03	.314
Phase x Gestational age	1 , 72	.11	.745
Phase x Intervention x Gestational age	1 , 72	.00	.97

Note: HF, High-frequency

LF/HF ratio across the procedure

The mean, standard error and confidence intervals for the ratio LF to HF is displayed in Table 39.

Table 39 - LF/HF ratio by intervention group and gestational age

				95% CI	
Intervention	Age group	*M*	*SE*	Lower limit	Upper limit
Sucrose	All n= 37	6.98	1.30	4.38	9.57
	< 32 weeks n= 16	5.96	1.96	2.05	9.86
	≥ 32 weeks n= 21	7.99	1.71	4.59	11.40
S+KMC	All n= 43	9.48	1,28	6.94	12.02
	< 32 weeks n= 14	9.90	2.10	5.72	14.07
	≥ 32 weeks n= 29	9.06	1.46	6.16	11.97

There was no overall effect of intervention and gestational age on LF/HF ratio (Pillai's Trace= .01, $F(1, 76)= .98$, $p= .33$).

There was no main effect of intervention or gestational age, or an interaction effect between intervention and gestational age. There were no significant interactions of intervention, gestational age or both, with phase. There was no main effect of phase, (see Table 40), the ratio LF to HF not being very different between the manipulation phase ($M= 8.54$, $SE= .81$) and the rest phase ($M= 7.92$, $SE= 1.31$).The mean difference between manipulation and rest failed to reach significance ($MD= .62$, $SE= 1.19$, 95% CI [-1.75, 3.00]).

Table 40 - Results of repeated-measures ANOVA for LF/HF

Factors	df	F	p
Between subjects			
Intervention	1, 76	1.89	.173
Gestational age	1, 76	.11	.742
Intervention x Gestational age	1, 76	.43	.433
Within subjects			
Phase	1, 76	.27	.603
Phase x Intervention	1, 76	2.47	.12
Phase x Gestational age	1, 76	.89	.35
Phase x Intervention x Gestational age	1, 76	.98	.326

Note: LF/HF, ratio between low-frequency and high-frequency peaks

In summary, low frequency peaks were significantly less in infants below 32 weeks gestational age compared to older infants. They were also significantly less during the manipulation phase compared to the rest phase. No main effects of group or gestational age were found on HF peaks and on LF/HF ratio.

7.2.7 Recovery time

The results of the analysis of recovery time by intervention and gestational age group will be presented as well as the results of the correlation tests with other variables.

Recovery time, as defined before, is the period, measured in seconds, elapsed from the end of the procedure, when the neonate is left quiet, until the heart rate returns to the initial values for five or more consecutive beats.

The recordings after the end of the procedure had a variable duration. By the end of the recording, some infants had recovered baseline heart rate values and some had not. Only the infants who had returned to baseline before the end of the recording were retained for analysis. When the recording was still on at 5 minutes after the end of the procedure (300 seconds) and the infant had not returned to baseline, the value was prorated to 310 seconds. This happened in 14 infants, representing 16.28% of the cases under analysis.

The hypothesis was that infants in the S+KMC group would have a shorter recovery time than infants in the Sucrose group. A two-way analysis of covariance (ANCOVA) was conducted, with intervention and gestational age as between-subjects factors, phase as within-subjects factor, and duration of needle stick as covariate. Mean, standard error and confidence intervals for recovery time by intervention group and gestational age group are displayed in Table 42. The mean recovery time of infants in Sucrose was slightly shorter (M= 121.43, SE= 18.03) than that of infants in the S+KMC group (M= 143.27, SE= 16.91) but the difference was not significant.

Table 41 - Recovery time by intervention group and gestational age

Intervention		*M*	*SE*	95% CI Lower limit	95% CI Upper limit
Sucrose	All n= 35	121.43	18.03	85.56	157.31
	< 32 weeks n= 16	125.43	26.57	72.56	178.31
	≥ 32 weeks n= 19	117.43	24.03	69.61	165.26
S+KMC	All n= 51	143.27	16.91	109.61	176.93
	< 32 weeks n= 13	195.82	29.03	138.04	253.60
	≥ 32 weeks n= 38	90.72	17.09	56.71	124.72

The results of the two-way ANCOVA are displayed in Table 43. A significant main effect of gestational age was found on recovery time, $F(1, 80)= 5.34$, $p= .023$ and the interaction between gestational age and group was close to significance $F(1, 80)= 3.94$, $p= .050$.

Older infants recovered faster, $M= 104.07$, $SE= 14.68$ than younger infants $M= 160.63$, $SE= 19.55$, $MD= 56.55$, $SE= 24.47$, 95% CI [7.86, 105.24].

Table 42 - Results of Two-way ANCOVA for recovery time

Factors	df	F	p	r	OP
	Between subjects				
Intervention	1, 80	.76	.385		.14
Gestational age	1, 80	5.34	.023*	.25	.63
Intervention x Gestational age	1, 80	3.94	.05		.50

Note: [a] The Greenhouse-Geisser correction was used. * Significant for α< .05. OP, observed power.

Dividing the rest phase in 30 seconds blocks, it was possible to calculate the number of infants who had recovered heart rate baseline values within each 30 seconds block and whether this was related to the intervention. To test the hypotheses that recovery at each block was associated with intervention, the Pearson Chi-Square test was performed for each of these periods.

No significant association was found when analyzing the sample as a whole but when looking at gestational age groups separately, recovery was associated with intervention at R60 and R90 for infants 32 weeks and more, and at R180 and R210 for infants below 32 weeks (see Table 44). There was a significant association between in-

tervention and whether or not infants 32 weeks and more had recovered at 60 and 90 seconds after the end of the procedure. Based on the odds ratio, older infants in the S+KMC group were 3.03 times more likely to have recovered heart rate baseline values at 60 seconds and 2.96 times of doing so at 90 seconds than infants in the Sucrose group. In infants below 32 weeks gestational age, the association between intervention and whether or not they had recovered was significant at 180 and 210 seconds. The odds ratio, though, was too small to be considered clinically significant.

Table 43 - Association between recovery and intervention, by age group (only significant results are displayed)

			Sucrose	S+KMC		
Gestational age	Time epoch	Recovered	n %	n %	Chi-Square	Odds ratio
< 32 weeks	R180	Yes	12 70.59	4 28.57	$\chi^2_{(1)}$= 5.427 *p*= .020*	.17
		No	5 29.41	10 71.43		
	R210	Yes	12 75.00	5 35.71	$\chi^2_{(1)}$= 4.693 *p*= .030*	.19
		No	4 25.00	9 64.29		
≥32 weeks	R60	Yes	6 23.08	20 47.62	$\chi^2_{(1)}$= 4.096 *p*= .043*	3.03
		No	20 76.92	22 52.38		
	R90	Yes	9 36.00	25 62.50	$\chi^2_{(1)}$= 4.331 *p*= .037*	2.96
		No	16 64.00	15 37.50		

* Significant for α< .05

The relationship between recovery time and infant and procedure variables was examined using a bivariate Pearson product-moment correlation. No correlation was found with postnatal age, number of previous painful procedures, time since last meal, total duration of the procedure and duration of pain phases.

In summary, a significant main effect of gestational age was found on recovery time, younger infants taking longer to recover. Although recovery time was not significantly different between the two intervention groups when the whole sample was considered, infants 32 weeks gestational age and older who received S+KMC were more likely than infants who received sucrose, to have recovered at 60 seconds and at 90 seconds after the end of the procedure.

7.2.8 Summary of pain responses to the interventions

The overall results of the hypothesis testing for each pain response as well as the estimated effect size are presented in Table 41.

Table 44 - Summary of significants results for pain responses

Outcome		Results	p	Effect size r
PIPP[a]		Main effect of Phase	.000	.32
Heart rate[a]	Maximum	Main effect of Phase	.000	.28
		Interaction effect Phase x Gestational age	.008	.12
	Average	Main effect of Gestational age	.000	.28
		Main effect of Phase	.000	.39
		Interaction effect Phase x Gestational age	.016	.11
	Minimum	Main effect of Gestational age	.000	.44
		Main effect of Phase	.000	.28
Oxygen saturation[a]	Maximum	Main effect of Phase	.02	.14
		Interaction effect Phase x Gestational age	.02	.14
Brow bulge[a]		Main effect of Intervention	.026	.22
		Main effect of Phase	.000	.31
		Interaction effect Phase x Intervention	.037	.12
Eye squeeze[a]		Main effect of Intervention	.015	.24
		Main effect of Phase	.000	.36
		Interaction effect Phase x Intervention	.022	.15
Nasolabial furrow[a]		Main effect of Phase	.000	.31
State[b]		Significant association between intervention and state		
Heart rate variability[a]	Low-frequency	Main effect of Gestational age	.006	.31
		Main effect of Phase	.017	.27
Recovery time[c]		Main effect of Gestational age	.02	.25

Notes: [a]Two-way repeated-measures ANOVA; [b]Chi-Square test; [c]Two-way ANCOVA.

The effect of intervention was found for brow bulge and eye squeeze with a small to medium effect size. A significant effect of gestational age was found for average and minimum heart rate, and for low-frequency peaks. The effect of phase was found for all the outcomes except minimum and average oxygen saturation, and high-frequency and LF/HF ratio.

7.3 Maternal anxiety and infants' pain responses

A secondary objective of this study was to explore the relation between maternal anxiety and the pain responses of infants who had S+KMC.

Maternal anxiety level was measured using the state questionnaire of the State-Trait Anxiety Inventory (Spielberger *et al.*, 1983; Silva, 2003) and was low in average (M= 39.64, SD= 9.82). The scores of maternal anxiety measured with the STAI by intervention and gestational age group are displayed in Table 45. When the two intervention groups were analyzed together, there were significant differences regarding mater-

nal anxiety, the mothers in the sucrose group revealing a higher level of anxiety (*M*= 43.48, *SD*= 9.82) than the mothers in the S+KMC group (*M*= 37.78, *SD*= 9.13, *t*(87)= 2.65), *p*= .009. When the two subgroups of gestational age were analyzed separately, this difference was only present in the group of infants 32 weeks gestational age and above.

Table 45 - Maternal anxiety (STAI score) for each intervention group by gestational age

Gestational age		All N= 89	S n= 29	S+KMC n= 60	Test
All	Mean	39.64	43.48	37.78	*t(87)*= 2.65
N= 89	SD	(9.82)	(10.23)	(9.13)	*p*= .009**
	Median	39	45	36.5	*MD*= 5.70
	Range	20-63	27-63	20-56	[1.43, 9.97]
< 32 weeks	Mean	39.56	41.88	38.47	*t(23)*= .80
n= 25	SD	9.92	7.77	10.82	*p*= .435
	Median	39.00	44.50	39.00	*MD*= 5.70
	Range	22-56	32-52	2-56	[-5.46, 12.27]
≥ 32 weeks	Mean	39.67	44.10	37.51	*t(62)*= 2.62
n= 64	SD	9.86	11.13	8.50	*p*= .011*
	Median	38.50	45.00	36.00	*MD*= 5.70
	Range	20-63	27-63	8.50	[1.57, 11.60]

Abbreviations: MD, Mean Difference. *Significant for α< .05; **Significant for α< .01.

The relationship between maternal anxiety and infants' pain response, namely PIPP scores, heart rate, oxygen saturation, facial actions, behavioral state, heart rate variability and infants' recovery time was examined only in the infants who had S+KMC.

Maternal anxiety and infants' PIPP scores

Pearson product-moment correlations between mothers' anxiety measured with the STAI and PIPP scores in different phases of the procedure were calculated. No significant correlations were found looking at the two gestational age groups together or examining each gestational age group separately (see APPENDIX O, Table 1).

Maternal anxiety and infants' heart rate

Pearson product-moment correlations between mothers' anxiety and maximum, average and minimum heart rate values of infants who had S+KMC were calculated for the different phases of the procedure. Considering all infants together, a significant weak to moderate negative correlation was found between the STAI and maximum heart rate at compression and rest. In infants below 32 weeks gestational age, correlations were not significant at any point in time. Conversely, in older infants a significant moderate negative correlation was found between the STAI and maximum heart rate during preparation, needle stick, compression, and rest; between the STAI and aver-

age heart rate at preparation, needle stick and compression; and between the STAI and minimum heart rate at preparation and compression. Significant correlations are displayed on Table 46 (For complete table see Appendix O, Table 2).

The coefficient of determination was calculated (r^2) to estimate the variability of one variable that is accounted for by the other variable. The maximum coefficient of determination was 23% at rest phase, representing a small effect size.

Table 46 - Pearson product-moment correlation coefficients (r) between mothers' anxiety and maximum heart rate, in infants who had S+KMC, and corresponding coefficients of determination (r^2) (only significant correlations are displayed)

	Gestational age	Baseline	Preparation	Needle Stick	Compression	Rest
			Maximum heart rate			
STAI	All		ns	ns	*r*= -.28	*r*= -.35
					p= .033*	*p*= .010*
					n= 58	n= 55
					r^2= .08	r^2= .12
	< 32 weeks		ns	ns	ns	ns
	≥ 32 weeks		*r*= -.32	*r*= -.37	*r*= -.37	*r*= -.48
			p= .042*	*p*= .017*	*p*= .017*	*p*= .002**
			n= 41	n= 41	n= 41	n= 40
			r^2= .10	r^2= .14	r^2= .14	r^2= .23
			Average heart rate			
		Baseline	Preparation	Needle Stick	Compression	Rest
	All	ns	ns	ns	ns	ns
	< 32 weeks	ns	ns	ns	ns	ns
	≥ 32 weeks		*r*= -.34	*r*= -.32	*r*= -.31	
			p= .031*	*p*= .040*	*p*= .046	
			n= 40	n= 41	n= 41	
			r^2= .12	r^2= .10	r^2= .10	
			Minimum heart rate			
		Baseline	Preparation	Needle Stick	Compression	Rest
	All	ns	ns	ns	ns	ns
	< 32 weeks	ns	ns	ns	ns	ns
	≥ 32 weeks	ns	*r*= .37	ns	ns	*r*= -.38
			p= .02*			*p*= .016*
			n= 40			n= 40
			r^2= .14			r^2= .14

Note: * Significant for α< .05; ** Significant for α< .01

Maternal anxiety and infants' oxygen saturation

Bivariate correlations between the STAI and minimum, average and maximum oxygen saturation levels were examined. Significant correlations were found only at rest,

in infants below 32 weeks. These correlations were moderate and negative between the STAI and minimum oxygen saturation (r= -.60, p= .02); and between the STAI and average oxygen saturation (r= -.57, p= .03) (see APPENDIX O, Table 3).

Maternal anxiety and infants' facial actions

To explore the relation between maternal anxiety and each facial action, bivariate correlations were computed, revealing no significant correlations between maternal anxiety and facial actions at any point in time (see APPENDIX O, Table 4)

Maternal anxiety and infants' behavioral state

As presented before, the majority of infants in the S+KMC group were in sleep states. The Independent Student t test was conducted to explore the difference in mean STAI scores between infants in sleep states and infants in wake states. No significant differences were found in the sample as whole or when considering each gestational age group separately (see APPENDIX O, Table 5).

Maternal anxiety and heart rate variability

The correlation between the STAI and indices of heart rate variability of infants in the S+KMC group were examined for each phase of the procedure using Pearson product-moment correlation. No significant correlations were found in any phase of the procedure (see Appendix O, Table 6).

Maternal anxiety and infants' recovery time

A Pearson correlation test was conducted, revealing no significant correlation between recovery time and maternal anxiety (r= .04, p= .76). Analysis by gestational age group yielded the same results: r= .19, p= .54 in infants below 32 weeks and r= -.17, p= .31 in infants 32 weeks gestational age and above.

In summary, maternal anxiety was significantly different between intervention groups, mothers in the Sucrose group showing higher levels of anxiety than mothers in the S+KMC group.

In infants in the S+KMC group 32 weeks gestational age and above, maternal anxiety was negatively correlated to maximum heart rate, with very low coefficients of determination.

7.4 Mothers' perceptions of doing Kangaroo Care

To explore mothers' perceptions of doing Kangaroo Care during the painful procedure, mothers were interviewed within a week of the blood draw in Kangaroo Care.

The introductory question was "Please tell me, how was it for you?" The questions that followed tried to clarify the issues raised by the mothers, such as their feelings during the procedure, their thoughts about themselves, the baby and the situation, and to recall the baby's reactions. After that, two final questions were asked: "Would you repeat this, if you had the choice?" and "Would you recommend it? If another mother was hesitating to do it, what would you tell her, and why?"

Seven mothers were not interviewed because they were discharged and two mothers did Kangaroo Care with two infants from a triplet, so a total of fifty-two interviews were analyzed.

The characteristics of mothers that were interviewed and their neonates are presented on Table 47, although they are similar from the rest of the sample presented on section 1 of the Results.

Table 47 - Characteristics of mothers (n= 52) that were interviewed and their neonates

Mothers' characteristics	
Age, Mean (*SD*)	31 (5)
First child (%)	41
Caesarean delivery (%)	59
1st time in Kangaroo Care (%)	69
Neonates' characteristics	
Gender (% males)	63
Gestational age in weeks, Mean (*SD*)	32 (2)
Birth weight in grams, Mean (*SD*)	1694 (480)
Postnatal age in days, Mean (*SD*)	7 (4)

A content analysis of the interviews was performed, as described in the Methods chapter, to allow for mothers' feelings, thoughts and observations to emerge. Main themes were identified and units of analysis within these themes were grouped in categories, according to their meaning. The frequency of these categories, representing the number of mothers who shared the same concept, is presented below. Findings are presented in tables if a theme has many categories, and recording units were selected to illustrate the diversity of mothers' expressions for each category. Each recording unit carries a code representing its source.

Three main themes were brought up by the mothers: 1) Expectations at the beginning of the event; 2) Doing Kangaroo Care; and 3) The blood draw.

Answers to the two final questions are presented at the end of the section.

Expectations at the beginning of the event

The expectations at the beginning of the event were recalled by 38 mothers, 26 recalling a negative expectation, 10 a positive expectation and 2 a neutral expectation.

Negative expectations. Mothers with a negative expectation recalled being nervous, expecting the baby to cry, being afraid the baby would have pain, afraid to see the baby being poked and afraid to hold the baby.

Some mothers made the reasons for being nervous at the beginning more clear, by explaining that they expected the baby to cry: *"I thought she was going to cry her hearth out..."* (119B2), and they were afraid they wouldn't be able to touch and comfort the baby. Most of the mothers who expected the baby to cry were positively impressed by the fact that the baby cried less than they expected.

Being afraid that the baby would have pain was recalled by five mothers like this one: *"I was a little afraid. I was scared that he would have pain."* (114A2).

Watching the baby being punctured was another reason for being nervous at first: *"I was a little nervous because a tiny little thing being poked in front of me...if you're not there, you don't see it, you don't feel it. There, I would watch it and feel it."* (113B2). *At this thought, one mother admits she nearly gave up participating in the study: "I thought it was going to be worse, I was scared. (...) I even thought I would give up, so I wouldn't see him suffer."* (105A2).

Being scared to hold a small and fragile baby for the first time was expressed by two mothers.

Positive expectations. Ten mothers had a positive expectation from the beginning. One states that she was calm because she knew she was in good hands. Another, relates being calm with not being afraid of needles. For some mothers, the dominant feeling was a positive anxiety, a feeling of happiness for being able to hold the baby: *"I was a little anxious because I knew I was going to hold him for the first time... I had not held him yet and that's what I wanted most. I wasn't at all nervous (...) There was this anxiousness to touch him."* (234B2).

Neutral expectations. For two mothers, the expectation could be defined neither as positive, nor as negative. They were just expectant to see what was going to happen (see Table 48).

Table 48 - Maternal expectations at the beginning of the event

Theme: EXPECTATIONS AT THE BEGINNING OF THE EVENT	
Categories (n)	Recording units
Negative (26)	I thought she was going to cry her heart out. 119B2 I expected him/her to cry 118A2; 117B2; 219B2; 223A2 I was afraid he would cry more. 102B2 What's going to happen... what are they going to do, he's gonna cry a lot, I can't touch him, will I handle it?232B2 I thought he would show less, or something, but he would have some reaction. Never thought there would be none. 241B2 I thought the baby would cry and I would get upset. 246B2 I was a little afraid. I was scared that he would have pain. 114A2; 205A2; 211A2; 252B2 I thought it was going to be a little more painful. 109B2 I thought it might hurt him a lot. 111A2 At first I was very nervous 110A2; 111A2; 119B2 At first I was a little worried because I didn't know how she was going to react. 211A2 I thought it would be harder for me than it really was. I actually liked it but at first I thought I wouldn't, I thought it was going to be worse. 246B2 I was afraid because it's hard for me to see him being poked, it's a little hard. 114A2 I was a bit depressed...that they were piercing her. 219A2 I was a little nervous because a tiny little thing being poked in front of me...if you're not there, you don't see it, you don't feel it. There, I would watch it and feel it.113B2 I thought it was going to be worse, I was scared.(...) I even thought I would give up so I wouldn't see him suffer. 105A2 First I was very anxious, ... holding the baby... 250B2 First, because he is fragile...how do I take him, how do I hold him, but afterwards...113B2
Positive (10)	I was calm... I knew I was in good hands 116A2 I wasn't nervous because I'm not afraid of needles208B2 I always thought I was going feel something good (...)I wasn't even worried. 253B2 The connection I was having with him was so intense; (...) it wasn't going to be that bad. 235B2 Actually, I was relaxed; I believed it was going to be alright because he was so calm...256B2
Neutral (2)	You're always expectant: how is he going to behave? Whether he was going to panic, whether he was going to yell, or scream...257B2 I wasn't afraid. 217A2 I wasn't very worried. I was...I didn't know if in my arms...he was quiet but I didn't know if he was going to cry. 223A2

Note: Numbers in brackets represent mothers included in the category.

Doing Kangaroo Care

The experience of doing Kangaroo Care was recalled by 45 mothers with verbal and nonverbal expressions, as being a very positive one. A first impulse to say how hard it was to describe such an overwhelming experience was shared by nine mothers with

expressions like: *"I have no words..."* or *"Holding them like that, you can't describe it..."*

Two different issues emerged as mothers described their experience: how they felt and how they perceived their babies' feelings.

Mothers' feelings. Often, the first expression that came out was an overall impression of the experience, qualified with adjectives such as good, wonderful, great, remarkable, and fantastic. Encouraged to go into detail, mothers recall feeling a strong emotion, which made some of them cry, as well as feeling very calm, comfortable and relaxed to the point of being sleepy. Actually, during the period of Kangaroo Care before the procedure, some mothers slept for a few minutes.

Being happy was mainly because they were holding the baby, but also because this would help the baby during the procedure: *"I felt happy because I believed she would tolerate [the pain] better."* 111A2.

For some mothers, time had stopped; they wanted to be there with the baby forever. Occasionally, the environment was not as quiet as required. In spite of this, one mother called it *"a magic moment"*.

Holding the baby skin-to-skin gave mothers a feeling of empowerment: *"The best thing I had from Kangaroo was to think a little like a mother. Think of how important we are for our children."* 111A2. This was said to compensate for the moments where mothers care for them in the incubator but don't really feel in control. Close to this was the feeling of being in possession of the baby, feeling the baby really belonged to them, as a mother of triplets, who did Kangaroo care with two of the babies, explains: *"When they were inside me, they were only mine. Now, the baby is himself, everybody touches him; it's not as if only I am allowed to do it. (...) At that moment we were together again."* 250B2.

Mothers recall feeling a strong connection with the infant, as if they were just one, and to some, it reminded them of the time they were pregnant.

Feeling rewarded and soothed by holding the baby was also described by some mothers, and they got more energy and courage to face the situation of being separated (see Table 49).

Mothers' perceptions of how the baby felt. Forty mothers talked about what the baby must have felt (see Table 50). The mothers' body and presence were referred to by twenty-eight mothers: the smell, the warmth, the voice, the sound of the heart:

"When they feel the mother again, (...) for them it's like... 'Hey! There's something here that I know, there's something here that... I'm back home!'" 109B2

Table 49 - Maternal feelings during Kangaroo Care

Theme: DOING KANGAROO CARE Sub-theme: How they felt (42)	
Categories (n)	Recording units
A good sensation (25)	I felt very good. It's a wonderful thing. 102B2 It's a great sensation. 217A2 It's a good feeling. 109B2
The baby's body (23)	To have them close to us... 241B2 He was right there... skin-to-skin. 242B2 Feel him warm (...) It looks as if our hearts are beating at the same time. 224B2 The two of us, feeling his heart on my skin and feeling his skin on mine. 257B2 Having him there, feeling him close to me... holding him tight. 212A2 Being in contact with her. 234B2 I felt her breathing, her movements, as if she was still in my womb. 205A2 Feeling his smell is very, very good. 114A2 To feel her body...the weight, the volume... 117B2 (I thought) he is so small, he fits in here. 106B2
Calm (11)	He passed me this calmness....I felt calm. 106B2 I was calm because he was so calm. 109B2 He fell asleep, he was calm, I was calm. 110A2 I hadn't been that calm yet. 212A2 That day I went home feeling calmer after that bit. 256B2 I was calm, I liked it. 205B2
Happy (11)	I 6)was thrilled, very happy to hold him like that. 109B2 I felt happy because I believed she would tolerate [the pain] better. 111A2 I was happy because I had never held such a tiny baby. 210B2 I felt a great joy. 201A2
Rewarded (10)	It was very rewarding to do kangaroo care (...) You forget about your problems. 111A2 It gives you strength. 201A2 Very comforting. 215B2 It gave me a new life, I can't explain... 219A2
A strong connection (7)	It was such an intimate moment... 106B2 I guess I didn't think of anything ...it is just our moment. 207A2 There is a big complicity. 215B2 It was just the two of us. So many people around and it seemed as if there was no one. 234B2 The connection I was having with him was so intense... 235B2 When I was holding the baby, I felt more affection for the babies (...) It looked as if we were just one person. 250B2
Like a mother (7)	You feel more like a mother, to be with her like that. Not 'til then...These moments where you do Kangaroo (...) you feel you're a mother, that your baby is there. 241B2 The best thing I had from Kangaroo was to think a little like a mother. Think of how important we are for our children. 111A2
In ecstasy (6)	At that time there was no before or after. That was all there was. 110B2 I was enjoying the moment, thinking of the baby. 253B2 I could stay there... like that... 106B2 It felt like staying with him like that forever. 109B2 You have him here and you want to hold him for a long time. 113B2 I wanted more [time]. 213B3 I want more. I want to be with her 24 hours. 247B2

In possession (6)	She is just ours, at that moment. 117B2 That he is ours, really ours. 241B2 It is something from inside us that is there. 252B2 It was something... for me to see that he was there, that he was mine, he was nested there. 242B2 The feeling that the baby is really mine. 250B2
As if still pregnant (5)	It was like having her inside me again 117B2 For me it was as if she was inside me. 205A2 When I had him on my breast it made me think that he and I are just one.... as if we were just one. It reminded me of when I was pregnant. As if we were still just one. 235B2 It was almost as if he was back inside [laughs] my womb. 242B2
Relaxed, sleepy, comfortable (5)	It was very relaxing. I was getting sleepy, it felt like sleeping. 224B2 I was completely relaxed. 253B2 I felt vey comfortable, like a kangaroo. 242B2
A strong emotion (4)	It's very touching. 109B2 I was moved with both of them. 111A2 I started crying with the emotion. 252B2 You get touched...I was. So touched that you have to go through it (to know what it feels like). 257B2

Table 50 - Maternal perceptions of the baby's feelings during Kangaroo Care

Theme: DOING KANGAROO CARE Sub-theme: How the baby felt (40)	
Categories (n)	Recording units
Mother's presence (28)	At least the warmth, the affection, and maybe the heart beat, right? That he could hear just a few weeks ago inside here...106B2 She felt I was cuddling her. 109B2 I think he likes to feel the smell of our skin and the warmth, he likes being snuggled. 113B2 She felt "My mom's here". She heard my heart again, that she knew well. 117B2 When he is on my lap he is always sleeping; sometimes he looks at me, sees I'm there and relaxes. (...) He feels I'm there, it's obvious.118B2 Mom is their home. 211A2 When they feel the mother again, (...) for them it's like... 'Hey! There's something here that I know, there's something here that... I'm back home!'109B2.
Safe/Protected/ Supported (17)	On the mothers' lap they must feel safe. 'Oh, nothing bad can happen here' right? 110A2 They feel they're on our lap. They feel safer. 114A2 He was in contact with me, he felt protected. 225B2 He cried, but he was there, huddled in mother, quiet. It gives a very different feeling of protection. 212A2 We are supporting, she knows we're there and she feels it. 252B2
Quiet, relaxed (17)	When she lay against me... at once... she was very quiet. 232B2 I noticed he was hyper calm, he didn't even move. 256B2 I thought he was quiet because he was with me. 212A2 When I hold him, he always sleeps. Sometimes he looks at me, sees that I'm there and relaxes. 118B2
As still inside of me (9)	He may hear some noise and remember the good time of intra-uterine life. 106B2 We pass them this peace, this feeling of being safe; they must feel like in the mother's womb, which is where they should still be. 114A2
Happy (6)	He was so happy! 114A2 He was enjoying it. 116A2 Even she liked it, she was all happy. 219A2
Comfortable (4)	He always falls asleep on my lap, he feels comfortable. 118B2

The blood draw

The blood draw took place after 30 minutes in Kangaroo Care. Encouraged to focus on the moment of the procedure, mothers talked about their feelings, about their coping strategies and about what the baby felt.

Mothers' feelings during the blood draw. For most of the mothers, the baby's behavior was determinant to the way they felt comforted during the blood draw. To see that the baby didn't react or didn't have pain was a relief for them: *"He was calm, and he calmed me down."* 217A2.

The behavior of the baby was a surprise, since many mothers expected the baby to cry. Their expectations at the beginning did not come true and mothers felt comforted by the baby's behavior.

Mothers were happy to feel they eased the baby's pain. It made them feel important. Many of them emphasized being able to protect their baby from pain. Some mothers referred they felt they were sharing a moment which was difficult for the baby and the positive feeling of being there for the baby was very salient. Being there, also gave mothers a feeling of reassurance, of being in control: they could see if the baby cried, if the baby suffered, rather than imagining what might happen (see Table 51).

Table 51 - Maternal feelings during the blood draw

Theme: THE BLOOD DRAW Sub-theme: How they felt	
Categories (n)	Recording units
Comforted by the baby's reaction (26)	To see that he didn't feel it, it was very good. It's very good to see your children don't suffer.114A2 You see the baby is calm... you're ok. 116A2 Nerves disappeared as I saw that she wasn't reacting.119B2 I was relieved to see she behaved. 201B2 He was calm, and he calmed me down. 217A2 He didn't cry, he was sleeping, he was normal, he was in my arms, I was happy. 118B2 I'm happy as long as he's happy. 258B2 It's always very important for mothers: the less they cry, the less they seem to suffer, the better. 112B2
Happy to ease the baby's pain (15)	I am happy that I reduced her pain. 201B2 Even if it hurt he would stay there looking for comfort to forget the pain of the stick. 106B2 At that moment I felt I was... important for her. (...)They don't suffer alone. We help them go through that. 111A2 I was sharing a moment where he might cry. It was good to share the moment, the two of us. And see that he didn't suffer. 114A2 I felt safe because I was holding him and he wasn't alone. Being able to comfort him... 216B2 I feel it's good (...) you seem to feel that small pain he's feeling. Which is not that...[bad]. Ok, it's not pleasant, I also don't like needles that much, but at that moment, you feel that little thing that he's feeling. 250B2

Protective (11)	I see it like... something that has to be done (the stick) and a way to ease the pain that he might have. 242B2 ... protected there, under my wing. 117B2 I am protecting him, helping him, as if I'm giving him the courage not to cry and stay quiet.256B2 It's a very good feeling to have him tight against me, 'I'm protecting you, you're being poked, hurt, but I'm here, close to you, don't you worry'... It's good, it's very good.257B2
In control (10)	It's reassuring. Because I'm feeling his movements, watching if he is ok or not, if he cries or not, there, closer. In the crib it would be different. 110B2 The mother is reassured (...) when they are taking out blood from the child; at that moment, she can feel what the baby is feeling. 250B2 It was better than sending me away and having me listening to him yelling, this was better. I liked it better. 257B2
Surprised (7)	It was quieter than I thought. 212A2 I never thought he would be so quiet having a stick, well... without crying. 225B2 It was surprising that Kangaroo worked like that. That it would make her so calm; and she felt so good that she didn't even show they were taking out blood. It really surprised me, in a positive way.241B2

Along the interview, the idea of mother and infant sharing the same feeling, of being in communion, was often there in sentences such as: "It wasn't just good for him; it was good for me too." 225B2; and "He was ok, I was ok" 116A2; or "We calmed down and that helped a little with pain" 111A2.

Dealing with the blood draw. Because of the baby's modest reaction to the needle stick, some mothers stated it wasn't hard for them to see the blood draw (see table 52). A few, though, admit that it was hard to see the baby being poked or even that they felt the pain of the stick: *"I was...ouch... (...) as if they were poking me."* 103A2.

Table 52 - Maternal feelings about the blood draw

Theme: THE BLOOD DRAW Sub-theme: Dealing with the blood draw	
Categories (n)	Recording units
Not hard at all to cope (15)	It wasn't hard at all. 118B2 Of course, you know they are being poked, right? But no... It didn't bother me. 107A2 Honestly, it didn't impress me. 232B2 It didn't bother me at all. Maybe if he had cried... 201A2 I don't remember (the needle stick). He didn't cry so I don't remember. I completely forgot the main objective was to see if it (Kangaroo Care) relieved (the pain of the needle stick) ...106B2.
Hard to cope (7)	It's hard to see the needle sticking. 2242 When the blood draw started I was a little nervous. I didn't really know how it worked and the needle was in and out, in and out until the blood came out, so I was a bit nervous. At that moment I was a bit nervous but it was better than having him in the cot. 110B2 It was a little hard to see him being poked. 120B2 Of course, it's a little hard (to see the stick) but it's for their own good. (...) What's hard to see is the needle stick. 224B2 I was sorry for the baby. I was moved. 210B2 During the sticks I felt a lot of pain. 109A2 I was...'ouch'... (...) as if they were poking me.103A2

In order to deal with the moment of the needle stick, which some described as not being a pleasant one, some mothers spontaneously mentioned their coping strategies and others were encouraged to talk about them.

Although only seven mothers had mentioned it had been hard to see the needle stick, nineteen mothers in total shared what they did, making clear why they were not negatively impressed by the stick.

Most of the mothers avoided the sight of the needle stick and the blood draw; they looked away: *"I tried not to look at the stick."* (109B2).

Try to enjoy the moment and not think about the blood draw were other strategies described by these two mothers: *"It didn't (bother me) because I wasn't thinking much about it: whether they were taking out blood or not. So it didn't bother me. (...) I also tried to enjoy the moment and not think about that part."* (253B2); *"If you concentrate on the baby and isolate from the other side..."* (209B2).

Trying to keep calm and trying to keep the baby calm was also used by some mothers: *"I tried to keep calm so he wouldn't feel I was nervous."* (216B2).

Table 53 - Maternal perceptions of the baby's reactions and pain during the blood draw

Theme: THE BLOOD DRAW Sub-theme: The baby's reaction and pain	
Categories (n)	Recording units
Didn't react (30)	When they poked him, he didn't cry. 103A2 He became calm and fell asleep. 106B2 I was looking at him, at his expression, and he was ok. 114A2 She gave no signs of suffering. (...) She always gives a small sign or cries a little, but there you couldn't notice a thing. 207A2 When they poked him, he didn't cry. 103B2 The baby, they poked her and she didn't even... sometimes in the crib she starts crying but that day she didn't even cry. 217A2 She wasn't even startled by the stick. Not at all. 117B2
Reacted just a little (8)	She behaved beautifully. (...) During the stick she didn't move, at all. (...) When they were pressing a little, then she moved a little, very very little. Even the reaction on her face was almost nothing. She almost didn't react. She moved a little, because she could feel someone was doing a small pressure, but otherwise, during the stick, especially the stick, she didn't even move. 211A2 He just gave a small cry, (...). 113B2 She cried a little bit from the stick but that is normal, her mother also cries.109B2 He was always quiet. There was a moment when he complained a little, he stretched, but then he went back in place. 110B2 You could only tell [what was happening] from the facial expression. 223A2 She was very calm and almost didn't move, didn't react. 211A2
Didn't notice the stick (9)	He was sleeping, he was quiet, I think he didn't notice what they were doing to him.113B2 I think she didn't even notice it. 117B2 The baby, it looked as if he didn't realize they were taking out the blood. 116A2 [He] didn't realize he was having a stick.(...) He was so calm, so calm, he didn't realize what was going on. 225B2

Had no pain (6)	I guess (...) if it hurt or if he felt something, he would start crying and yelling. 113B2 Doing Kangaroo Care they don't suffer at all. She was there, very calm, didn't cry or anything. 241B2 No, she had no pain at all. 252B2 He didn't suffer with that little stick. 234B2
Didn't feel much (6)	I didn't hear her cry, I don't think she felt much, because she was so quiet... 112B2 I don't think they suffered a lot. 109A2 He felt the warmth and that reduced the pain. 110B2 She was so well, that that was the least. 224B2 It reduced her suffering. 111A2 That pain, finally, she almost didn't feel it because she was so calm. 217A2

The baby's reactions and pain. Mothers also talked about the baby's reaction during the blood draw and the pain they perceived the baby to feel. They describe a quiet, sleeping baby, a baby that was so nested in their breast that showed no reaction or nearly, to the needle stick. Most of the mothers felt the baby hardly had any pain, if at all (see Table 53).

Repeating the blood draw in Kangaroo Care

The last two questions were introduced after a few interviews had already been conducted. Forty-four mothers were asked about whether they would like to do Kangaroo Care again in case the baby needed another venepuncture or, if they had the choice, what would it be. All mothers said they would repeat Kangaroo Care for the purpose of a needle stick. A few mothers answered with a laconic *yes* but, based on the intonation, on the enthusiasm of the answer or on the explanations that were spontaneously given, it is possible to say that the majority of mothers were very affirmative about it, like in these two cases:

- *"If she needed another stick, yes, it could be like this, no doubt."* 234B2;

- *"Yes, no problem. I wouldn't mind repeating it. I don't see any inconvenience for me or for him. In fact, it's only convenient (...). Since he didn't react, nothing can be better."* 253B2.

Given the choice, the preference would be to hold the baby: *"I would rather hold her."* 122A2

In the unit where Kangaroo Care is not part of standard care, some mothers, although willing to repeat the experience, were not sure whether they would have a chance to do it: *"If possible, I'd like to do it every time, because I think it helped [the baby] a lot to be by my side,"* 211A2; and *"If there is an opportunity, I think it's great to go through this."* 252B2.

Imagining that the baby was alone, i.e., without the mother, while he was having

a needle stick, was unbearable for some mothers: *"I prefer to hold him, have him calm and have them take the blood. It's better than if I'm coming in [to the Unit] and he is crying and I see they're ... oh no. I'd rather hold him and have them take blood while I'm holding him."* 116A2.

Some mothers put the condition of no risk, since for many babies, this was their first time out of the incubator, which was a concern expressed by two mothers: "*Yes. If there was no risk"* 205A2.

Some mothers even anticipated doing Kangaroo Care again for a painful procedure: *"I'll do it more, I'll do it again"* 235B2. A mother of two twins was very sure about it: *"Next time, next time I know (...) I'll put them in here and try that they don't realize something is being done to them that hurts"* 118A2.

Doing Kangaroo Care out of the Hospital, in the Health Centre, where children have their immunizations was also considered: *"I wouldn't mind at all to repeat it every time he has a vaccine or a stick... I'd be willing to, I would. (...) I want more. This way I spare my son and I'm more relaxed too."* 225B2; *"For now, I don't know how it works in the Health Centre but when I go there, I'll ask"* 256B2.

Recommending Kangaroo Care for venepuncture

The final question of the interview was "Would you recommend it to other mothers?" or, "If another mother was hesitating to do it, what would you tell her?"

The answers to this question ranged from a very strong encouragement of other women to do kangaroo care for a painful procedure to a less categorical recommendation.

Most mothers (n= 37) were definite about recommending it to other women in the same situation, frequently using expressions like *"No doubt, I would"* 111A2; or, *"I'd tell her to do it, of course. (...) I'd be totally in favor."* 253B2.

As for the reasons, some brought up the baby: *"It's much better for the baby to be in touch with the mother and feel safe."* 116A2; and *"In face of his reaction when he was poked in kangaroo, I think it should be recommended. If somebody asked me, I'd recommend it, no doubt"* 212A2; or *"They should grab the opportunity to minimize the pain. Because the baby doesn't feel. I am sure about that. I am sure."* 225B2.

Another set of reasons was related to the mother: *"I would recommend it. I would really advise her to do it. It's much more tranquilizing (...). I think the mother will be more relaxed if she does kangaroo than otherwise."* 235B2; and also *"I would tell her to do it because it's a unique experience: to see that the baby is not crying... it's great"* 219B2.

A few mothers were able to articulate benefits for both the baby and the mother: *"I'd tell her it's very important for the mothers to be there when it might be painful for the*

baby. And I think it helps the baby a lot, to feel cuddled and safe next to the mother who, as I said before, is their home. I think it's important for both." 211A2.

Only a few mothers (n= 10) were not as keen about convincing others to do Kangaroo Care for venepuncture, although they wouldn't discourage them. Their main point was that others should try it and judge for themselves, like in the case of an 18 year-old mother who advocates *"It's something that you have to think for yourself and not be told to do or not to do."* 110B2. This is what she would say: *"Make the test. If you like it, you like it. If you don't like it 'does [the baby] like it or not?' If he doesn't like it, you don't do it again."* 110B2.

Another mother put straightforward what she would tell another woman: *"Try it. It's easy"* 118A2.

Their own experience was often used as reference: *"Considering it was a good experience for me, and especially for the baby, who should be in the first place, I'd tell the mother to go on."* 232B2.

Mothers also left their recommendations to professionals: *"...they should plan the care so that we can do it [kangaroo care]. Whenever there's a blood draw, try to have the parents there: the father or the mother."* 111A2; and *"They should put it in practice everywhere."* 241B

Being aware that having the baby in kangaroo for a blood draw was part of a research protocol, some mothers made suggestions for the continuity of kangaroo care for painful procedures:

- *"They should continue to do it [the blood draw] like this. At least they won't hear the babies' cry, they're quiet."* 113B2;

- *"It would be good if this continued. Even for immunizations."* 119B2;

- *"It would be great if a pilot-project could start in Health Centres, from the results of this study"* 209B2.

In conclusion, the experience of doing kangaroo care was described by mothers as overwhelming. Their initial apprehension related to the fear that the baby would cry or suffer was quickly replaced by a feeling of relief related to the perception that the baby's reaction was one of no pain. They took so much pleasure in holding the baby skin-to-skin and to contribute to ease the baby's pain that even mothers who were less comfortable with needles and blood enjoyed the experience. Being able to share what the baby was feeling, to protect him/her and be a part of the baby's pain relief was mentioned by the mothers as salient aspects. Even those mothers for whom dealing with the needle

stick was hard, revealed that they would repeat it, because it was worthwhile. Although varying in their conviction, all mothers would encourage other women in a similar situation to hold the baby in kangaroo care for the blood draw.

CHAPTER 8.
Discussion

CHAPTER 8. Discussion

In the previous chapter the results of this research were presented. Now the findings will be discussed examining each outcome, in order to respond to the objectives of the study. Strengths and limitations will be pointed out. Theoretical issues and implications for clinical practice and research will be presented.

8.1 Kangaroo care, sucrose and pacifier vs sucrose and pacifier

The main hypothesis of the study stated that during venepuncture, the pain responses of preterm neonates who receive S+KMC are less than the pain responses of preterm neonates who receive Sucrose. To test this hypothesis, a two-factor repeated-measures ANOVA was conducted. Intervention was one of the between-subjects factors and the other was gestational age, considering that a difference in pain responses might exist between younger and older preterm infants. Phase of procedure was the within-subjects factor for repeated-measures. This model was used to test the effects on PIPP scores, heart rate, oxygen saturation, facial actions and heart rate variability.

Pain responses across the procedure

In both groups, pain responses, namely the PIPP score, facial actions, heart rate and oxygen saturation varied across phases of the procedure, changing from baseline as soon as the infant was manipulated for skin inspection and cleansing, changing again in the same direction at needle stick, and changing in the opposite direction towards baseline at compression and rest. These changes were statistically significant for all the indicators except oxygen saturation and indicate infants' reactivity to a painful stimulus and to its cessation. The magnitude of the change, for all the indicators, was bigger from baseline to needle stick than from baseline to any other phase showing that preterm infants react with signs of stress to non-painful stimulation caused by holding and disinfecting the hand with a wet cold swab, as well as by compression after withdrawing the needle but, most importantly, they are able to discriminate needle stick phase

as the most distressing phase of the procedure. This pattern of reactivity to a painful procedure has been reported in all the studies that examine the responses across the procedure.

The effect of intervention

Significant differences between intervention groups were found in facial actions, behavioral state and recovery time. Neonates in the S+KMC group displayed less brow bulge and eye squeeze than neonates in the Sucrose group; more neonates remained asleep during the procedure in the S+KMC group compared to the Sucrose group; neonates 32 weeks gestational age and older who received S+KMC were more likely than infants who received Sucrose to have recovered heart rate baseline values at 60 seconds and at 90 seconds after the end of the procedure.

No significant differences were found in PIPP scores, heart rate, oxygen saturation levels, and indices of heart rate variability.

The effect of gestational age

Gestational age had a main effect on some of the pain responses, namely average and minimum heart rate, low-frequency peaks of heart rate variability, and recovery time, corroborating that this was an important variable to consider. No effect of gestational age was found on PIPP scores or facial actions, although according to the literature facial actions vary with gestational age, infants below 28 weeks displaying the same but more subtle responses (Gibbins *et al.*, 2008a; Johnston, Stevens, Craig, & Grunau, 1993). The fact that this study did not include infants below 28 weeks may explain the lack of differences related to gestational age.

Facial actions

The difference found between intervention groups in the percentage of time displaying facial actions has been described in other studies under the effect of kangaroo care (Castral *et al.*, 2008; Johnston *et al.*, 2008b; Johnston *et al.*, 2003), sucrose (Gibbins *et al.*, 2002) and sucrose/dextrose with pacifier (Akman *et al.*, 2002; Blass & Watt, 1999).

Facial actions are considered very specific indicators of pain (Gibbins *et al.*, 2008b; Stevens *et al.*, 2007; Johnston *et al.*, 1997b). The three facial actions recorded - brow bulge, eye squeeze and nasolabial furrow – have been reported to be highly correlated (Grunau & Craig, 1987). In this study too, the three facial actions were highly correlated, especially during the manipulative phases of the procedure, with 60 to 80% of shared variance. However, a significant difference between intervention groups was

found only for brow bulge and eye squeeze. The difference between the two groups regarding the percentage of time that the infants displayed nasolabial furrow was smaller than for the other facial actions, and lacked statistical significance. A possible reason for this finding could be that, in this study, infants were given a pacifier and therefore, sucking movements may have hindered the display of nasolabial furrow.

An incidental finding was that there was a difference in facial actions display between boys and girls, boys displaying more brow bulge than girls in the compression phase. Although the effect size was low (r= .19), it deserves to be discussed. This finding is in agreement with Grunau & Craig (1987), who found that boys showed shorter time to display facial action than girls. In animal studies too, long before puberty, the effect of low levels of testosterone, as a result of prenatal stress, seems to be related to the increased responses of male rat pups to inflammatory pain (Butkevich, Barr, & Vershinina, 2007). In recent studies, however, female term and preterm newborns of all gestational ages displayed more expressions of pain during heel lance than male newborns (Guinsburg *et al*., 2000). In adults, sex differences have been shown in the expression of opioid receptors, pain sensitivity and perception, and in the response to analgesia in both animal and human studies, yet with conflicting results both in clinical and experimental research (Fillingim, King, Ribeiro-Dasilva, Rahim-Williams, & Riley, III, 2009).

Behavioral state

The relation between intervention and behavioral state was tested using Pearson Chi-square. It is well known that KMC promotes sleep state. It is therefore not surprising that the proportion of infants in sleep states was higher in the S+KMC group. Although at baseline the majority of infants in both intervention groups were asleep, as soon as the infants were disturbed for skin preparation, which is a non-painful stimulation, the proportion of infants awake and asleep became nearly the same in the Sucrose group, but not in the S+KMC group, where most children remained asleep. At needle stick again, while half the infants in the Sucrose group were awake, only less than one third of the infants in the S+KMC group were so. It is possible then to state that KMC during a painful procedure reduces changes in behavioral state from sleep to wake states, therefore favoring energy conservation, so important for preterm infants.

Differences in sleep state between infants in kangaroo care and in the incubator have been reported at baseline and post-stick, infants being predominantly in deep sleep during kangaroo care, as opposed to active sleep in the incubator (Ludington-Hoe *et al*., 2005).

A significant negative moderate to strong correlation was found between behavioral state and facial actions at all phases except baseline. The correlation during needle stick was stronger, with a coefficient of determination (r^2= .39) meaning that a significant proportion of the variability of one of the variables is explained by the other (Cohen, 1988). These findings are in agreement with the seminal observations of Grunau and Craig (1987) that awake-alert but inactive infants respond to pain with the most facial activity, and infants in quiet sleep show the least facial reaction.

Recovery time

After the disruption caused by an aversive stimulus triggering a response from the autonomic nervous system, namely an acceleration of heart rate, physiological parameters tend to return to their initial values signaling the infants' capacity to maintain homeostasis (Johnston *et al.*, 2008b). With increasing age and in appropriate for gestational age neonates, the ability to respond sooner is accompanied by the ability to quickly return to the values before disruption (Galland *et al.*, 2006). The higher the infants' ability to maintain physiological stability, the shorter the time needed to recover from the stress of pain.

Recovery time has been studied as an outcome in a number of trials comparing interventions for pain control during procedures. Johnston and colleagues (2008b), in a cross-over trial with 61 infants 28 to31 weeks and 6 days gestational age, comparing kangaroo care to incubator care for heel lance, found a 70 seconds difference in recovery time between those who were in KC during the heel lance (M= 123 seconds, 95%CI [103,142]) and those who were in the incubator (M= 193 seconds, 95% CI [158, 227]), F (61, 1)= 13.6, p< .001. A shorter recovery time of heart rate has also been reported by Bucher *et al.* (1995) who compared sucrose to placebo for heel lance in infants 27 to 34 weeks gestational age. The median difference was 53 seconds, in favor of the sucrose group.

In this study, there was no difference between groups in the mean time to recover baseline values. This may be due to the fact that the recording time was not the same for all infants and did not always suffice for infants to reach baseline, therefore increasing the risk of a Type II error. However, there was a main effect of gestational age, older infants recovering faster than younger infants, and a significant difference between the two groups was found in terms of the increased likelihood of infants 32 weeks gestational age and above, in the S+KMC group, to recover heart rate baseline values at 60 and 90 minutes after the end of the procedure, compared to infants in the Sucrose group. This difference in recovery between the two gestational age groups is similar to the one found by Butt and Kisilevsky (2000), studying the effect of music for heel lance

pain in two age groups (less and greater than 31 weeks gestational age). They found a more rapid return of heart rate to baseline values in infants above 31 weeks but not in younger infants, in the presence of music compared to the absence of music.

These results confirm that older infants have a better capacity to recover physiological stability and take clear benefit from these interventions.

PIPP scores

It is worth noticing that PIPP scores at needle stick, the most painful phase of the procedure, did not exceed a mean of 7.18 in the sucrose group and 6.21 in the S+KMC group. These values are much lower than those reported by Johnston and colleagues (2003) at 30 seconds after the procedure when kangaroo care was used alone in neonates 32 weeks and above (10.1); and those reported in very preterm infants at 90 seconds after the procedure (8.87) (Johnston *et al.*, 2008b). Mean PIPP scores in this study, all phases together, were 4.85 for the Sucrose and 5 for the S+KMC groups. These scores are also lower than those reported by Stevens and team (1999) for pacifier with water (8.44) and for pacifier with sucrose (7.87). Those studies, however, have examined pain caused by heel lance, which is known to be higher than that caused by venepuncture, at least in term infants (Shah & Ohlsson, 2007). For that reason, it is more pertinent to compare the PIPP score obtained in this study by sucrose with pacifier (M= 4.85, 95% CI [4.26, 5.83]) and by S+KMC (M= 5.0, 95% CI [3.96, 5.38]) with the score obtained by Taddio and colleagues (2009) using sucrose during venepuncture (M= 6.8, 95% CI [5.7, 7.9]). The scores in the present study are lower.

The PIPP scores obtained in this study can be considered as no pain or minimal pain scores, indicating that both interventions are useful in keeping pain levels low. Although the comparison was not made in this study, the results suggest that the combined use of sucrose, pacifier and kangaroo care reduces pain responses to lower levels than those attained when each of these interventions is used alone.

Heart rate

In terms of reactivity, neonates responded to stimulation with changes in heart rate. The direction of this change, as expected from an autonomic response, was an increase in heart rate. The magnitude of the increase from baseline was bigger in the needle stick phase than in any of the other phase of the procedure. In the compression and rest phases, heart rate returned back to baseline values showing the neonate's regulation capacity.

Neonates reacted to skin preparation, a non-painful stimulation caused by holding the hand and swabbing with moistened cotton (room-temperature alcoholic so-

lution) with significant changes in maximum, average and minimum heart rate. This finding does not match the observation of Bartocci and co-workers (2006), who report that skin disinfection, unlike needle stick, caused no significant change in heart rate, although it produced changes in cortical activity.

Lack of statistical difference in heart rate between intervention groups was also found by Castral and colleagues (2008) comparing kangaroo care to no intervention. Johnston and team (2003) reported no differences at 30 and 60 seconds after heel lance and in very preterm infants a significant difference only at 90 seconds (Johnston *et al.*, 2008b). This period of time was not examined in the present study.

The change in maximum heart rate from baseline to needle stick was two-fold bigger in infants ≥ 32 weeks, in the sucrose group, than in infants in S+KMC, yet this difference was not statistically significant, probably due to a very large variability.

Minimum and average heart rates were significantly higher in younger infants, a predictable finding, given that heart rate values normally decrease with increasing postconceptional age. This has also been reported by researchers comparing pain responses in different gestational ages (Gibbins *et al.*, 2008a)

Maximum heart rate was positively correlated with postnatal age, with a moderate effect size in the Sucrose group (r^2= .31) and a small effect size in the S+KMC group (r^2= .17). This could indicate that in infants under S+KMC, postnatal age did not increase pain reactivity as much as in the sucrose group.

All the infants in this study had non-nutritive sucking, which has been reported to decrease heart rate significantly, both in the absence of stimulations and in the presence of painful stimuli (Shiao, Chang, Lannon, & Yarandi, 1997). The increase in heart rate triggered by a needle insertion or heel stick is also smaller when comparing infants who are using pacifier to infants who are not (Campos, 1989; Field & Goldson, 1984; Miller & Anderson, 1993). Sucking may have blunted the effect on heart rate although the effect of non-nutritive sucking on heart rate is far from being clear. According to DiPietro, Cusson, Caughy and Fox (1994), there is evidence to suggest that non-nutritive sucking lessens behavioral distress but does not alter physiological responsiveness.

Oxygen saturation levels

Unlike minimum and average oxygen saturation levels, which did not change significantly across phases of the procedure, maximum oxygen saturation levels changed significantly between needle stick and rest, with an interaction effect of gestational age, younger infants having slightly lower oxygen saturation levels than older infants.

The drop in oxygenation described in other studies (Ludington-Hoe *et al.*, 2005) was not observed in this study, suggesting that pain was reasonably controlled in the two groups of neonates.

The lack of difference between the groups regarding oxygen saturation has been found in other studies. In a systematic review about the effects of sucrose (Stevens, Yamada, & Ohlsson, 2004), none of the five studies examined, that used oxygen saturation as an outcome, reported significant differences between different intervention groups. Taking into account that these studies were comparing sucrose to placebo or no intervention and found no difference in oxygen saturation, it is not surprising that this study, where effective interventions were being compared, has not found such a difference.

Heart rate variability

No effect of intervention was found on LF, HF or LF/HF. There was a main effect of phase on LF, with significantly less LF peaks during the manipulative phases of the procedure than at rest. LF peaks are a result of the influence of both sympathetic and parasympathetic systems, so the change in LF is consistent with the reported high values of heart rate during needle stick and their decrease at rest. No significant changes were found from one phase to another in HF and LF/HF ratio. In the presence of pain, the parasympathetic system withdraws (Sweet & McGrath, 1998) and it is expected to find less HF peaks and a higher ratio LF/HF. The infants in both intervention groups had a pain relieving intervention so this might be the reason for the lack of difference between phases.

Heart rate variability is a function of the Autonomous Nervous System (ANS) maturation, so the main effect of gestational age on LF, older infants having more LF peaks than younger infants, may provide an explanation. On the other hand, the maturity of the ANS has been shown to be different in small for gestational age infants and appropriate for gestational age infants (Galland *et al.*, 2006). In this study, the relation weight/gestational age was not controlled for, making it more difficult to interpret the results.

The absence of differences in heart rate variability is consistent with the lack of differences found in other physiological variables.

Safety of the combined intervention

Adverse events may occur in the course of blood draw, related to the pain caused by the procedure or, eventually, to the interventions used. Our safety criteria were de-

rived from those used by Gibbins and co-workers (2002).

In this study, there were no cases of sustained tachycardia or desaturation during the procedure. However, 7.3% of the neonates needed a second attempt to succeed the blood draw but a relation with pain control intervention could not be found. From the procedural point of view, blood draw in kangaroo care was significantly quicker than when the infant was lying down in the incubator.

8.2 Maternal anxiety and the pain responses of neonates in KMC

The level of maternal anxiety was generally low to moderate (*M*= 39.64, *SD*= 9.82), similar to the anxiety level of a sample (N= 284) of adult women from the Portuguese population measured with the S-STAI (*M*= 38.2, *SD*= 10.77) (Silva, 2003). It was lower than in two samples of mothers during the NICU stay in the US (*M*= 47.80, *SD*= 14.63) and in the UK (*M*= 52.63, *SD*= 13.71) (Franck, Cox, Allen, & Winter, 2005), and also lower than the anxiety level demonstrated at discharge by mothers whose babies had been severely ill (*M*= 43.2, *SD*= 13.1) (Allen *et al.*, 2004). The fact that babies in this sample were not in critical conditions may have accounted for this low level of anxiety.

In exploring the relationship between maternal anxiety and intervention, mothers of infants in the S+KMC group had significantly lower levels of anxiety before the procedure than mothers of infants in the Sucrose group. The STAI was used immediately before infants and mothers were placed in kangaroo care, so mothers knew whether they were going to hold their baby or not. This is a possible explanation for the difference between the two groups: higher anxiety in mothers who were not going to hold the baby could be related to feeling powerless. The protective role assumed by parents in normal circumstances is hindered when they are unable to participate in the care of their infants, which causes them distress (Franck *et al.*, 2004). On the other hand, in mothers in the kangaroo care group, the anticipation of holding the baby skin-to-skin might have elicited the release of oxitocyn which has a role in reducing stress (Matthiesen *et al.*, 2001). An improvement on this design would be to have all mothers complete the STAI prior to randomization, as a baseline measure.

The relationship between the level of maternal anxiety and infants" pain responses was examined only in the S+KMC group. No correlation was found with PIPP scores, facial actions, indices of heart rate variability or recovery time. When examining both gestational age groups together, a significant moderate negative correlation was found at compression and rest between the STAI and maximum heart rate. In infants 32 weeks gestational age and above, a significant moderate negative correlation was found between the STAI and maximum heart rate during preparation, needle stick, compression

and rest. This result seems to be a paradox, contradicting the expected co-regulation between mother and infant, and a reasonable explanation is hard to find. It could be that the sample size of this sub-group (n= 60) was underpowered.

The STAI was moderate and negatively correlated with minimum and average oxygen saturation levels at rest, in infants below 32 weeks, indicating that higher levels of maternal anxiety were related to lower minimum and average oxygenation of the infant, as might be expected. The fact that this relation was observed only at the rest phase, after the end of the procedure, may indicate that younger infants are still having a delayed response to the procedure.

8.3 Mothers' perceptions of doing KMC during venepuncture

A first indicator of mothers' attitude towards kangaroo care during blood draw was the rate of consent. Two hundred and eleven mothers were approached and only six refused (2,8%). In the interviews, mothers reported only positive feelings about doing kangaroo care during venepuncture for blood draw.

The experience of holding the baby skin-to-skin was positive. The 30 minutes in kangaroo care before the blood draw gave them time to relax, enjoy their baby, and feel in communication. Many of these women had been in hospital for bed-rest, weeks before the delivery, fearing to lose the baby. After the delivery, they were separated from their newborn and, in some cases, they had to face the uncertainty of having an ill or medically unstable baby and even the possibility of loss. None of the infants in our sample had any severity of illness criteria when they participated in the study but many had been ventilated during the first days of life. Even in more benign situations such as jaundice, being under phototherapy can be a cause for mothers' anxiety. In this context, being able to do kangaroo care even for a few minutes was, as mothers described it, soothing: it represented a unique gratifying moment within a bad period of weeks or months. The state of calmness and peacefulness described by mothers during kangaroo care, during which they were totally focused on their baby, may be a clear demonstration of maternal behavior induced by the release of oxitocyn as a consequence of infant's proximity (Porter, 2004).

When urged in the interview to focus on the blood draw, mothers talked about their surprise and how comforted they were by the baby's behavior, which they interpreted as no pain or a very small pain only. They referred feeling protective and in control of what was happening: *"like a mother"*, to use their own words.

Mothers emphasized the fact that babies hardly cried or even noticed the needle stick. The accuracy of this recollection is sustained by three elements: the researcher's

observation during the procedure that crying occurred rarely; the results of the measurement of facial actions; and published reports like the one of Blass and Watt (1999) on the efficacy of suckling and sucrose, reporting that they saw no behavioral indication in nine of the ten infants that the heel lance had even occurred, and that grimacing was reduced to almost nothing.

The fact that this was a first time in doing skin-to-skin for slightly more than two-thirds of the mothers may have accounted for the enthusiasm they demonstrated when they talked about this experience: mothers were so positively overwhelmed by holding the baby for the first time that the blood draw became secondary. Yet, this keenness about kangaroo care was shared by mothers who had experienced providing kangaroo care previously, confirming the findings of studies about mothers' perceptions of kangaroo care in the absence of painful procedures (Furlan *et al.*, 2003). Mothers statements about feeling so well and happy to hold the baby reinforce the findings of other researchers that mothers' mood is improved by kangaroo care (de Macedo, Cruvinel, Lukasova, & D'Antino, 2007).

Being able to share what the baby was feeling, to protect him or her, and to be a part of the baby's pain relief, elements of parental role (Franck *et al.*, 2004), were clearly articulated by mothers. The enhancement of their feeling *"like a mother"* is a consequence of the exposure to interaction with the baby, and it has been described in studies on early intervention in postnatal care (Gomes-Pedro *et al.*, 1989). It will likely have contributed to reinforce their self-esteem and self-confidence which were naturally shaken by a premature delivery (Eriksson & Pehrsson, 2005).

The desire declared by mothers to protect their baby and to share their pain seems to have a counterpart in animals' mother-newborn relation: there is evidence from animal studies that maternal care is influenced by offspring's exposure to pain, maternal grooming being higher when rat pups are exposed to pain versus no pain (Walker, Kudreikis, Sherrard, & Johnston, 2003).

These findings point out that mothers long for the opportunity to hold the baby and that kangaroo care gives them a privileged chance to interact with their newborn and to actualize, sometimes for the first time, that they have, indeed, become mothers. They are willing to endure the unpleasantness of seeing their babies undergo a potentially painful procedure, if they have the opportunity, even for a short moment, of fulfilling their parental protective role, a genetically imprinted behavior in most animal species, by comforting them, and above all, reducing their pain by holding them skin-to-skin.

8.4 Strengths and limitations

This is the first study to examine the combination of kangaroo care, sucrose and pacifier and the need to research combined interventions to improve the pain relieving effect of Non-pharmacological interventions has been reiterated in the literature (Gibbins *et al*., 2002; Stevens *et al*., 2010). This is also the first study to measure maternal anxiety prior to KMC and to examine in detail mothers' perceptions of doing kangaroo care during a painful procedure. Differences between the comparison groups were found in facial action, a very specific indicator, and in recovery time, suggesting that this combination is effective in reducing pain. No adverse effects occurred, indicating that this intervention can be used safely. Finally, kangaroo care is very much appreciated by mothers even if they have to bear seeing the needles and the blood..

Regarding the painful stimulus, the large majority of studies used heel-stick as a model of procedural pain. There was a need to study the effect of these interventions to reduce pain from venepuncture.

As for the type study, randomized-controlled trials are considered to provide the most reliable evidence on intervention efficacy since potentially confounding factors may be controlled (Polit Hungler, 1991). But other aspects were also taken into consideration when designing this study, to ensure the quality of the study. More than one site was used which increases generalizability; the sample size was calculated so that the results would have statistical power; strict criteria of inclusion and exclusion were defined to guarantee that the infants would be in similar conditions regarding severity of illness; digital recording of data increased the precision of measurement compared to studies where recording is observational, in real time. Although this was not a double-blinded study, because the kangaroo care condition cannot be concealed, there was blinding of the assessors of facial coding.

In terms of study design, trying to find relevant differences in pain responses by adding kangaroo care to two well known potent pain relief interventions – sucrose and pacifier – instead of comparing the combined intervention to placebo, as most studies have done so far, was challenging since the effect size would be small. The sample size was previously calculated to warrant statistical power and find these differences. Yet, having used estimates from studies of intervention versus no intervention or placebo, it is possible that the sample was underpowered to detect a small statistical difference in some of the outcomes. In clinical trials, besides looking at statistical significance, clinical significance is also of interest and a 30% reduction in numerical ratings is considered clinically meaningful (Rowbotham, 2001). In this study, brow bulge and eye squeeze

were statistically significantly lower in infants who received the combined intervention. If the difference is considered in terms of proportion, it corresponds to a 54% reduction in brow bulge and a 57% reduction in eye squeeze, which can be considered clinically significant.

The absence of a third intervention group of kangaroo care, pacifier and placebo instead of sucrose might be considered a limitation, in that having the three groups would also have allowed the comparison between sucrose and kangaroo care. However, given that the use of sweet solutions for minor painful procedures is part of standard care in the study units, not only this was not feasible for ethical reasons, but the pertinence of the comparison is questionable.

The experimental protocol and the selection of phases of the procedure that were examined involve both limitations and strengths. First of all, the baseline period was considered to be the 30 seconds before the venepuncture. By doing so, infants were already in a different condition: either in kangaroo care or in the incubator. It can be argued that this is not a true baseline (Johnston *et al.*, 2008b) and in this study, the fact that more babies were asleep at baseline in the S+KMC group than in the Sucrose group may have prevented any differences to be found. On the other hand, in the present study, we have included the phase of skin preparation, which includes holding the infant's hand to inspect the vein and disinfect the skin with a wet, cold swab. This allowed us to observe that the undisturbed infant quickly reacted to this non-painful stimulus by significant changes in behavioral state and in heart rate, confirming that heart rate is an indicator of stress and not specifically of pain.

In terms of analysis, it is important to consider that the selection of the time epochs to be examined was arbitrarily designated in accordance to other similar studies (Johnston *et al.*, 2008a; Johnston *et al.*, 2008b; Johnston *et al.*, 2003; Johnston *et al.*, 1997). However, it is possible that the peak of the response in each phase of the procedure may not have been achieved for all infants, especially the younger group, in the first 30 seconds.

The selected outcomes are yet another strength of this study. The curves representing the different outcomes across the procedure, all with a quadratic trend, suggest that the indicators examined are useful to measure preterm infants' reactivity to stress and pain.

Regarding the instruments used in this study, they were validated measures: the PIPP has been extensively used in neonatal pain research and the STAI has been used in many studies to measure parental anxiety in the context of neonatal care. In this way,

one of the major difficulties encountered in conducting systematic reviews and meta-analysis, related to the diversity of measuring tools was avoided (Clarke, 2007).

There are a few other limitations of this study related to the clinical data collected and to the procedure. Regarding clinical data, the number of previous painful procedures might have been underestimated. Although it accurately reflects what was recorded in the clinical charts, there are instances where more than one try is made before the actual procedure is achieved, like in a blood draw or a catheter placement, and all those attempts may not have been recorded. The lack of relation between infants' pain responses and the number of previous painful procedures in this study, unlike other studies (Grunau, Oberlander, Whitfield, Fitzgerald, & Lee, 2001; Holsti *et al.*, 2005; Goffaux *et al.*, 2008; Johnston & Stevens, 1996), could be related to this fact. Another possible explanation is that the mean number of painful procedures in this study is low (M= 2.56/infant/day), compared to Carbajal and colleagues (2008), who found a mean of 12/infant/day, and Cignacco & team (2009), who report a mean of 22.9/infant/day, to cite only two recent studies. This may be due to differences in the type of painful procedure included in the studies but also, as speculated before, to the fact that unsuccessful attempts are not always recorded. Staff should therefore be encouraged to document all attempts made, whether successful or not, so that in future studies this variable may be measured more accurately.

Whether any of the infants was being treated with caffeine was not included in the study, which could modify the autonomic response and state. Another variable that was not considered was mothers' medication during pregnancy, namely treatment of depression with selective serotonin reuptake inhibitors (SSIR) and benzodiazepines, which decrease behavioral responses to pain and increase parasympathetic modulation during recovery after a noxious event (Oberlander & Saul, 2002).

Regarding the procedure, the needle stick was performed by different nurses, therefore introducing differences in the needle gauge chosen, in the positioning of the hand, in the pressure applied during the compression phase, among others. However, the sample size and high number of nurses should have reduced the potential confounding effect of these variables and this diversity has been considered as increasing the generalizability since it approaches the "real world" (Taddio *et al.*, 2008, p.42).

It was not always possible to control for environmental noise. It is known that neonatal wards can be very busy and noisy places. The beeps and alarms of other infants, the voices of staff, an inadvertedly call for a name will have been heard by the infants and might have interfered with their state and heart rate. These are known shortcomings of research in the natural, clinical field.

8.5 Theoretical issues

The combined intervention used in this study is multidimensional: it involves the oro-gustatory stimulation provided by sucrose; the oro-tactile stimulation brought by sucking a pacifier and, by adding kangaroo care, the multisensorial stimulation from kangaroo care which includes at least swaddling, touch by skin-to-skin contact, warmth, smell, auditory recognition of maternal heart beat and vestibular stimulation from mothers' respiratory movements, that is, the "hidden regulators" of infant physiology and behavior, as Hofer (1994) calls them.

The mechanisms behind these interventions, as described earlier, are complex and diverse in nature. They are also not completely understood. There is evidence of the involvement of the opioid system with the use of sweet solutions, namely sucrose (Blass & Ciaramitaro, 1994), although in one study, an intravenous injection of naloxone, an opioid antagonist, has not diminished the pain reducing effect of glucose during heel stick (Gradin, 2005).

Pacifiers may relieve pain through two distinct but complementary mechanisms: sensory dominance and self-regulation (Carbajal *et al.*, 1999). The sensory dominance hypothesis postulates that the sucking activity and the oro-tactile stimulation associated with it are powerful sources of perceptual information that would compete with pain for the limited attention resources of the neonate. The self-regulation hypothesis considers that by engaging in the sucking activity, neonates would be able to control by themselves one source of incoming stimulation, thus facilitating self-regulation.

In the case of kangaroo care, the contact comfort seems to be regulated by non-opioid systems, since naloxone does not revert the soothing effect of contact in animal studies (Blass & Ciaramitaro, 1994). The effect of mothers' touch and smell, on the other hand, seems to involve oxitocynergic mechanisms (Lund *et al.*, 2002) which have an antinociceptive action (Uvnas-Moberg, Bruzelius, Alster, Bileviciute, & Lundeberg, 1992). It is possible that contact, during which touch and smell are present, elicits the release of neurotransmitters related to pleasant stimuli, such as dopamine and serotonin. Swaddling, also present in the kangaroo care position, is another component that promotes self-regulation by reducing limb activity and motor disorganization caused by stress (van Sleuven *et al.*, 2007).

Interventions that combine multiple stimuli have been studied before in term and preterm infants (28-35 weeks) showing that human contact (massage, voice and eye contact) associated to sweet solutions and pacifier are more efficacious than sweet solutions with pacifier (Bellieni *et al.*, 2001).

According to the results of this study, infants as young as 28 weeks gestational age benefit from the combination of interventions. Little is known, however, about infants below this gestational age.

The findings of this study, namely the responses of neonates to the interventions provided, support that the experience of pain can be modulated by a variety of stimuli as stated by the Gate-Control theory. The tactile, vestibular, olfactory, auditory and gustatory stimuli provided by maternal skin-to-skin contact combined with sucrose and pacifier compete with the painful stimulus of needle stick and activate both ascending and descending gating mechanisms.

Three main theoretical questions are raised by this study: 1) what is the most efficacious combination of stimuli?; 2) at what gestational age are preterm infants able to integrate these different mechanisms and obtain a synergistic effect of the combined use of Non-pharmacological interventions? and 3) is there is a limit in the amount of different stimuli that preterm infants are able to tolerate, when the interventions combined work through different mechanisms?

In order to respond to these questions, further research is needed on younger infants and exploring this and other combinations of Non-pharmacological interventions.

8.6 Implications for clinical practice

Giving sucrose with or without pacifier to preterm infants before a painful procedure has now become a common practice in many NICUs. The results of this study indicate that kangaroo care may be added safely to these two interventions with further reduction in pain. The main grounds in favor of the use of this combined intervention that can be drawn from this study are related to the decrease in infants' pain responses, the safety of the combination, the technical advantages and maternal satisfaction.

Regarding infants' pain responses, the combined intervention studied significantly reduces facial expressions of pain (brow bulge and eye squeeze); less infants change from sleep to wake states at needle stick, with more deep sleep in kangaroo care than in the incubator; the probability of having recovered baseline heart rate at 60 and 90 seconds after the end of procedure is higher.

In terms of safety, no adverse events such as choking, tachycardia or desaturation are to be expected. This has sometimes been reported with sucrose in preterm neonates (Gibbins & Stevens, 2003). Technically, blood draw by venepuncture is quicker in kangaroo care compared to incubator.

Considering the advantages for mothers, those who know they will hold the baby

are less anxious before the venepuncture and mothers appreciate doing kangaroo care, even if just for venepuncture, not only for the pleasure of holding the baby but also to fulfill their maternal protective role.

In order to be beneficial, a minimum amount of time in kangaroo care is required. After a period of 20 minutes of skin-to-skin contact, there is a fall in circulating β-endorphin (p= 0.008) as well as a reduction in cortisol levels (P= 0.002) (Mooncey, Giannakoulopoulos, Glover, Acolet, & Modi, 1997), which are normally secreted in neonates in response to stress (Anand, Sippell, & Aynsley-Green, 1987). This suggests that skin-to-skin contact reduces the activity of the hypothalamic-pituitary-adrenal axis. In healthy neonates, crying time was significantly reduced during intra-muscular injection after 10 minutes of kangaroo care (Kashaninia *et al.*, 2008), and during heel stick after 10 to 15 minutes (Gray *et al.*, 2000). Pain scores of healthy term neonates were also lower during immunization after 2 minutes of kangaroo care (Chermont, Falcao, de Souza Silva, de Cassia Xavier Balda, & Guinsburg, 2009). In preterm infants, studies have found a significant reduction in neurobehavioral stress signs after 10 minutes (Ferber& Makhoul, 2008), and a decrease in pain scores after 10 minutes (Freire *et al.*, 2008), 15 minutes (Castral *et al.*, 2008; Johnston *et al.*, 2008b) and 30 minutes (Johnston *et al.*, 2003; Akcan *et al.*, 2009). Only one study provided 3 hours of kangaroo care. Considering these studies and the current results, there is consistent evidence to support that at least 10 to15 minutes of kangaroo care should be offered before the painful procedure.

In neonatal units where kangaroo care is not a regular practice, for reasons that are beyond the scope of this discussion, these results reinforce other studies on the benefits of KMC, encouraging nurses to use it more often and plan routine care in order to allow the addition of kangaroo care to standard pain relief provided by sweet solutions and pacifiers. In units where kangaroo care is offered to mothers and infants as part of standard care, our results show that there is no reason why it should be interrupted or postponed, as it is often done, to perform a blood draw in the incubator. In both cases, along with pain relief, infants and mothers will benefit from the other immediate positive effects of kangaroo care, namely on the quality of infants' sleep (Lehtonen & Martin, 2004) and on mothers' self-confidence (Furlan *et al.*, 2003).

The importance of implementing an effective pain-prevention program in every neonatal unit is stressed by the American Academy of Pediatrics and collaborators (2006) and there are in Portugal national recommendations for analgesia in neonates from the Secção de Neonatologia da Sociedade Portuguesa de Pediatria (Rocha *et al.*,

2004). Taking these into consideration, the results of our study reinforce the inclusion of kangaroo care combined with sucrose and pacifier in local guidelines for the management of pain in minor procedures.

8.7 Implications for research

A few reflections emerge from this study with implications for research. These are concerned with the selection of pain indicators and mediating variables, the subpopulations that are included in studies of this kind, the pain situations that need investigation and parental outcomes.

This study confirms the usefulness of using a validated pain scale as well as analyzing behavioral and physiological indicators separately, since the regulation of these two types of indicators is different (Castral *et al.*, 2008). In fact, the correlation between changes in heart rate and facial actions has been reported as low and not significant (Morison *et al.*, 2001). In this study, significant differences were found in facial actions and state but not in heart rate. The results of studies using physiological variables are not convergent, as discussed earlier. Therefore, the suggestion of Pereira and co-wokers (1999) that these indicators should be used as supplement and not as main indicators is supported. In fact, when a factorial analysis of indicators was performed to analyze the structure of acute pain responses in vulnerable neonates, physiological variables including heart rate variability and oxygen saturation added 8 to 26% to the variance resulting from facial actions which ranged from 29 to 39% (Stevens *et al.*, 2007).

Other indicators have been explored more recently, which are part of the Neonatal Individualized Developmental Care Assessment Program (NIDCAP) such as finger splay, arm and leg extension, torso movements, among others (Holsti *et al.*, 2004) and a new pain scale has been developed, the BIIP (Holsti, Grunau, Oberlander, & Osiovich, 2008; Holsti & Grunau, 2007). Attention should be paid to the results of trials using these indicators in the next future.

An unexplored issue in this study was the quality of sucking. It has been reported that the analgesic effect of sucking an unflavored pacifier occurred only when the rate of sucking was over 30 sucks/minute (Blass & Watt, 1999). It would be interesting to explore the relation between rate of sucking, behavioral state, PIPP score, facial actions, heart rate and heart rate variability. The analysis of the differences between gestational age groups in sucking behavior and the correlation with pain outcomes may worth examining in future studies or as a secondary analysis of this data set.

The differences found in this study between boys and girls regarding facial actions, and the fact that they are in the opposite direction of other studies, deserve further research.

There is a need to study pain relieving interventions in other age groups and subpopulations, namely infants below 28 weeks and neonates who are ventilated. Infants at risk of neurological impairment have been receiving attention from researchers in the last years, concerning their particular behavioral and physiological responses (Stevens *et al.*, 2006) but studies about their responses to interventions are scarce. These infants who are more vulnerable are usually excluded from research for ethical reasons and to control for confounding factors. An effort has to be made in the future to design studies that will respect their vulnerability albeit providing evidence to respond to their specific needs.

Another unexplored issue is the effect of repeated interventions to reduce procedural pain. While the effects of repeated use of sucrose have been studied (Johnston *et al.*, 2007b; Stevens *et al.*, 2005; Johnston *et al.*, 2002; Johnston *et al.*, 1999), little is known about the effects of repeated kangaroo care and combined interventions for pain relief. Not only efficacy over time needs to be examined but there have been some concerns that interventions involving mothers, like kangaroo care, might create some associative memories between pain and maternal holding. Arguments against this potential adverse effect are that the input to the brain originated by close contact and suckling, in animal studies, would prevent cortical activation and therefore associative memory (Ludington-Hoe *et al.*, 2005). Besides, if kangaroo mother care is used regularly outside of any painful situation, the comfort obtained by the infant should remain as a pleasant memory predominating over the few painful moments. So much as, in older children, there is no evidence of deleterious effect of parental support during painful procedures on infant-parent relationship since the feeling of protection predominates. However, in order to discard this remote possibility, well designed longitudinal studies to address this issue are required if kangaroo care or other interventions involving parents are to be used repeatedly for painful procedures such as blood draw and immunizations.

Non-pharmacological interventions have been studied mainly for procedural pain. For long-lasting pain, attention has been given to pharmacological agents. There is no reason why a number of Non-pharmacological interventions, namely maternal comfort by kangaroo care, would not be useful to complement their action.

In this study, only maternal anxiety before kangaroo care and mothers' perceptions were explored. In future studies, other parental outcomes may be interesting to fo-

cus on, namely the effects of kangaroo care as a pain control intervention on overall maternal stress during hospitalization, maternal mood and mother-infant interaction and bonding.

The purpose in raising these issues is to draw the attention to the main gaps in knowledge regarding non-pharmacological pain interventions, which need to be investigated.

CONCLUSION

CONCLUSION

Pain is a protective mechanism with a very early set off in phylogenetic and ontogenetic evolution, designed to signal tissue damage and trigger defense. When deprived of its warning function, as it is the case in medical care, pain is a cause of unnecessary suffering with deleterious immediate, short-term and long-term effects.

As discipline and profession, nursing is concerned with human responses to health problems, diseases and life processes, such as pain. The aim of this research was therefore to study the effect of nursing interventions to reduce pain in preterm neonates during invasive procedures in the Neonatal Intensive Care Unit.

This is the first study to date in the literature to assess the efficacy of the combination of kangaroo mother care, sucrose and pacifier compared to that of sucrose and pacifier, during venepuncture for blood draw in preterm infants; to examine the relation between maternal anxiety and the pain responses of the babies who had kangaroo care; and to give a voice to mothers who passed through the experience of holding the baby skin-to-skin during a painful event.

In order to frame these objectives, in the first part of this dissertation, through the introduction and the literature review, the need to study combined interventions with parental involvement to reduce pain from minor procedures in neonatal intensive care was argued.

In the second part of the dissertation, in spite of the limitations of the study, it was possible to conclude that the design, the outcome measures and the research protocol were appropriately selected to attain the objectives. The hypothesis that by adding kangaroo care, an effective intervention to reduce pain responses in neonates, to the standard effective intervention composed by sucrose and pacifier, lower pain scores during venepuncture would be observed was supported by the results. The mixed approach to the analysis of variance allowed examination of the effects of intervention and gestational age on the pain responses, as well as the analysis of the infants' pain reactivity across the procedure.

The results demonstrated that:

• compared to the use of sucrose with pacifier, the combination of kangaroo care, sucrose and pacifier reduced brow bulge and eye squeeze; and reduced recovery time in infants 32 weeks gestational age and above;

• mothers' anxiety level before kangaroo care for venepuncture did not interfere with the pain responses of the infants; it was low to moderate and significantly lower in mothers who were randomized to the kangaroo care group than in mothers who were randomized to the sucrose group;

• mothers enjoyed doing kangaroo care, they felt that their motherhood was realized and that their maternal role of protecting the baby from pain was fulfilled.

This study adds to the body of knowledge about pain management and neonatal nursing. It is also a contribution to nursing research in Portugal which does not have a tradition in clinical trials. This study shows that it is possible in our clinical environment to conduct research about the efficacy of nursing interventions and this is a step towards producing evidence that can guide nursing practice.

We are aware that pain is a subjective experience and that we can only infer the infants' pain experience from the objective changes in behavioral and physiological changes observed. The main theoretical questions raised by this study concern the most efficacious combination of interventions, the amount of stimulation that can be tolerated by preterm infants, and the age at which combinations of interventions that act through different mechanisms can be effectively integrated to reduce preterm infants' pain responses.

Major implications for clinical practice are that guidelines for neonatal pain management in stable preterm infants 28 weeks gestational age and above may include recommendations based on the findings of this study, namely, the involvement of parents in pain management by the addition of kangaroo care to sweet solutions and pacifier for venepuncture; it also suggests that when continuous kangaroo care is standard practice, it should not be interrupted to perform blood draw by venepuncture since infants show less pain, mothers are happy and blood harvesting is quicker.

The combination of sweet-solution, pacifier and kangaroo care has potential to reduce pain from other procedures as well, and kangaroo care on its own may be useful in established and prolonged pain conditions. The effect of its repeated use, the application to other painful situations, and the impact on parental outcomes deserves to be addressed in future research.

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Appendixes

Premature Infant Pain Profile

PROCESS	INDICATOR	0	1	2	3	SCORE
Chart	Gestational Age (at time of observation)	36 weeks and more	32 weeks to 35 weeks, 6 days	28 weeks to 31 weeks, 6 days	less than 28 weeks	
Observe Infant 15 sec *Observe baseline:* *Heart Rate*_____ *Oxygen saturation* _____	Behavioral State	active/awake eyes open facial movements crying (with eyes open or closed)	quiet/awake eyes open no facial movements	active/sleep eyes closed facial movements	quiet/sleep eyes closed no facial movements	
Observe Infant 30 sec	Heart Rate Max. _____	0 to 4 beats/ minute increase	5 to 14 beats/ minute increase	15 to 24 beats/ minute increase	25 beats/minute or more increase	
	Oxygen Saturation Min. _____	0 to 2.4% decrease	2.5 to 4.9% decrease	5.0 to 7.4% decrease	7.5% or more decrease	
	Brow Bulge	None 0-9% of time	Minimum 10-39% of time	Moderate 40-69% of time	Maximum 70% of time or more	
	Eye Squeeze	None 0-9% of time	Minimum 10-39% of time	Moderate 40-69% of time	Maximum 70% of time or more	
	Nasolabial Furrow	None 0-9% of time	Minimum 10-39% of time	Moderate 40-69% of time	Maximum 70% of time or more	
					TOTAL SCORE	

Appendix B - State-Trait Anxiety Inventory (STAI)

QUESTIONARIO DE AUTO-AVALIAÇAO

de Charles D. Spielberger

com a colaboração de

R.L. Gorsuch, R. Lushene, P. R. Vagg e G. A. Jacobs

STAI Forma Y-1

Data: ...../...../.....

INSTRUÇÕES: Encontram-se em baixo um certo número de expressões que as pessoas usam para se descreverem a si próprias. Leia cada uma delas e a seguir assinale o circulo apropriado à direita indicando como se sente *agora*, isto é, *neste preciso momento*. Não há respostas certas ou erradas. Não gaste demasiado tempo em cada pergunta, dê a resposta que lhe parece descrever melhor a maneira como actualmente se sente.

	NÃO	UM POUCO	MODERADAMENTE	MUITO
1. Sinto-me calmo(a)	①	②	③	④
2. Sinto-me seguro(a)	①	②	③	④
3. Estou tenso(a)	①	②	③	④
4. Sinto-me sob pressão	①	②	③	④
5. Sinto-me à vontade	①	②	③	④
6. Estou preocupado(a) com possíveis contratempos	①	②	③	④
7. Sinto-me satisfeito(a)	①	②	③	④
8. Sinto-me com medo	①	②	③	④
9. Sinto-me confortável	①	②	③	④
10. Sinto-me uma pilha de nervos	①	②	③	④
11. Estou descontraído(a)	①	②	③	④
12. Sinto-me contente	①	②	③	④
13. Estou preocupado(a)	①	②	③	④
14. Sinto-me confuso(a)	①	②	③	④
15. Sinto-me sereno(a)	①	②	③	④
16. Sinto-me assustado(a)	①	②	③	④
17. Sinto-me inquieto(a)	①	②	③	④
18. Sinto-me perturbado(a)	①	②	③	④
19. Estou relaxado(a)	①	②	③	④
20. Estou tranquilo(a)	①	②	③	④

QUESTIONARIO DE AUTO-AVALIAÇAO

STAI Forma Y-2

Data:…../…../…..

INSTRUÇÕES: Encontram-se em baixo um certo número de expressões que as pessoas usam para se descreverem a si próprias. Leia cada uma delas e a seguir assinale o círculo apropriado à direita indicando como *habitualmente se sente*. Não há respostas certas ou erradas. Não gaste demasiado tempo em cada pergunta, dê a resposta que lhe parece descrever melhor a maneira como habitualmente se sente.

	QUASE NUNCA	ALGUMAS VEZES	FREQUENTEMENTE	QUASE SEMPRE
21. Sinto-me bem disposto(a)	①	②	③	④
22. Sinto-me nervoso(a) e agitado(a)	①	②	③	④
23. Estou satisfeito(a) comigo mesmo	①	②	③	④
24. Gostava de poder ser tão feliz como os outros parecem ser	①	②	③	④
25. Sinto-me um "falhado(a)"	①	②	③	④
26. Sinto-me descansado(a)	①	②	③	④
27. Sou "calmo(a), Indiferente e sereno(a)"	①	②	③	④
28. Sinto que as dificuldades se amontoam de maneira que não consigo ultrapassá-las	①	②	③	④
29. Preocupo-me com coisas que na realidade não têm importância	①	②	③	④
30. Sou feliz	①	②	③	④
31. Tenho pensamentos que me perturbam	①	②	③	④
32. Tenho falta de confiança em mim próprio(a)	①	②	③	④
33. Sinto-me seguro(a)	①	②	③	④
34. Tomo facilmente decisões	①	②	③	④
35. Sinto-me incapaz	①	②	③	④
36. Sinto-me satisfeito(a)	①	②	③	④
37. Alguns pensamentos sem importância passam pela minha cabeça e aborrecem-me	①	②	③	④
38. Tomo as contrariedades tão a sério que não consigo deixar de pensar nelas	①	②	③	④
39. Sou uma pessoa imperturbável	①	②	③	④
40. Quando penso nos assuntos que tenho entre mãos fico tenso e a "ferver por dentro"	①	②	③	④

(Edição Experimental - Tradução e adaptação de Emanuel Ponsano)

Appendix C - Authorization from the Administration Boards of the hospitals

S. R.

MINISTÉRIO DA SAÚDE
HOSPITAIS DA UNIVERSIDADE DE COIMBRA
Av. Bissaya Barreto
e
Praceta Prof. Mota Pinto
3000-075 COIMBRA

Telefones: Bloco Central/Bloco de Celas 239 400 400 / 239 400 500 / 239 400 600 / 239 403 939
Maternidade Dr. Daniel de Matos- 239 403 060
Telefax n.º 239 823 907

COMISSÃO DE ÉTICA PARA A SAÚDE
Presidente: Prof. Doutor José Joaquim Sousa Barros; Vice-Presidente: Dr. David Amador Rocha; Vogais: Dra. Maria Odete Isabel; Prof. Doutor Carlos Alberto Fontes Ribeiro; Enfermeiro: José Mendes Bajanca; Jurista: Prof. Doutor José de Faria e Costa; Padre: José António Afonso Pais

Exma. Senhora:
Enfª Natália Oliveira
Digmª Enfermeira Directora dos HUC

N/Refª	Ofício Nº	Data
CES	363	09.06.2006

ASSUNTO: *Projecto de Investigação (Tese de Doutoramento) - "Estudo da eficácia do contacto Materno pele-a-pele em combinação com a sacarose na redução da resposta dos recém-nascidos pré-termo a procedimentos dolorosos."* - Enf.ª Ananda Maria Fernandes (Enfermeira Especialista de Saúde Infantil e Pediátrica, Mestre em Ciências de Enfermagem pela Universidade do Porto e Professora Coordenadora da Escola Superior de Enfermagem Dr. Ângelo da Fonseca) - Trabalho a realizar na Unidade de Cuidados Intensivos ao Recém-Nascido da Maternidade Dr. Daniel de Matos.

Cumpre-me informar Vossa Ex.ª que, na reunião da Comissão de Ética para a Saúde dos Hospitais da Universidade de Coimbra de 6 de Junho de 2006, com a presença da maioria dos seus membros e após análise pormenorizada do projecto mencionado em epígrafe, foi emitido **parecer favorável**.

S. R.

MINISTÉRIO DA SAÚDE
HOSPITAIS DA UNIVERSIDADE DE COIMBRA
Av. Bissaya Barreto
e
Praceta Prof. Mota Pinto
3000-075 COIMBRA

Telefones: Bloco Central/Bloco de Celas 239 400 400 / 239 400 500 / 239 400 600 / 239 403 939
Maternidade Dr. Daniel de Matos- 239 403 060
Telefax n.º 239 823 907

O presente parecer apenas se torna exequível após inclusão no consentimento informado do destino a dar às filmagens após a conclusão do estudo, e depois de obtidas as autorizações dos Directores dos Serviços onde o mesmo decorrerá.

Com os melhores cumprimentos,

O PRESIDENTE DA COMISSÃO DE ÉTICA

Prof. Doutor José Joaquim Sousa Barros

SERVIÇOS CENTRAIS
CENTRO HOSPITALAR COIMBRA

Exm[a]
Srª Enfermeira Ananda Maria Fernandes

Luzeiro
S. Romão

3020 – 261 Coimbra

Vossa Data:	Vossa Referência	Data:	Nossa Referência
05-04-2006		23/05/2006	793/Sec

Assunto: Pedido de autorização para realização de estudo

Em resposta à solicitação de V. Ex[as], cumpre-nos informar de que, face ao parecer favorável da Comissão de Ética, o *«Estudo da eficácia do contacto materno pele-a-pele em combinação com a sacarose na redução da resposta dos recém-nascidos pré-termo a procedimentos dolorosos»*, foi autorizado.

Nesta data foi também dado conhecimento ao Director do Unidade de Cuidados Intensivos Neonatais,

Com os melhores cumprimentos,

O Presidente do Conselho de Administração do
Centro Hospitalar de Coimbra

(Dr. Rui Pato)

SERVIÇOS CENTRAIS
DO CENTRO HOSPITALAR
DE COIMBRA
Quinta dos Vales
S. Martinho do Bispo
3041-853 COIMBRA
Tel. 239 800 100
Fax. 239 442 620

HOSPITAL GERAL DA C.
PORTUGUESA DO BRASIL
Quinta dos Vales
S. Martinho do Bispo
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/IP

HOSPITAL PEDIÁTRICO
DE COIMBRA
Avenida Bissaya Barreto
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Tel. 239 480 300

MATERNIDADE
BISSAYA BARRETO
Rua Angelina
3030-061 COIMBRA
Tel. 239 480 400

APARTADO 7005
3041-853 COIMBRA

www.chc.min-saude.pt

Ministério da Saúde
Med.1160 - 12.2004 - Ed.01 - Rev.0

Appendix D - Information sheet for parents

Caros Pais:

Como sabem, durante o internamento há necessidade de realizar aos bebés alguns exames, como a colheita de sangue, que podem causar alguma dor.

É nossa preocupação reduzir o mais possível a dor durante esses procedimentos.

Por essa razão, está a ser realizado nas Unidades de Neonatalogia de Coimbra um estudo sobre duas formas de reduzir a dor dos bebés durante a colheita de sangue, para o qual é solicitada a vossa colaboração. Uma delas é a colocação do bebé em contacto com o peito da mãe; a outra é a colocação na boca de uma pequena quantidade de água açucarada. Em estudos anteriores, não se verificou qualquer risco para o bebé ou a mãe. O estudo é dirigido pela Enfermeira Ananda Fernandes, Especialista em Enfermagem de Saúde Infantil e Pediátrica, professora da Escola Superior de Enfermagem de Coimbra e estudante de Doutoramento na Universidade de Lisboa.

Se aceitarem participar, procederemos da seguinte forma:

1. Será atribuída ao bebé, ao acaso, a letra A ou B.

2. Uma das vezes em que houver necessidade de colher sangue ao bebé, a colheita será feita da forma habitual mas para tentar reduzir a dor será feito o seguinte:

- Se tiver a letra A, 2 minutos antes do exame será colocada na boca a chupeta molhada em água açucarada, como é habitual fazer-se neste serviço;

- Se tiver a letra B, o bebé será colocado em contacto directo com o peito da mãe durante 30 minutos antes da colheita de sangue e aí permanecerá durante a mesma; 2 minutos antes do exame será colocada na boca a chupeta molhada em água açucarada, como é habitual fazer-se neste serviço.

A face do bebé (apenas a face do bebé) será filmada durante o procedimento para analisarmos a sua reacção. A filmagem será feita de forma a que a mãe não seja identificada e será arquivada no final do estudo. Antes e depois da colheita será feita uma pequena

entrevista à mãe. Esse filme será utilizado exclusivamente para fins científicos e de ensino, e se desejarem, ser-vos-á oferecida, gratuitamente uma cópia. Iremos também observar os batimentos cardíacos e a respiração para o que serão colocados eléctrodos idênticos aos que já tem.

Os vossos nomes não constarão no registo dos dados do estudo e os vossos dados pessoais não serão em circunstância alguma divulgados.

Em qualquer momento do estudo poderão desistir, sem ter que dar qualquer justificação, bastando para tal dizê-lo à enfermeira que cuida do vosso filho.

Se não quiserem participar, não há qualquer problema. Estão no vosso pleno direito e respeitaremos a vossa decisão, que não terá qualquer influência nos cuidados ao bebé.

Se quiserem saber mais sobre o estudo, poderão falar com a Sra. Enfermeira-Chefe ou com as Sras. Enfermeiras _____ e _____

Se aceitarem participar, devem assinar a "Declaração de Consentimento Informado" e entregá-la à enfermeira.

Em todo o caso desejamos que tudo corra pelo melhor e que possam regressar a casa, com o vosso bebé, brevemente.

Coimbra, Janeiro de 2007

Appendix E - Parents' consent form

DECLARAÇÃO DE CONSENTIMENTO INFORMADO

Declaro que aceito participar, com o meu filho, neste estudo sobre as formas de diminuir a dor dos bebés durante a colheita de sangue.

Para isso, autorizo que, numa das vezes em que haja necessidade de colher sangue ao bebé, a colheita seja realizada da forma habitual, mas para tentar reduzir a dor seja feito o seguinte:

- Dois minutos antes do exame será colocada na boca a chupeta molhada em água açucarada, como é habitual fazer-se neste serviço;

ou

- O bebé será colocado em contacto directo com o colo da mãe durante 30 minutos antes da colheita de sangue e aí permanecerá durante a mesma; 2 minutos antes do exame será colocada na boca a chupeta molhada em água açucarada, como é habitual fazer-se neste serviço.

Autorizo igualmente que:

- o bebé seja filmado e o filme seja utilizado para fins científicos e de ensino, ficando arquivado no final do estudo para o mesmo fim;

- sejam colocados no bebé eléctrodos semelhantes aos que já tem, para registar os batimentos do coração, a respiração e os movimentos.

Consinto em ser entrevistada antes e depois da colheita de sangue.

Os nossos nomes não serão em qualquer circunstância revelados.

Em qualquer momento do estudo poderei desistir, sem ter que dar justificações e bastando para tal dizê-lo à enfermeira que cuida do meu filho.

Coimbra, _____/_____/_____

Assinatura_____

Appendix F - Protocol

ESTUDO SOBRE AS RESPOSTAS DE DOR NOS RECÉM-NASCIDOS PRÉTERMO - Ananda Fernandes

PROTOCOLO

I FASE – Selecção da amostra

	QUEM	QUEM
1.	Em cada criança que dá entrada, proceder à verificação dos critérios de inclusão/ exclusão;	Enfermeira/ Ananda
2.	Guardar os excluídos na pasta; colocar os restantes no respectivo processo da criança;	
3.	Explicar o estudo aos pais oralmente e dar carta informativa; pedir que a resposta seja dada durante o mesmo dia ou no dia seguinte;	
4.	Se a mãe consentir, dar declaração a assinar e guardá-la na pasta; se recusar, perguntar, sem insistir, se tem algum motivo que queira dizer, e registar a resposta;	
5.	Nas crianças que têm folha de inclusão, quando estiver prevista colheita, desde que não haja emergência, avisar enfermeira.	Elementos da equipa
6.	Reavaliar os critérios de inclusão/exclusão;	Enfermeira/ Ananda
7.	Se excluído definitivamente, retirar alerta e colocar folha na pasta; se não, manter folha no processo, com o alerta;	
8.	Se a criança for incluída avisar Ananda - 917500541	
9.	Determinar o grupo utilizando a tabela de randomização	
10.	Proceder à aplicação do protocolo de colheita de dados	

II – FASE – Colheita de dados

	PREPARAÇÃO	
11.	Relembrar a mãe acerca do estudo	
12.	Colocar eléctrodos suplementares e sensor de saturação no bebé	
13.	Instalar a mãe e bebé em Canguru: cadeirão a 60º, mãe sem blusa ou camisola, apenas a bata, bebé com fralda, coberto pela bata da mãe e com manta nas costas, mãos da mãe sobre as costas do bebé;	
14.	Ligar Somté, preparar câmara;	Ananda
15.	Instruir a mãe para não encostar o queixo à cabeça do bebé e não falar durante a colheita;	
16.	Manter Canguru durante 30 minutos;	
17.	Dois minutos antes da colheita, colocar solução prevista sobre a chupeta e colocar chupeta no bebé	
	REALIZAÇÃO	
18.	Desinfectar a pele, puncionar, colher o sangue e comprimir veia	Enfermeira

Appendix G - Data collection form

ESTUDO SOBRE AS RESPOSTAS DE DOR
NOS RECÉM-NASCIDOS PRETERMO
Ananda Fernandes

AVISAR QUANDO FOR PROGRAMADA COLHEITA

Nº Ordem _____

Nº do processo _____ **Data de nascimento** ____/____/____ **Admissão** ____/____/____

AVALIAÇÃO À ENTRADA Data _____/_____/____

CRITÉRIOS DE EXCLUSÃO		
Idade gestacional	**< 28 sem** **> 36 e 6 dias**	**≥ 28 sem** **< 37 sem**
Apgar aos 5 minutos	**≤ 6**	**> 6**
Mãe **diabética**	**SIM**	**NÃO**
Com **anomalia congénita major**	**SIM**	**NÃO**
Mãe não sabe ler e escrever **Português**	**Não sabe**	**Sabe**

SE ALGUM SIM, EXCLUIR DEFINITIVAMENTE (Arquivar)

SE TODOS NÃO, PEDIR CONSENTIMENTO

CONSENTIMENTO: SIM (Prosseguir estudo)

NÃO (Arquivar na pasta) Motivo invocado _____

REAVALIAÇÃO QUANDO PROGRAMADA A COLHEITA Data ____/____/____

CRITÉRIOS DE EXCLUSÃO	**SIM**	**NÃO**
1. Submetido a **cirurgia**		
2. Com **hemorragia intra-ventricular** > grau 2 ou LPV		
3. **Gravemente doente**		
4**. Ventilado**		
5. Administrados **analgésicos** nas 12 horas anteriores		
5. Administrados **sedativos** nas 48 horas anteriores		
7. Submetido a **procedimento doloroso** nas 12 horas anteriores;		

Se algum sim nos critérios 1, 2 e 3, EXCLUIR definitivamente

Se algum sim nos critérios 4, 5, 6 e 7, REAVALIAR aquando da próxima colheita

PROSSEGUIR ESTUDO

Março 2007

FICHA DE REGISTO DE DADOS INDIVIDUAIS Nº Ordem _____

1. **Grupo**	1 S 2 SK

Criança:	
2. **Sexo**	1 Masc. 2 Fem
2. **Idade gestacional ao nascer**	Semanas
3. **Gestação**	I II III IV V VI
4. **Paridade**	I II III IV V VI
5. **Ordem na fratria**	1 2 3 4 5 6
6. **Tipo de parto**	1 Eutócico 2 Instrumentado 3 Cesariana
7. **Apgar**	/ /
8. **Peso de Nascimento**	Gramas
9. **Diagnóstico principal**	
10. **Score CRIB**	
11. **Idade materna**	
12. **Número de vezes de Canguru Materno anteriores**	12.1 com este_____ ou 12.2 outro filho_____

Data da colheita	
13. **Idade pós-natal**	Dias
14**. Idade Gestacional actual**	
15. **Acesso vascular**	1 Sim 2 Não
16. **Sonda**	1 Naso-gástrica 2 Oro-gástrica
17. **O2 suplementar**	1 Sim 2 Não
18. **Número intervenções dolorosas anteriores**	
19. **Estado comportamental**	0 – Acordado, activo, olhos abertos, movimentos faciais, choro 1 – Acordado, calmo, sem movimentos faciais 2 – Sono leve, olhos fechados, movimentos faciais 3 – Sono calmo, olhos fechados, sem movimentos faciais
20. **Baseline FC**	
21. **Baseline Sat O2**	

Observações:

Dose:

Última refeição:

Colheita para:

Março 2007

Appendix H - The Neonatal Facial Coding System

Grunau, Ruth Eckstein; Fitzgerald, Colleen E.; Ellwood, Ann-Louise Craig,

Kenneth D. (2007). *Neonatal Facial Coding System Training Manual.* Vancouver: Early Human Experience Unit, Centre for Community Child Health Research, Child & Family Research Institute.

Action	Description
Brow Bulge	Bulging, creasing and/or vertical furrows above and between brows occurring as a result of lowering and drawing together of the eyebrows.
Eye Squeeze	Squeezing and/or bulging of the eyelids
Naso-labial Furrow	Pulling upwards and deepening of the naso-labial furrow (a line or wrinkle which begins adjacent to the nostril wings and runs down and outwards beyond the lip corners).
Mouth Open	Mouth open more than relaxed lips apart. Many babies lips are apart even when their face is relaxed. Comparison is the individual baby's usual relaxed face. Jaw drop may be visible as a cue.
Vertical Mouth Stretch	Characterized by a tautness at the lip corners coupled with a pronounced downward pull on the jaw. Often stretch mouth is seen when an already wide mouth is opened a fraction further by an extra pull at the jaw.
Horizontal Mouth	This appears as a distinct horizontal stretch pull at the corners of the mouth sometimes accompanied by a taut upper lip.
Taut Tongue	Raised, cupped tongue with sharp tensed edges. The first occurrence of taut tongue is usually easy to see, often occurring with a wide open mouth. After this first occurrence, the mouth may close slightly. Taut tongue can be scored on the basis of the still visible tongue edges.
Chin Quiver	An obvious high frequency up-down motion of the lower jaw.
Tongue Protrusion (see page 7 for inclusion criteria)	Action of the tongue moving forward but not always beyond the infant's lips.

Appendix I - Comparison between final sample and lost cases

Table 1 – Group Statistics

		N	Mean	Std. Deviation	Std. Error Mean
Postnatal age	Lost	20,00	7,45	4,95	1,11
	Final sample	110,00	6,51	3,99	,38
Birth weight	Lost	20,00	1492,75	394,38	88,19
	Final sample	110,00	1657,15	437,32	41,70
Last_meal	Lost	20,00	92,00	51,77	11,58
	Final sample	99,00	108,31	69,02	6,94
Previous_painful_procedures	Lost	20,00	14,10	18,65	4,17
	Final sample	106,00	16,65	19,38	1,88
Maternal_age	Lost	20,00	30,65	4,50	1,01
		110,00	30,29	4,67	,45

Table 2 – Independent t test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
						Sig	Mean	Std. Error	95% Confidence Interval of the Difference	
		f	Sig	t	df	(2-tailed)	Difference	Difference	Lower	Upper
Birth_weight	Equal variances assumed	0.864	0.354	-1.568	128	.119	-164.39545	104.82205	-371-804	43.011288
	Equal variances not assumed			-1.685	28.200	.103	-164.39545	97.54611	-364.146	35.35502
Last_meal	Equal variances assumed	1.974	0.163	-1.000	117	.319	-16.31313	16.30870	-48.61167	15.98540
	Equal variances not assumed			-1.209	34.240	.235	-16.31313	13.49517	-43.73154	11.10528
Maternal_ weight	Equal variances assumed	0.302	0.584	.318	128	.751	.35909	1.12863	-1.87409	2.59227
	Equal variances not assumed			.326	26.985	.747	.35909	1.10001	-1.89801	2.61619
STAI	Equal variances assumed	3.773	0.055	.682	99	.497	2.19288	3.21673	-4.18981	8.57558
	Equal variances not assumed			.505	12.374	.623	2.19288	4.34552	-7.24359	11.62936

Table 3 - Mann-Whitney Test ranks

	Group	N	Mean Rank	Sum of Ranks
Gestational_age	Lost	62	62,57	3879,50
	Final sample	68	68,17	4635,50
days	Lost	62	62,02	3845,50
	Final sample	68	68,67	4669,50
Previous_painful_procedures	Lost	59	64,14	3784,50
	Final sample	67	62,93	4216,50

Test Statistics(a)

	Gestational_age	Postnatal days	Previous_painful_procedures
Mann-Whitney U	1,926,500	1,892,500	1,938,500
Wilcoxon W	3,879,500	3,845,500	4,216,500
Z	-,856	-1,011	-,186
Asymp. Sig. (2-tailed)	,392	,312	,852

a Grouping Variable: Group

Appendix J - PIPP scores across phases of the procedure

Table 1 - PIPP scores across phases of the procedure by intervention and gestational age group(GA)

Phase	Intervention	GA	M	SD	N
Preparation	Sucrose	< 32	4,25	2,79	16
PIPP		=>32	4,75	3,13	28
		Total	4,57	2,99	44
	S+KMC	< 32	5,20	1,21	15
		=>32	4,56	1,63	41
		Total	4,73	1,54	56
	Total	< 32	4,71	2,19	31
		=>32	4,64	2,34	69
		Total	4,66	2,28	100
S30	Sucrose	< 32	6,31	4,09	16
PIPP		=>32	7,68	4,36	28
		Total	7,18	4,27	44
	S+KMC	< 32	6,07	2,58	15
		=>32	6,27	3,81	41
		Total	6,21	3,50	56
	Total	< 32	6,19	3,39	31
		=>32	6,84	4,07	69
		Total	6,64	3,87	100
C30	Sucrose	< 32	4,94	2,54	16
PIPP		=>32	4,11	2,69	28
		Total	4,41	2,64	44
	S+KMC	< 32	5,07	1,16	15
		=>32	4,39	2,28	41
		Total	4,57	2,05	56
	Total	< 32	5,00	1,97	31
		=>32	4,28	2,44	69
		Total	4,50	2,32	100
R30	Sucrose	< 32	3,69	1,45	16
PIPP		=>32	3,04	1,17	28
		Total	3,27	1,30	44
	S+KMC	< 32	4,67	,98	15
		=>32	3,78	1,24	41
		Total	4,02	1,23	56
	Total	< 32	4,16	1,32	31
		=>32	3,48	1,26	69
		Total	3,69	1,31	100

		M		SE	N
		95% CI			
		LL UL			
Total	Sucrose	4.85		0.29	44
		4.26	5.43		
	S+KMC	5		0.28	56
		3.96	5.38		

CI= Confidence Interval LL= Lower Limit; UL= Upper Limit

Table 2 - Tests of Within-Subjects Contrasts for the PIPP

Measure:PIPP						
Source	Phase	Type III Sum of Squares	df	Mean Square	F	Sig.
Phase	Level 1 vs. Level 2	302,371	1	302,371	30,847	,000
	Level 2 vs. Level 3	323,464	1	323,464	27,407	,000
	Level 3 vs. Level 4	58,629	1	58,629	13,994	,000
Phase * Group	Level 1 vs. Level 2	30,867	1	30,867	3,149	,079
	Level 2 vs. Level 3	22,604	1	22,604	1,915	,170
	Level 3 vs. Level 4	9,090	1	9,090	2,170	,144
Phase * GA	Level 1 vs. Level 2	15,390	1	15,390	1,570	,213
	Level 2 vs. Level 3	49,941	1	49,941	4,232	,042
	Level 3 vs. Level 4	,005	1	,005	,001	,972
Phase * Group * GA	Level 1 vs. Level 2	,003	1	,003	,000	,985
	Level 2 vs. Level 3	9,183	1	9,183	,778	,380
	Level 3 vs. Level 4	,797	1	,797	,190	,664
Error(Phase)	Level 1 vs. Level 2	941,016	96	9,802		
	Level 2 vs. Level 3	1132,997	96	11,802		
	Level 3 vs. Level 4	402,213	96	4,190		

Table 3 - Pairwise comparisons for the PIPP

							95% Confidence Interval for Differencea	
(I) Time		(J) Time		Mean Difference (I-J)	Std. Error	Sig.a	Lower Bound	Upper Bound
dimension1	1	dimension2	2	-1,891*	,341	,000	-2,809	-,974
			3	,065	,311	1,000	-,774	,904
			4	,898*	,229	,001	,282	1,514
	2	dimension2	1	1,891*	,341	,000	,974	2,809
			3	1,956*	,374	,000	,950	2,963
			4	2,789*	,406	,000	1,695	3,883
	3	dimension2	1	-,065	,311	1,000	-,904	,774
			2	-1,956*	,374	,000	-2,963	-,950
			4	,833*	,223	,002	,233	1,433
	4	dimension2	1	-,898*	,229	,001	-1,514	-,282
			2	-2,789*	,406	,000	-3,883	-1,695
			3	-,833*	,223	,002	-1,433	-,233

Based on estimated marginal means

*. The mean difference is significant at the ,05 level.

a. Adjustment for multiple comparisons: Bonferroni.

Appendix K - Infants' charcteristics ans PIPP scores

Table 1 - Correlation between infants' characteristics and PIPP scores

	PIPP score at Needle stick	
	Sucrose	S+KMC
Birth weight	*r*= .30	*r*= .20
	p= .01	*p*= .13
	n= 46	n= 61
Postnatal age	*r*= .53	*r*= .27
	p= .00	*p*= .04
	n= 46	n= 61
Time since last meal	*r*= -.06	*r*= -.03
	p= .70	*p*= .84
	n= 45	n= 54
Number of previous painful procedures	*r*= -.04	*r*= -.07
	p= .78	*p*= .62
	n= 46	n= 60

Appendix L - Hearth rate across the procedure

Table 1 - Maximum heart rate (Max HR) in beats per minute across phases of the procedure by intervention and age group (GA)

Time epoch	Intervention	GA	M	SD	N
Baseline	Sucrose	< 32	167.63	10.27	16
Max HR		=>32	168	15.92	27
		Total	167.86	13.95	43
	S+KMC	< 32	174.73	10.91	15
		=>32	165.1	14.36	39
		Total	167.78	14.08	54
	Total	< 32	171.06	11.01	31
		=>32	166.29	14.97	66
		Total	167.81	13.95	97
Preparation	Sucrose	< 32	173.19	14.08	16
Max HR		=>32	176	18.62	27
		Total	174.95	16.95	43
	S+KMC	< 32	180.8	9.37	15
		=>32	171.87	15.13	39
		Total	174.35	14.27	54
	Total	< 32	176.87	12.45	31
		=>32	173.56	16.63	66
		Total	174.62	15.43	97
S30	Sucrose	< 32	175.13	17.34	16
Max HR		=>32	183.11	19.59	27
		Total	180.14	18.98	43
	S+KMC	< 32	182.8	13.26	15
		=>32	175.51	17.8	39
		Total	177.54	16.87	54
	Total	< 32	178.84	15.74	31
		=>32	178.62	18.79	66
		Total	178.69	17.79	97
C30	Sucrose	< 32	173.25	13.95	16
Max HR		=>32	171.63	26.29	27
		Total	172.23	22.32	43
	S+KMC	< 32	177.13	10.11	15
		=>32	167.26	18.11	39
		Total	170	16.79	54
	Total	< 32	175.13	12.2	31
		=>32	169.05	21.75	66
		Total	170.99	19.36	97
R30	Sucrose	< 32	171.69	24.45	16
Max HR		=>32	161.59	16.3	27
		Total	165.35	20.06	43
	S+KMC	< 32	171.8	11.07	15
		=>32	160.18	15.61	39
		Total	163.41	15.32	54
	Total	< 32	171.74	18.87	31
		=>32	160.76	15.78	66
		Total	164.27	17.51	97

			M			
			95% CI		SE	N
		LL		UL		
Total	Sucrose		172.12		2.283	43
		167.587		176.655		
	S+KMC		172.72		2.199	54
		168.353		177.085		

CI= Confidence Interval LL= Lower Limit; UL= Upper Limit

Table 2 - Tests of Within-Subjects Contrasts for Maximum heart rate

Measure:Max

Source	Phase	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Phase	Level 1 vs. Level 2	3,632,497	1	3,632,497	54,437	,000	,369	54,437	1,000
	Level 2 vs. Level 3	1,124,792	1	1,124,792	12,694	,001	,120	12,694	,941
	Level 3 vs. Level 4	3,879,045	1	3,879,045	15,469	,000	,143	15,469	,973
	Level 4 vs. Level 5	3,004,879	1	3,004,879	10,824	,001	,104	10,824	,902
Phase * Group	Level 1 vs. Level 2	2,752	1	2,752	,041	,840	,000	,041	,055
	Level 2 vs. Level 3	60,526	1	60,526	,683	,411	,007	,683	,129
	Level 3 vs. Level 4	1,673	1	1,673	,007	,935	,000	,007	,051
	Level 4 vs. Level 5	3,426	1	3,426	,012	,912	,000	,012	,051
Phase * GA	Level 1 vs. Level 2	51,396	1	51,396	,770	,382	,008	,770	,140
	Level 2 vs. Level 3	242,067	1	242,067	2,732	,102	,029	2,732	,373
	Level 3 vs. Level 4	775,356	1	775,356	3,092	,082	,032	3,092	,413
	Level 4 vs. Level 5	544,243	1	544,243	1,961	,165	,021	1,961	,283
Phase * Group * GA	Level 1 vs. Level 2	15,690	1	15,690	,235	,629	,003	,235	,077
	Level 2 vs. Level 3	65,048	1	65,048	,734	,394	,008	,734	,136
	Level 3 vs. Level 4	256,638	1	256,638	1,023	,314	,011	1,023	,170
	Level 4 vs. Level 5	236,158	1	236,158	,851	,359	,009	,851	,150
Error (Phase)	Level 1 vs. Level 2	6,205,794	93	66,729					
	Level 2 vs. Level 3	8,240,579	93	88,608					
	Level 3 vs. Level 4	23,321,260	93	250,766					
	Level 4 vs. Level 5	25,817,003	93	277,602					

Table 3 - Pairwise Comparisons for Maximum heart rate

Measure: Max

(I) Phase		(J) Phase		Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Differencea	
							Lower Bound	Upper Bound
dimension1	1	dimension2	2	-6,600*	,894	,000	-9,172	-4,027
			3	-10,272*	1,395	,000	-14,284	-6,260
			4	-3,452	1,695	,445	-8,326	1,422
			5	2,550	1,313	,551	-1,224	6,325
	2	dimension2	1	6,600*	,894	,000	4,027	9,172
			3	-3,672*	1,031	,006	-6,636	-,708
			4	3,147	1,539	,437	-1,279	7,574
			5	9,150*	1,259	,000	5,530	12,770
	3	dimension2	1	10,272*	1,395	,000	6,260	14,284
			2	3,672*	1,031	,006	,708	6,636
			4	6,820*	1,734	,002	1,834	11,806
			5	12,822*	1,565	,000	8,321	17,324
	4	dimension2	1	3,452	1,695	,445	-1,422	8,326
			2	-3,147	1,539	,437	-7,574	1,279
			3	-6,820*	1,734	,002	-11,806	-1,834
			5	6,002*	1,824	,014	,756	11,249
	5	dimension2	1	-2,550	1,313	,551	-6,325	1,224
			2	-9,150*	1,259	,000	-12,770	-5,530
			3	-12,822*	1,565	,000	-17,324	-8,321
			4	-6,002*	1,824	,014	-11,249	-,756

Based on estimated marginal means

*. The mean difference is significant at the ,05 level.

[a]. Adjustment for multiple comparisons: Bonferroni.

Table 4 - Average heart rate (Avg HR) in beats per minute across phases of the procedure by intervention and gestational age group (GA)

Time epoch	Intervention	GA	M	SD	N
Baseline	Sucrose	< 32	158.94	8.87	16
Avg HR		=>32	155.33	15.65	27
		Total	156.67	13.52	43
	S+KMC	< 32	161.53	14.48	15
		=>32	152.44	12.07	39
		Total	154.96	13.29	54
	Total	< 32	160.19	11.78	31
		=>32	153.62	13.6	66
		Total	155.72	13.35	97
Preparation	Sucrose	< 32	165.5	11.55	16
Avg HR		=>32	165.22	14.67	27
		Total	165.33	13.45	43
	S+KMC	< 32	171.6	11.13	15
		=>32	160.28	13.2	39
		Total	163.43	13.56	54
	Total	< 32	168.45	11.58	31
		=>32	162.3	13.93	66
		Total	164.27	13.47	97
S30	Sucrose	< 32	168.44	15.27	16
Avg HR		=>32	171.56	15.55	27
		Total	170.4	15.34	43
	S+KMC	< 32	174.47	10.91	15
		=>32	164.23	15.59	39
		Total	167.07	15.07	54
	Total	< 32	171.35	13.47	31
		=>32	167.23	15.87	66
		Total	168.55	15.2	97
C30	Sucrose	< 32	163.81	13.11	16
Avg HR		=>32	158.78	18.26	27
		Total	160.65	16.55	43
	S+KMC	< 32	167.93	10.79	15
		=>32	154.28	15.56	39
		Total	158.07	15.57	54
	Total	< 32	165.81	12.03	31
		=>32	156.12	16.73	66
		Total	159.22	15.98	97
R30	Sucrose	< 32	159.06	8.93	16
Avg HR		=>32	151.04	16.33	27
		Total	154.02	14.46	43
	S+KMC	< 32	163.07	9.92	15
		=>32	147.21	12.94	39
		Total	151.61	14.06	54
	Total	< 32	161	9.49	31
		=>32	148.77	14.43	66
		Total	152.68	14.21	97
			M	SE	N
			95% CI		
		LL	UL		
Total	Sucrose		161.77	1.9	43
		158	165.54		
	S+KMC		161.7	1.83	54
		158.07	165.33		

CI= Confidence Interval LL= Lower Limit; UL= Upper Limit

Table 5 - Tests of Within-Subjects Contrasts for average heart rate

Measure: Avg

Source	Phase	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Powera
Phase	Level 1 vs. Level 2	6,155,493	1	6,155,493	78,456	,000	,458	78,456	1,000
	Level 2 vs. Level 3	1,348,833	1	1,348,833	18,115	,000	,163	18,115	,988
	Level 3 vs. Level 4	5,984,953	1	5,984,953	31,557	,000	,253	31,557	1,000
	Level 4 vs. Level 5	3,112,082	1	3,112,082	41,061	,000	,306	41,061	1,000
Phase * Group	Level 1 vs. Level 2	11,133	1	11,133	,142	,707	,002	,142	,066
	Level 2 vs. Level 3	31,428	1	31,428	,422	,517	,005	,422	,099
	Level 3 vs. Level 4	4,419	1	4,419	,023	,879	,000	,023	,053
	Level 4 vs. Level 5	1,561	1	1,561	,021	,886	,000	,021	,052
Phase * GA	Level 1 vs. Level 2	6,375	1	6,375	,081	,776	,001	,081	,059
	Level 2 vs. Level 3	104,519	1	104,519	1,404	,239	,015	1,404	,216
	Level 3 vs. Level 4	697,556	1	697,556	3,678	,058	,038	3,678	,475
	Level 4 vs. Level 5	141,001	1	141,001	1,860	,176	,020	1,860	,271
Phase * Group * GA	Level 1 vs. Level 2	160,380	1	160,380	2,044	,156	,022	2,044	,293
	Level 2 vs. Level 3	27,906	1	27,906	,375	,542	,004	,375	,093
	Level 3 vs. Level 4	116,985	1	116,985	,617	,434	,007	,617	,122
	Level 4 vs. Level 5	3,175	1	3,175	,042	,838	,000	,042	,055
Error(Phase)	Level 1 vs. Level 2	7,296,614	93	78,458					
	Level 2 vs. Level 3	6,924,568	93	74,458					
	Level 3 vs. Level 4	17,638,047	93	189,656					
	Level 4 vs. Level 5	7,048,688	93	75,792					

a. Computed using alpha= ,05

Table 6 - Pairwise Comparisons for average heart rate

Measure:Avg

(I) Phase		(J) Phase		Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a] Lower Bound	Upper Bound
dimension1	1	dimension2	2	-8,591*	,970	,000	-11,380	-5,802
			3	-12,613*	1,469	,000	-16,837	-8,389
			4	-4,141*	1,376	,034	-8,097	-,186
			5	1,967	1,122	,828	-1,259	5,194
	2	dimension2	1	8,591*	,970	,000	5,802	11,380
			3	-4,022*	,945	,000	-6,739	-1,305
			4	4,450*	1,224	,005	,930	7,970
			5	10,558*	1,057	,000	7,518	13,599
	3	dimension2	1	12,613*	1,469	,000	8,389	16,837
			2	4,022*	,945	,000	1,305	6,739
			4	8,471*	1,508	,000	4,135	12,808
			5	14,580*	1,430	,000	10,469	18,691
	4	dimension2	1	4,141*	1,376	,034	,186	8,097
			2	-4,450*	1,224	,005	-7,970	-,930
			3	-8,471*	1,508	,000	-12,808	-4,135
			5	6,109*	,953	,000	3,367	8,850
	5	dimension2	1	-1,967	1,122	,828	-5,194	1,259
			2	-10,558*	1,057	,000	-13,599	-7,518
			3	-14,580*	1,430	,000	-18,691	-10,469
			4	-6,109*	,953	,000	-8,850	-3,367

Based on estimated marginal means

*. The mean difference is significant at the ,05 level.

[a]. Adjustment for multiple comparisons: Bonferroni.

Table.7 - Minimum heart rate (Min HR) in beats per minute across phases of the procedure by intervention and gestational age group (GA)

Time epoch	Intervention	GA	M	SD	N
Baseline	Sucrose	< 32	149.31	9.38	16
Min HR		=>32	141.59	17.75	27
		Total	144.47	15.52	43
	S+KMC	< 32	155.6	11.18	15
		=>32	139.26	12.88	39
		Total	143.8	14.37	54
	Total	< 32	152.35	10.61	31
		=>32	140.21	14.98	66
		Total	144.09	14.81	97
Preparation	Sucrose	< 32	157.63	11.48	16
Min HR		=>32	152.93	16.09	27
		Total	154.67	14.58	43
	S+KMC	< 32	164.2	9.23	15
		=>32	144.36	16.11	39
		Total	149.87	17	54
	Total	< 32	160.81	10.81	31
		=>32	147.86	16.53	66
		Total	152	16.07	97
S30	Sucrose	< 32	159.63	14.99	16
Min HR		=>32	156.11	19.77	27
		Total	157.42	18.03	43
	S+KMC	< 32	164.33	10.65	15
		=>32	148.74	17.66	39
		Total	153.07	17.41	54
	Total	< 32	161.9	13.08	31
		=>32	151.76	18.76	66
		Total	155	17.73	97
C30	Sucrose	< 32	151.87	15.24	16
Min HR		=>32	142.96	21.4	27
		Total	146.28	19.63	43
	S+KMC	< 32	157.2	13.49	15
		=>32	138.13	17.59	39
		Total	143.43	18.56	54
	Total	< 32	154.45	14.43	31
		=>32	140.11	19.23	66
		Total	144.69	18.99	97
R30	Sucrose	< 32	148.31	9.39	16
Min HR		=>32	136.44	18.31	27
		Total	140.86	16.51	43
	S+KMC	< 32	151.87	11.59	15
		=>32	134.18	14.12	39
		Total	139.09	15.57	54
	Total	< 32	150.03	10.49	31
		=>32	135.11	15.87	66
		Total	139.88	15.93	97

			M		SE	N
			95% CI			
		LL		UL		
Total	Sucrose		149.68		1.91	43
		145.88		153.48		
	S+KMC		149.79		1.84	54
		146.13		153.45		

CI= Confidence Interval LL= Lower Limit; UL= Upper Limit

Table 8 - Tests of Within-Subjects Contrasts for minimum heart rate

Measure:MinHR

Source	Phase	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Powera
Phase	Level 1 vs. Level 2	5,796,957	1	5,796,957	32,093	,000	,257	32,093	1,000
	Level 2 vs. Level 3	490,766	1	490,766	2,237	,138	,023	2,237	,316
	Level 3 vs. Level 4	7,785,360	1	7,785,360	21,360	,000	,187	21,360	,996
	Level 4 vs. Level 5	1,954,333	1	1,954,333	10,810	,001	,104	10,810	,902
Phase * Group	Level 1 vs. Level 2	184,120	1	184,120	1,019	,315	,011	1,019	,170
	Level 2 vs. Level 3	2,321	1	2,321	,011	,918	,000	,011	,051
	Level 3 vs. Level 4	51,703	1	51,703	,142	,707	,002	,142	,066
	Level 4 vs. Level 5	3,327	1	3,327	,018	,892	,000	,018	,052
Phase * GA	Level 1 vs. Level 2	1,184	1	1,184	,007	,936	,000	,007	,051
	Level 2 vs. Level 3	154,058	1	154,058	,702	,404	,007	,702	,132
	Level 3 vs. Level 4	411,051	1	411,051	1,128	,291	,012	1,128	,183
	Level 4 vs. Level 5	12,871	1	12,871	,071	,790	,001	,071	,058
Phase * Group * GA	Level 1 vs. Level 2	221,470	1	221,470	1,226	,271	,013	1,226	,195
	Level 2 vs. Level 3	49,003	1	49,003	,223	,638	,002	,223	,075
	Level 3 vs. Level 4	19,137	1	19,137	,053	,819	,001	,053	,056
	Level 4 vs. Level 5	98,210	1	98,210	,543	,463	,006	,543	,113
Error (Phase)	Level 1 vs. Level 2	16,798,627	93	180,630					
	Level 2 vs. Level 3	20,405,038	93	219,409					
	Level 3 vs. Level 4	33,897,372	93	364,488					
	Level 4 vs. Level 5	16,813,909	93	180,795					

a. Computed using alpha= ,05

Table 9 Pairwise Comparisons for minimum heart rate

Measure: MinHR								
							95% Confidence Interval for Difference[a]	
(I) Phase		(J) Phase		Mean Difference (I-J)	Std. Error	Sig.[a]	Lower Bound	Upper Bound
dimension1	1	dimension2	2	-8,337*	1,472	,000	-12,569	-4,105
			3	-10,763*	1,984	,000	-16,467	-5,058
			4	-1,101	1,870	1,000	-6,479	4,277
			5	3,740*	1,291	,047	,026	7,453
	2	dimension2	1	8,337*	1,472	,000	4,105	12,569
			3	-2,426	1,622	1,000	-7,090	2,238
			4	7,236*	1,631	,000	2,547	11,925
			5	12,077*	1,586	,000	7,516	16,637
	3	dimension2	1	10,763*	1,984	,000	5,058	16,467
			2	2,426	1,622	1,000	-2,238	7,090
			4	9,662*	2,091	,000	3,650	15,673
			5	14,502*	1,863	,000	9,146	19,859
	4	dimension2	1	1,101	1,870	1,000	-4,277	6,479
			2	-7,236*	1,631	,000	-11,925	-2,547
			3	-9,662*	2,091	,000	-15,673	-3,650
			5	4,841*	1,472	,014	,607	9,075
	5	dimension2	1	-3,740*	1,291	,047	-7,453	-,026
			2	-12,077*	1,586	,000	-16,637	-7,516
			3	-14,502*	1,863	,000	-19,859	-9,146
			4	-4,841*	1,472	,014	-9,075	-,607

Based on estimated marginal means

*. The mean difference is significant at the ,05 level.

[a]. Adjustment for multiple comparisons: Bonferroni.

Appendix M – Oxygen saturation across the procedure

Table 1 - Minimum oxygen saturation levels (Min SpO2) (%) across phases of the procedure by intervention and gestational age group(GA)

Time epoch	Intervention	GA	M	SD	N
Baseline	Sucrose	< 32	95.81	2.59	16
Min SpO2		=>32	97.05	2.54	20
		Total	96.5	2.6	36
	S+KMC	< 32	97.36	1.69	14
		=>32	96.65	2.7	37
		Total	96.84	2.47	51
	Total	< 32	96.53	2.32	30
		=>32	96.79	2.63	57
		Total	96.7	2.52	87
Preparation	Sucrose	< 32	95.63	2.9	16
Min SpO2		=>32	96.85	2.35	20
		Total	96.31	2.64	36
	S+KMC	< 32	96.71	2.13	14
		=>32	95.65	3.16	37
		Total	95.94	2.93	51
	Total	< 32	96.13	2.58	30
		=>32	96.07	2.93	57
		Total	96.09	2.8	87
S30	Sucrose	< 32	95.5	4.4	16
Min SpO2		=>32	96.2	2.07	20
		Total	95.89	3.28	36
	S+KMC	< 32	96	2.15	14
		=>32	96.11	2.23	37
		Total	96.08	2.19	51
	Total	< 32	95.73	3.48	30
		=>32	96.14	2.16	57
		Total	96	2.68	87
C30	Sucrose	< 32	95.56	4.76	16
Min SpO2		=>32	96.7	2.27	20
		Total	96.19	3.58	36
	S+KMC	< 32	96	2.35	14
		=>32	96.41	2.25	37
		Total	96.29	2.27	51
	Total	< 32	95.77	3.78	30
		=>32	96.51	2.25	57
		Total	96.25	2.87	87
R30	Sucrose	< 32	96.06	1.91	16
Min SpO2		=>32	96.95	2.56	20
		Total	96.56	2.31	36
	S+KMC	< 32	96.86	1.51	14
		=>32	95.81	3.93	37
		Total	96.1	3.45	51
	Total	< 32	96.43	1.76	30
		=>32	96.21	3.53	57
		Total	96.29	3.03	87

		M		SE	N
		95% CI			
		LL	UL		
Total	Sucrose	96.23		0.39	51
		95.47	97		
	S+KMC	96.36		0.36	87
		95.64	97.07		

CI= Confidence Interval LL= Lower Limit; UL= Upper Limit

Table 2 - Average oxygen saturation levels (Avg SpO2) (%) across phases of the procedure by intervention and gestational age group (GA)

Time epoch	Intervention	GA	M	SD	N
Baseline	Sucrose	< 32	96.59	2.57	16
Avg SpO2		=>32	97.77	2.01	20
		Total	97.24	2.32	36
	S+KMC	< 32	97.83	1.44	14
		=>32	97.54	2.26	37
		Total	97.62	2.05	51
	Total	< 32	97.16	2.18	30
		=>32	97.62	2.16	57
		Total	97.46	2.16	87
Preparation	Sucrose	< 32	96.46	2.51	16
Avg SpO2		=>32	97.5	2.04	20
		Total	97.04	2.29	36
	S+KMC	< 32	97.53	1.65	14
		=>32	97.25	1.96	37
		Total	97.32	1.87	51
	Total	< 32	96.96	2.19	30
		=>32	97.33	1.98	57
		Total	97.21	2.05	87
S30	Sucrose	< 32	96.44	3.5	16
Avg SpO2		=>32	96.99	2.05	20
		Total	96.74	2.76	36
	S+KMC	< 32	97.12	1.54	14
		=>32	97.09	1.95	37
		Total	97.1	1.83	51
	Total	< 32	96.76	2.74	30
		=>32	97.05	1.97	57
		Total	96.95	2.25	87
C30	Sucrose	< 32	96.34	4.76	16
Avg SpO2		=>32	97.46	2.2	20
		Total	96.96	3.56	36
	S+KMC	< 32	96.8	2.36	14
		=>32	97.38	1.84	37
		Total	97.22	1.99	51
	Total	< 32	96.55	3.78	30
		=>32	97.4	1.95	57
		Total	97.11	2.73	87
R30	Sucrose	< 32	97.29	1.53	16
Avg SpO2		=>32	97.63	2.29	20
		Total	97.48	1.97	36
	S+KMC	< 32	97.87	0.94	14
		=>32	96.95	3	37
		Total	97.2	2.62	51
	Total	< 32	97.56	1.3	30
		=>32	97.19	2.77	57
		Total	97.32	2.36	87

			M		SE	N
			95% CI			
		LL		UL		
Total	Sucrose		96.23		0.33	51
		96.38		97.71		
	S+KMC		97.33		0.33	87
		96.72		97.95		

CI= Confidence Interval LL= Lower Limit; UL= Upper Limit

Table 3 - Maximum oxygen saturation levels (Max SpO2) (%) across phases of the procedure by intervention and gestational age group (GA)

Time epoch	Intervention	GA	M	SD	N
Baseline	Sucrose	< 32	97.38	2.66	16
Max SpO2		=>32	98.55	1.64	20
		Total	98.03	2.2	36
	S+KMC	< 32	98.5	1.29	14
		=>32	98.24	1.59	37
		Total	98.31	1.5	51
	Total	< 32	97.9	2.17	30
		=>32	98.35	1.6	57
		Total	98.2	1.82	87
Preparation	Sucrose	< 32	97.38	2.13	16
Max SpO2		=>32	98.2	1.47	20
		Total	97.83	1.81	36
	S+KMC	< 32	98.21	1.37	14
		=>32	98.3	1.49	37
		Total	98.27	1.44	51
	Total	< 32	97.77	1.83	30
		=>32	98.26	1.47	57
		Total	98.09	1.61	87
S30	Sucrose	< 32	97.38	2.5	16
Max SpO2		=>32	97.65	2.06	20
		Total	97.53	2.24	36
	S+KMC	< 32	98.07	1.44	14
		=>32	97.84	1.91	37
		Total	97.9	1.78	51
	Total	< 32	97.7	2.07	30
		=>32	97.77	1.95	57
		Total	97.75	1.98	87
C30	Sucrose	< 32	96.94	4.77	16
Max SpO2		=>32	98.2	2.14	20
		Total	97.64	3.55	36
	S+KMC	< 32	97.64	2.41	14
		=>32	98.27	1.68	37
		Total	98.1	1.9	51
	Total	< 32	97.27	3.81	30
		=>32	98.25	1.83	57
		Total	97.91	2.7	87
R30	Sucrose	< 32	98.5	1.03	16
Max SpO2		=>32	98.3	2.2	20
		Total	98.39	1.76	36
	S+KMC	< 32	98.79	0.97	14
		=>32	97.95	2.28	37
		Total	98.18	2.04	51
	Total	< 32	98.63	1	30
		=>32	98.07	2.24	57
		Total	98.26	1.92	87

			M		SE	N
			95% CI			
		LL		UL		
Total	Sucrose		97.85		0.29	36
		97.28		98.42		
	S+KMC		98.18		0.27	36
		97.65		98.71		

CI= Confidence Interval LL= Lower Limit; UL= Upper Limit

Appendix N - Facial actions across the procedure

Table 1 - Percentage of brow bulge across phases of the procedure by intervention and age group (GA)

Time epoch	Intervention	GA	M	SD	N
Baseline	Sucrose	< 32	1.4	4.14	17
Brow bulge		=>32	0.5	2.37	28
		Total	0.84	3.14	45
	S+KMC	< 32	1.43	4.76	15
		=>32	0.43	2.12	42
		Total	0.69	3.02	57
	Total	< 32	1.41	4.37	32
		=>32	0.46	2.2	70
		Total	0.76	3.06	102
Preparation	Sucrose	< 32	5.05	17.32	17
Brow bulge		=>32	11	20.97	28
		Total	8.75	19.68	45
	S+KMC	< 32	0.88	3.41	15
		=>32	4.02	12.83	42
		Total	3.19	11.2	57
	Total	< 32	3.1	12.83	32
		=>32	6.81	16.79	70
		Total	5.65	15.68	102
S30	Sucrose	< 32	23.22	37.08	17
Brow bulge		=>32	34.92	34.93	28
		Total	30.5	35.8	45
	S+KMC	< 32	6.95	16.39	15
		=>32	20.3	29.74	42
		Total	16.78	27.39	57
	Total	< 32	15.59	29.98	32
		=>32	26.15	32.48	70
		Total	22.84	31.95	102
C30	Sucrose	< 32	11.04	27.42	17
Brow bulge		=>32	6.29	14.03	28
		Total	8.08	19.99	45
	S+KMC	< 32	0.98	3.8	15
		=>32	7.52	21.32	42
		Total	5.8	18.57	57
	Total	< 32	6.32	20.51	32
		=>32	7.03	18.64	70
		Total	6.81	19.14	102
R30	Sucrose	< 32	2.22	4.78	17
Brow bulge		=>32	1.16	3.37	28
		Total	1.56	3.94	45
	S+KMC	< 32	1.42	3.08	15
		=>32	1	3.51	42
		Total	1.11	3.38	57
	Total	< 32	1.85	4.03	32
		=>32	1.06	3.43	70
		Total	1.31	3.63	102

Table 2 - Tests of Within-Subjects Contrasts for brow bulge

Measure: BB

Source	Time	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Powera
Phase	Level 1 vs. Level 2	1,598,860	1	1,598,860	6,874	,010	,066	,738
	Level 2 vs. Level 3	22,441,550	1	22,441,550	31,105	,000	,241	1,000
	Level 3 vs. Level 4	19,174,199	1	19,174,199	24,119	,000	,198	,998
	Level 4 vs. Level 5	2,167,222	1	2,167,222	5,931	,017	,057	,674
Phase * Group	Level 1 vs. Level 2	666,765	1	666,765	2,867	,094	,028	,389
	Level 2 vs. Level 3	2,109,369	1	2,109,369	2,924	,090	,029	,395
	Level 3 vs. Level 4	2,635,055	1	2,635,055	3,315	,072	,033	,438
	Level 4 vs. Level 5	333,623	1	333,623	,913	,342	,009	,157
Phase * GA	Level 1 vs. Level 2	653,254	1	653,254	2,809	,097	,028	,382
	Level 2 vs. Level 3	1,375,813	1	1,375,813	1,907	,170	,019	,277
	Level 3 vs. Level 4	2,922,002	1	2,922,002	3,675	,058	,036	,475
	Level 4 vs. Level 5	58,171	1	58,171	,159	,691	,002	,068
Phase * Group * GA	Level 1 vs. Level 2	39,831	1	39,831	,171	,680	,002	,069
	Level 2 vs. Level 3	107,506	1	107,506	,149	,700	,002	,067
	Level 3 vs. Level 4	501,947	1	501,947	,631	,429	,006	,123
	Level 4 vs. Level 5	612,703	1	612,703	1,677	,198	,017	,250
Error(Phase)	Level 1 vs. Level 2	22,794,439	98	232,596				
	Level 2 vs. Level 3	70,705,300	98	721,483				
	Level 3 vs. Level 4	77,909,613	98	794,996				
	Level 4 vs. Level 5	35,811,767	98	365,426				

Table 3 - Pairwise Comparisons for brow bulge

Measure: BB

(I) Phase		(J) Phase		Mean Difference (I-J)	Std. Error	Sig.a	95% Confidence Interval for Differencea	
							Lower Bound	Upper Bound
dimension1	1	dimension2	2	-4,300	1,640	,101	-9,010	,410
			3	-20,409*	3,241	,000	-29,718	-11,100
			4	-5,519	2,046	,082	-11,394	,357
			5	-,513	,437	1,000	-1,768	,743
	2	dimension2	1	4,300	1,640	,101	-,410	9,010
			3	-16,109*	2,888	,000	-24,404	-7,814
			4	-1,219	2,427	1,000	-8,188	5,750
			5	3,787	1,749	,328	-1,237	8,811
	3	dimension2	1	20,409*	3,241	,000	11,100	29,718
			2	16,109*	2,888	,000	7,814	24,404
			4	14,890*	3,032	,000	6,182	23,598
			5	19,896*	3,378	,000	10,196	29,597
	4	dimension2	1	5,519	2,046	,082	-,357	11,394
			2	1,219	2,427	1,000	-5,750	8,188
			3	-14,890*	3,032	,000	-23,598	-6,182
			5	5,006	2,056	,167	-,898	10,910
	5	dimension2	1	,513	,437	1,000	-,743	1,768
			2	-3,787	1,749	,328	-8,811	1,237
			3	-19,896*	3,378	,000	-29,597	-10,196
			4	-5,006	2,056	,167	-10,910	,898

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

*. The mean difference is significant at the ,05 level.

Table 4 - Percentage of eye squeeze across phases of the procedure by intervention and gestational age group (GA)

Time epoch	Intervention	GA	M	SD	N
Baseline	Sucrose	< 32	1,02	2,81	17
Brow bulge		=>32	1,09	3,72	28
		Total	1,07	3,37	45
	S+KMC	< 32	,90	3,49	15
		=>32	,10	,65	42
		Total	,31	1,86	57
	Total	< 32	,97	3,09	32
		=>32	,50	2,43	70
		Total	,64	2,65	102
Preparation	Sucrose	< 32	6,01	19,26	17
Brow bulge		=>32	9,27	19,52	28
		Total	8,04	19,27	45
	S+KMC	< 32	,63	2,43	15
		=>32	4,40	12,49	42
		Total	3,41	10,89	57
	Total	< 32	3,48	14,19	32
		=>32	6,35	15,73	70
		Total	5,45	15,26	102
S30	Sucrose	< 32	23,74	38,24	17
Brow bulge		=>32	34,31	31,46	28
		Total	30,31	34,15	45
	S+KMC	< 32	5,99	15,41	15
		=>32	19,81	30,18	42
		Total	16,18	27,64	57
	Total	< 32	15,42	30,71	32
		=>32	25,61	31,30	70
		Total	22,41	31,32	102
C30	Sucrose	< 32	6,51	17,85	17
Brow bulge		=>32	5,31	12,37	28
		Total	5,76	14,49	45
	S+KMC	< 32	,95	3,69	15
		=>32	4,94	14,37	42
		Total	3,89	12,56	57
	Total	< 32	3,91	13,36	32
		=>32	5,09	13,51	70
		Total	4,72	13,41	102
R30	Sucrose	< 32	1,71	3,79	17
Brow bulge		=>32	,48	1,66	28
		Total	,94	2,69	45
	S+KMC	< 32	,51	1,96	15
		=>32	,49	1,89	42
		Total	,50	1,89	57
	Total	< 32	1,14	3,08	32
		=>32	,49	1,79	70
		Total	,69	2,28	102

Table 5 - Tests of Within-Subjects Contrasts for eye squeeze

Measure: ES

Source	Phase	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Powera
Phase	Level 1 vs. Level 2	1,595,602	1	1,595,602	7,181	,009	,068	,756
	Level 2 vs. Level 3	21,830,026	1	21,830,026	29,867	,000	,234	1,000
	Level 3 vs. Level 4	23,643,102	1	23,643,102	33,232	,000	,253	1,000
	Level 4 vs. Level 5	1,140,162	1	1,140,162	6,176	,015	,059	,692
Phase * Group	Level 1 vs. Level 2	450,847	1	450,847	2,029	,158	,020	,292
	Level 2 vs. Level 3	2,612,539	1	2,612,539	3,574	,062	,035	,465
	Level 3 vs. Level 4	3,738,976	1	3,738,976	5,255	,024	,051	,622
	Level 4 vs. Level 5	121,690	1	121,690	,659	,419	,007	,127
Phase * GA	Level 1 vs. Level 2	325,778	1	325,778	1,466	,229	,015	,224
	Level 2 vs. Level 3	1,628,878	1	1,628,878	2,229	,139	,022	,315
	Level 3 vs. Level 4	2,524,434	1	2,524,434	3,548	,063	,035	,462
	Level 4 vs. Level 5	87,298	1	87,298	,473	,493	,005	,105
Phase * Group * GA	Level 1 vs. Level 2	10,268	1	10,268	,046	,830	,000	,055
	Level 2 vs. Level 3	40,582	1	40,582	,056	,814	,001	,056
	Level 3 vs. Level 4	20,223	1	20,223	,028	,866	,000	,053
	Level 4 vs. Level 5	85,338	1	85,338	,462	,498	,005	,103
Error (Phase)	Level 1 vs. Level 2	21,776,856	98	222,213				
	Level 2 vs. Level 3	71,627,814	98	730,896				
	Level 3 vs. Level 4	69,721,707	98	711,446				
	Level 4 vs. Level 5	18,093,187	98	184,624				

Table 6 - Pairwise Comparisons for eye squeeze

Measure:ES

(I) phase		(J) phase		Mean Difference (I-J)	Std. Error	Sig.a	95% Confidence Interval for Differencea Lower Bound	Upper Bound
dimension1	1	dimension2	2	-4,295	1,603	,086	-8,899	,308
			3	-20,183*	3,218	,000	-29,427	-10,940
			4	-3,649	1,462	,143	-7,848	,551
			5	-,018	,391	1,000	-1,141	1,106
	2	dimension2	1	4,295	1,603	,086	-,308	8,899
			3	-15,888*	2,907	,000	-24,237	-7,539
			4	,647	2,039	1,000	-5,209	6,502
			5	4,278	1,683	,126	-,555	9,110
	3	dimension2	1	20,183*	3,218	,000	10,940	29,427
			2	15,888*	2,907	,000	7,539	24,237
			4	16,535*	2,868	,000	8,297	24,772
			5	20,166*	3,294	,000	10,705	29,626
	4	dimension2	1	3,649	1,462	,143	-,551	7,848
			2	-,647	2,039	1,000	-6,502	5,209
			3	-16,535*	2,868	,000	-24,772	-8,297
			5	3,631	1,461	,146	-,565	7,827
	5	dimension2	1	,018	,391	1,000	-1,106	1,141
			2	-4,278	1,683	,126	-9,110	,555
			3	-20,166*	3,294	,000	-29,626	-10,705
			4	-3,631	1,461	,146	-7,827	,565

Based on estimated marginal means

[a]. Adjustment for multiple comparisons: Bonferroni.

*. The mean difference is significant at the ,05 level.

Table 7 - Percentage of nasolabial furrow across phases of the procedure by intervention and gestational age group (GA)

Time epoch	Intervention	GA	M	SD	N
Baseline	Sucrose	< 32	1,21	3,50	17
Brow bulge		=>32	,19	,77	28
		Total	,57	2,25	45
	S+KMC	< 32	,27	1,03	15
		=>32	,27	1,27	42
		Total	,27	1,21	57
	Total	< 32	,77	2,65	32
		=>32	,24	1,09	70
		Total	,40	1,74	102
Preparation	Sucrose	< 32	4,48	17,41	17
Brow bulge		=>32	9,32	20,06	28
		Total	7,50	19,04	45
	S+KMC	< 32	5,19	18,19	15
		=>32	6,85	14,88	42
		Total	6,41	15,66	57
	Total	< 32	4,82	17,49	32
		=>32	7,84	17,04	70
		Total	6,89	17,16	102
S30	Sucrose	< 32	20,76	35,06	17
Brow bulge		=>32	25,37	28,85	28
		Total	23,63	31,03	45
	S+KMC	< 32	9,26	23,59	15
		=>32	21,32	32,48	42
		Total	18,15	30,66	57
	Total	< 32	15,37	30,33	32
		=>32	22,94	30,93	70
		Total	20,57	30,79	102
C30	Sucrose	< 32	1,87	4,74	17
Brow bulge		=>32	5,39	14,39	28
		Total	4,06	11,76	45
	S+KMC	< 32	3,57	12,85	15
		=>32	7,66	20,21	42
		Total	6,58	18,54	57
	Total	< 32	2,67	9,32	32
		=>32	6,75	18,03	70
		Total	5,47	15,89	102
R30	Sucrose	< 32	,68	2,81	17
Brow bulge		=>32	,73	2,31	28
		Total	,71	2,48	45
	S+KMC	< 32	,99	3,85	15
		=>32	1,51	6,14	42
		Total	1,37	5,60	57
	Total	< 32	,83	3,29	32
		=>32	1,20	4,96	70
		Total	1,08	4,49	102

Table 8 - Tests of Within-Subjects Contrasts for nasolabial furrow

Measure: NLF

Source	Phase	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Powera
Phase	Level 1 vs. Level 2	3,091,201	1	3,091,201	10,286	,002	,095	,888
	Level 2 vs. Level 3	13,985,295	1	13,985,295	20,632	,000	,174	,994
	Level 3 vs. Level 4	18,319,899	1	18,319,899	25,395	,000	,206	,999
	Level 4 vs. Level 5	1,149,747	1	1,149,747	4,354	,040	,043	,542
Phase * Group	Level 1 vs. Level 2	4,420	1	4,420	,015	,904	,000	,052
	Level 2 vs. Level 3	1,027,992	1	1,027,992	1,517	,221	,015	,230
	Level 3 vs. Level 4	2,060,839	1	2,060,839	2,857	,094	,028	,387
	Level 4 vs. Level 5	44,827	1	44,827	,170	,681	,002	,069
Phase * GA	Level 1 vs. Level 2	305,675	1	305,675	1,017	,316	,010	,170
	Level 2 vs. Level 3	558,944	1	558,944	,825	,366	,008	,147
	Level 3 vs. Level 4	443,398	1	443,398	,615	,435	,006	,121
	Level 4 vs. Level 5	268,522	1	268,522	1,017	,316	,010	,170
Phase * Group * GA	Level 1 vs. Level 2	96,092	1	96,092	,320	,573	,003	,087
	Level 2 vs. Level 3	611,935	1	611,935	,903	,344	,009	,156
	Level 3 vs. Level 4	256,468	1	256,468	,356	,552	,004	,091
	Level 4 vs. Level 5	,050	1	,050	,000	,989	,000	,050
Error (Phase)	Level 1 vs. Level 2	29,452,883	98	300,540				
	Level 2 vs. Level 3	66,429,153	98	677,848				
	Level 3 vs. Level 4	70,696,195	98	721,390				
	Level 4 vs. Level 5	25,876,411	98	264,045				

Table 9 - Pairwise Comparisons for nasolabial furrow

Measure: NLF

(I) Phase		(J) Phase		Mean Difference (I-J)	Std. Error	Sig.a	95% Confidence Interval for Differencea	
							Lower Bound	Upper Bound
dimension1	1	dimension2	2	-5,979*	1,864	,018	-11,333	-,625
			3	-18,695*	3,299	,000	-28,171	-9,220
			4	-4,141	1,723	,181	-9,088	,806
			5	-,495	,518	1,000	-1,981	,992
	2	dimension2	1	5,979*	1,864	,018	,625	11,333
			3	-12,717*	2,800	,000	-20,757	-4,676
			4	1,838	1,906	1,000	-3,637	7,313
			5	5,484*	1,855	,039	,157	10,811
	3	dimension2	1	18,695*	3,299	,000	9,220	28,171
			2	12,717*	2,800	,000	4,676	20,757
			4	14,555*	2,888	,000	6,260	22,850
			5	18,201*	3,311	,000	8,692	27,710
	4	dimension2	1	4,141	1,723	,181	-,806	9,088
			2	-1,838	1,906	1,000	-7,313	3,637
			3	-14,555*	2,888	,000	-22,850	-6,260
			5	3,646	1,747	,395	-1,372	8,665
	5	dimension2	1	,495	,518	1,000	-,992	1,981
			2	-5,484*	1,855	,039	-10,811	-,157
			3	-18,201*	3,311	,000	-27,710	-8,692
			4	-3,646	1,747	,395	-8,665	1,372

Based on estimated marginal means

*. The mean difference is significant at the ,05 level.

[a]. Adjustment for multiple comparisons: Bonferroni.

Appendix O- Maternal anxiety and infants' pain responses

Table 1- Pearson product-moment correlation coefficients (r) between the STAI and PIPP scores in different phases of the procedure, in infants who had S+KMC, and corresponding coefficients of determination (r2) for significant correlations

		PIPP			
	Gestational age	Preparation	Needle Stick	Compression	Rest
STAI	All	*r*= .08	*r*= -.04	*r*= .07	*r*= .11
		p= .55	*p*= .78	*p*= .60	*p*= .44
		n= 60	n= 60	n= 59	n= 57
	< 32 weeks	*r*= -.042	*r*= .-.05	*r*= .13	*r*= .23
		p= .87	*p*= .86	*p*= .61	*p*= .41
		n= **17**	n= **17**	n= **17**	n= **15**
	≥ 32 weeks	*r*= **.12**	*r*= **-.03**	*r*= **.055**	*r*= **.04**
		p= **.45**	*p*= **.83**	*p*= **.74**	*p*= **.78**
		n= **43**	n= **43**	n= **42**	n= **42**

Table 2 - Maternal anxiety and infants' heart rate

	Gestational age	Baseline	Preparation	Needle Stick	Compression	Rest
			Maximum heart rate			
STAI	All	*r*= -.18	*r*= -.20	*r*= -.22	*r*= -.28	*r*= -.35
		p= .19	*p*= .13	*p*= .09	*p*= .03*	*p*= .010*
		n= 57	n= 57	n= 58	n= 58	n= 55
					r^2= .08	r^2= .12
	< 32 weeks	*r*= .10	*r*= .06	*r*= .11	*r*= -.11	*r*= -.18
		p= .72	*p*= .81	*p*= .68	*p*= .68	*p*= .53
		n= 17	n= 17	n= 17	n= 17	n= 15
	≥ 32 weeks	*r*= -.31	*r*= -.32	*r*= -.37	*r*= -.37	*r*= -.48
		p= .06	*p*= .042*	*p*= .02*	*p*= .02*	*p*= .00**
		n= 40	n= 41	n= 41	n= 41	n= 40
			r^2= .10	r^2= .14	r^2= .14	r^2= .23
			Average heart rate			
		Baseline	Preparation	Needle Stick	Compression	Rest
	All	*r*= -.02	*r*= -.13	*r*= -.14	*r*= -.18	*r*= -.23
		p= .86	*p*= .35	*p*= .30	*p*= .19	*p*= .09
		n= 57	n= 57	n= 58	n= 58	n= 55
	< 32 weeks	*r*= .44	*r*= .28	*r*= .27	*r*= .10	*r*= -.01
		p= .08	*p*= .28	*p*= .29	*p*= .69	*p*= .98
		n= 17	n= 17	n= 17	n= 17	n= 15
	≥ 32 weeks	*r*= -.30	*r*= -.34	*r*= -.32	*r*= -.31	*r*= -.43
		p= .06	*p*= .03*	*p*= .04*	*p*= .04*	*p*= .00**
		n= 40	n= 40	n= 41	n= 41	n= 40
			r^2= .12	r^2= .10	r^2= .10	
			Minimum heart rate			
		Baseline	Preparation	Needle Stick	Compression	Rest
	All	*r*= .06	*r*= -.14	*r*= -.02	*r*= -.14	*r*= -.16
		p= .65	*p*= .30	*p*= .90	*p*= .30	*p*= .26
		n= 57	n= 57	n= 58	n= 58	n= 55
	< 32 weeks	*r*= .54	*r*= .36	*r*= .35	*r*= .10	*r*= .17
		p= .03	*p*= .16	*p*= .17	*p*= .71	*p*= .56
		n= 17	n= 17	n= 17	n= 17	n= 15
	≥ 32 weeks	*r*= -.16	*r*= -.37	*r*= -.16	*r*= -.28	*r*= -.38
		p= .31	*p*= .02*	*p*= .32	*p*= .07	*p*= .02*
		n= 40	n= 40	n= 41	n= 41	n= 40
			r^2= .14			r^2= .14

Note: * Significant for α< .05; ** Significant for α< .01

Table 3 - Maternal anxiety and oxygen saturation

	Gestational age	Baseline	Preparation	Needle Stick	Compression	Rest
			Minimum oxygen saturation			
STAI	All	*r*= .04	*r*= .04	*r*= -.01	*r*= -.03	*r*= .01
		p= .76	*p*= .74	*p*= .97	*p*= .81	*p*= .93
		n= 60	n= 58	n= 59	n= 57	n= 53
	< 32 weeks	*r*= -.23	*r*= -.12	*r*= -.26	*r*= -.43	*r*= -.60
		p= .38	*p*= .64	*p*= .31	*p*= .10	*p*= .024*
		n= 17	n= 17	n= 17	n= 16	n= 14
						r^2= .22
	≥ 32 weeks	*r*= .12	*r*= .10	*r*= .12	*r*= .17	*r*= .11
		p= .45	*p*= .55	*p*= .46	*p*= .30	*p*= .53
		n= 41	n= 41	n= 42	n= 41	n= 39
			Average oxygen saturation			
		Baseline	Preparation	Needle Stick	Compression	Rest
	All	*r*= .04	*r*= .09	*r*= -.00	*r*= -.08	*r*= .06
		p= .78	*p*= .52	*p*= .98	*p*= .56	*p*= .67
		n= 58	n= 58	n= 59	n= 57	n= 53
	< 32 weeks	*r*= -.34	*r*= -.19	*r*= -.40	*r*= -.45	*r*= -.57
		p= .18	*p*= .48	*p*= .11	*p*= .08	*p*= .03*
		n= 17	n= 17	n= 17	n= 16	n= 14
						r^2= .33
	≥ 32 weeks	*r*= .16	*r*= .20	*r*= .17	*r*= .15	*r*= .14
		p= .32	*p*= .21	*p*= .30	*p*= .35	*p*= .39
		n= 41	n= 41	n= 42	n= 41	n= 39
			Maximum oxygen saturation			
		Baseline	Preparation	Needle Stick	Compression	Rest
	All	*r*= .00	*r*= .12	*r*= .00	*r*= -.05	*r*= .57
		p= .97	*p*= .37	*p*= .98	*p*= .69	*p*= .69
		n= 58	n= 58	n= 59	n= 57	n= 53
	< 32 weeks	*r*= -.37	*r*= -.15	*r*= -.44	*r*= -.41	*r*= -.48
		p= .14	*p*= .56	*p*= .07	*p*= .12	*p*= .08
		n= 17	n= 17	n= 17	n= 16	n= 14
	≥ 32 weeks	*r*= .15	*r*= .24	*r*= .19	*r*= .18	*r*= .15
		p= .34	*p*= .13	*p*= .24	*p*= .26	*p*= .37
		n= 41	n= 41	n= 42	n= 41	n= 39

Note: * Significant for α< .05

Table 4 - Maternal anxiety and infants' facial actions

	Gestational age	Baseline	Preparation	Needle Stick	Compression	Rest
				Brow bulge		
STAI	All	*r*= -.08	*r*= -.02	*r*= -.08	*r*= -.04	*r*= .01
		p= .53	*p*= .90	*p*= .54	*p*= .74	*p*= .93
		n= 60	n= 60	n= 60	n= 60	n= 57
	< 32 weeks	*r*= -.24	*r*= .02	*r*= -.12	*r*= -.15	*r*= -.09
		p= .93	*p*= .95	*p*= .65	*p*= .56	*p*= .76
		n= 17	n= 17	n= 17	n= 17	n= 15
	≥ 32 weeks	*r*= -.26	*r*= -.04	*r*= -.06	*r*= -.03	*r*= -.41
		p= .10	*p*= .80	*p*= .71	*p*= .83	*p*= .80
		n= 43	n= 43	n= 43	n= 43	n= 42
				Eye squeeze		
		Baseline	Preparation	Needle Stick	Compression	Rest
	All	*r*= -.12	*r*= -.02	*r*= -.11	*r*= -.06	*r*= .06
		p= .35	*p*= .87	*p*= .41	*p*= .68	*p*= .67
		n= 60	n= 60	n= 60	n= 60	n= 57
	< 32 weeks	*r*= -.15	*r*= -.21	*r*= -.18	*r*= -.15	*r*= -.11
		p= .56	*p*= .41	*p*= .49	*p*= .56	*p*= .69
		n= 17	n= 17	n= 17	n= 17	n= 15
	≥ 32 weeks	*r*= -.18	*r*= .06	*r*= -.09	*r*= -.05	*r*= .1
		p= .26	*p*= .70	*p*= .59	*p*= .77	*p*= .41
		n= 43	n= 43	n= 43	n= 43	n= 42
				Nasolabial furrow		
		Baseline	Preparation	Needle Stick	Compression	Rest
	All	*r*= .01	*r*= -.10	*r*= -.10	*r*= -.10	*r*= -.06
		p= .95	*p*= .43	*p*= .45	*p*= .44	*p*= .68
		n= 60	n= 60	n= 60	n= 60	n= 57
	< 32 weeks	*r*= -.35	*r*= -.14	*r*= -.26	*r*= -.21	*r*= -.19
		p= .18	*p*= .59	*p*= .32	*p*= .42	*p*= .50
		n= 17	n= 17	n= 17	n= 17	n= 15
	≥ 32 weeks	*r*= .14	*r*= -.08	*r*= -.04	*r*= -.07	*r*= -.02
		p= .37	*p*= .61	*p*= .80	*p*= .66	*p*= .91
		n= 43	n= 43	n= 43	n= 43	n= 42

Table 5 - Maternal anxiety and infants' behavioral state

			Behavioral state				
	Gestational age		Baseline	Preparation	Needle Stick	Compression	Rest
STAI	All	Awake	*M*= 34.00	*M*= 36.00	*M*= 36.56	*M*= 35.00	*M*= 38.33
			SD= 14.49	*SD*= 11.02	*SD*= 7.75	*SD*= 9.35	*SD*= 14.47
		Asleep	*M*=38.05	*M*= 38.02	*M*= 38.31	*M*= 38.27	*M*= 37.93
			SD= 8.77	*SD*= 8.95	*SD*= 9.71	*SD*= 10.00	*SD*= 8.93
		Student t test	t(58)= -.86	t(58)= -.55	t(58)= -.68	t(58)= -99	t(55)= .08
			p= .40	*p*= .59	*p*= .50	*p*= .33	*p*= .94
	< 32 weeks	Awake	*M*= 38.50	*M*= 40.67	*M*= 37.75	*M*= 39.50	*M*= 42.00
			SD= 23.33	*SD*= 16.92	*SD*= 14.10	*SD*= 21.92	*SD*= 18.38
		Asleep	*M*= 38.47	*M*= 38.00	*M*= 38.69	*M*= 38.33	*M*= 38.92
			SD= 9.75	*SD*= 9.94	*SD*= 10.31	*SD*= 9.97	*SD*= 10.10
		Student t test	t(1.1)= .00 *	t(15)= .38	t(15)= -.15	t(15)= .14	t(13)= .37
			p= .99	*p*= .71	*p*= .89	*p*= .89	*p*= .72
	≥ 32 weeks	Awake	*M*= 29.50	*M*= 32.50	*M*= 36.21	*M*= 33.71	*M*= 31
			SD= 2.12	*SD*= 3.70	*SD*= 5.66	*SD*= 5.28	*SD*= -
		Asleep	*M*= 37.90	*M*= 38.03	*M*= 38.14	*M*= 38.25	*M*= 37.61
			SD= 8.51	*SD*= 8.71	*SD*= 9.60	*SD*= 8.86	*SD*= 8.64
		Student t test	t(41)= -1.40	t(41)= -1.25	t(39)= -.82*	t(41)= -1.30	t(40)= -.76
			p= .18	*p*= .22	*p*= .42	*p*= .20	*p*= .45

Note: * Significant for α< .05; ** Significant for α< .01

Table 6 - Maternal anxiety and indices of heart rate variability

	Gestational age	Manipulation phase	Rest phase
		Low frequency	
STAI	All	*r*= -.01	*r*= .04
		p= .93	*p*= .82
		n= 55	n= 44
		High frequency	
	All	*r*= .08	*r*= .25
		p= .58	*p*= .10
		n= 53	n= 44
		LF/HF ratio	
	All	*r*= .10	*r*= -.07
		p= .45	*p*= .65
		n= 55	n= 44