KANGAROO CARE FOR ANALGESIA IN PRETERM INFANTS UNDERGOING HEEL STICK PAIN

by

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Table of Contents

Table of Contents (Continued)

List of Tables

List of Tables (Continued)

List of Figures

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Kangaroo Care for Analgesia in Preterm Infants Undergoing Heel Stick Pain

Abstract

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Heelstick is the most common painful procedure in hospitalized premature infants and may be associated with impaired physiological, neurologic and immunologic functioning. The purpose was to examine Kangaroo Care (KC) effect on heelstick pain as measured by the Premature Infant Pain Profile (PIPP) and heart rate variability (HRV) indices.

Twenty-eight preterm infants 30-32 weeks gestational age were tested in a cross-over design. Fourteen were randomized to group A (incubator heelstick [IH] on day1; KC heelstick [KCH] on day2) and 14 to group B (KCH on day1; IH on day2). The first 18 were tested in an 80-minute protocol and the last 10 in a 30-minute protocol.

During the Heel Stick phase, in the 30-min protocol subgroup, PIPP scores were lower in KCH (range 4.00-10.60) than IH (range 9.75-14.33) and the difference between KCH and IH were > 2 points (considered clinically important). Repeated-measures analysis showed that PIPP was lower in KCH than IH at 0.5 minute after the stick in the Total Sample, $p < .05$. Infants had an average of 27% less pain in KCH than IH and KC had a large effect on reducing pain.

During the Recovery phase**,** KCH infants had less pain at three time points ($p < .05$-$.001$) in the 30-minute protocol subgroup and at 2 time points ($p < .05$) in the Total

Sample. Pain was 21%-25% less in KCH than IH, and KC had medium effect on reducing pain.

In KCH compared to IH, low frequency (LF, sympathetic activity) and high frequency power (HF, parasympathetic activity) were higher during Baseline ($p < .05$-.01); LF was higher during Heel Stick ($p < .001$); and LF/HF ratio (balance of sympathetic-parasympathetic activity) was lower during Recovery ($p < .001$). Infants had better balanced autonomic activity in KCH than IH during their response to heelstick.

Infants also had a shorter duration of bradycardia, higher SaO_2, less decrement in SaO_2 from Baseline to Heel Stick, and shorter or no oxygen desaturation in KCH than IH. In conclusion, KC reduced bio-behavioral and autonomic pain responses in preterm infants undergoing heelstick pain during the first 2-9 days of life.

CAPTER ONE

Background and Significance

The survival of premature infants has risen steadily over the past decade (Alexander et al., 2003). During their neonatal intensive care unit (NICU) stay, infants are subjected to numerous invasive procedures as part of their care (D. P. Barker & Rutter, 1995; Evans, McCartney, Lawhon, & Galloway, 2005; Johnston, Collinge, Henderson, & Anand, 1997; Simons et al., 2003; Stevens et al., 1999; Stevens et al., 2003). That newborns, full-term or preterm, can detect, process, and respond to painful stimuli is now well established (Anand, 1998a; Anand & Carr, 1989; Anand & Hickey, 1987). Further, the preterm infant is hypersensitive to pain and is at even greater risk for pain because of immature mechanisms at birth to inhibit or dampen nociception (Fitzgerald & Beggs, 2001). Excessive and/or prolonged unrelieved pain in the infant causes adverse physiological effects in all major organ systems that can be life threatening and have long-term cumulative effects (Anand, 1998b; Grunau et al., 2005; Holsti, Grunau, Oberlander, & Whitfield, 2004; Mooncey, Giannakoulopoulos, Glover, Acolet, & Modi, 1997; Taddio, Shah, Gilbert-MacLeod, & Katz, 2002).

However, current interventions to alleviate preterm infant pain are inadequate and inconsistently used. A study even found that morphine given as a loading dose followed by continuous intravenous infusions does not appear to provide adequate analgesia for the acute pain caused by invasive procedures among ventilated preterm infants, despite its routine use in the NICU (Carbajal et al., 2005). Heel stick is the most common painful invasive procedure in the premature infant, and 40% to 90% of infants do not receive any preventive and/or effective treatment (Choonara, 1992; Lago et al., 2005; F. L. Porter &

Anand, 1998; Sabrine & Sinha, 2000; Stevens et al., 2003; Stevens, Taddio, Ohlsson, & Einarson, 1997; Walco, Cassidy, & Schechter, 1994).

To discover new and creative approaches to address the challenge of infant pain is a primary nursing focus (Halimaa, 2003). Non-pharmacological interventions are valuable alternatives for pain relief during minor procedures in neonates. Kangaroo Care (KC), also called skin-to-skin contact, has received favorable recognition as a promising infant care technique to improve the infants' physiological stability (Affonso, Wahlberg, & Persson, 1989; Charpak, Ruiz et al., 2005; Ludington-Hoe, Anderson, Swinth, Thompson, & Hadeed, 2004; Ludington-Hoe, Nguyen, Swinth, & Satyshur, 2000; Ludington-Hoe & Swinth, 1996). Although a few studies showed that KC reduced infants' pain responses (Gray, Miller, Philipp, & Blass, 2002; Gray, Watt, & Blass, 2000; Johnston et al., 2003; Kostandy, 2005; Ludington-Hoe, Hosseini, & Torowicz, 2005), additional studies are needed to confirm the effects of KC as a behavioral analgesic for preterm infants as indicated by multidimensional behavioral and physiological measures. The purpose of this cross-over quasi-experimental design study was to determine if KC blunts behavioral and physiological responses to heel stick pain better than the standard method wherein the infant of remains in an incubator for the heel stick. Chapter One includes the statement of problem, background to the problem, description of the KC intervention, the conceptual framework, assumptions, research questions, and hypotheses.

Statement of Problem

Premature infants in the high-tech isolation of the neonatal intensive care unit suffer from numerous painful invasive procedures, discomforting interventions, and a developmentally inappropriate intensive care environment throughout each day and over

a prolonged period – often without their mothers there to protect and soothe them. In several studies, preterm neonates had a mean of 10 to 15 painful procedures per day during their first two days of life; heel sticks and suctioning were the most common types of pain experienced in the NICU, and were rarely treated (< 2%) (D. P. Barker & Rutter, 1995; Evans et al., 2005; F. L. Porter & Anand, 1998; Simons et al., 2003; Stevens et al., 2003). One 23-week gestational age infant (birth weight 560 g) underwent 488 procedures during the NICU stay (D. P. Barker & Rutter, 1995). A sample of 239 Canadian infants in one NICU had a total of 2134 invasive procedures over the one-week study period, and medication was given for only 0.8% of the procedures (Johnston, Collinge et al., 1997). Another study found that during hospitalization (of weeks or months in length) preterm infants (< 28 weeks gestation) endured more than 700 multiple invasive procedures that would be considered painful by adults (F. L. Porter, Wolf, & Miller, 1999). However, 80% of infants do not have effective pain relief from these procedures (Carbajal et al., 2005; Choonara, 1992, 1999; Dodds, 2003; Walco et al., 1994). Preventing and eliminating pain in neonates has been a particular problem.

Background

Perception of Pain in Infants

Historically, newborn infants were believed to be decorticate beings without the capacity to feel or be affected by pain like adults. Infants were considered comparable to a semi-anesthetized adult (Swafford, 1968). Many surgical procedures, including circumcision, have been routinely performed on infants younger than three months without anesthesia or analgesia (Franck, 1987). As recently as a decade ago, common myths and assumptions about pain in infants prevailed. Infants were considered to be

neurologically immature, not able to perceive or locate pain, have no memory of pain, and infant pain was considered temporary (Burrows & Berde, 1993; McCready, MacDavitt, & O'Sullivan, 1991; Phillips, 1995; Purcell-Jones, Dormon, & Sumner, 1988; Sredl, 2003). Both physicians and nurses were reluctant to use anesthetic and analgesic agents in very young infants because they believed that narcotics could not be given safely to them (F. L. Porter, Wolf, Gold, Lotsoff, & Miller, 1997). Specific information on the experience of pain in newborns has been also notably absent in the literature. In standard pediatric and neonatology texts, the concept of infant pain had not even been mentioned in texts prior to 1998 (Cote, Morse, & James, 1991; Ferrell, Virani, Grant, Vallerand, & McCaffery, 2000; Phillips, 1995). Pain in neonates was a neglected subject in the research literature until the mid-1980s, but the number of articles published on this topic has increased sharply since 1985 (Banos, Ruiz, & Guardiola, 2001).

Our knowledge of pain in neonates has increased dramatically in the past two decades. Supported by an impressive body of neuroanatomical, neurochemical, and biobehavioral evidence, term and preterm infants possess the ability to perceive and respond to pain and remember pain experiences (Anand, 2000a; Anand & Carr, 1989; Anand & Hickey, 1987; Evans et al., 2005; Fitzgerald & Beggs, 2001; Holsti, Grunau, Oberlander, & Whitfield, 2005). Premature infants are more hypersensitive to nociceptive stimuli compared to full-term infants because immature sensory processing within the spinal cord leads to lower thresholds for excitation and sensitization, thereby potentially maximizing the central effects of tissue-damaging inputs (Taddio et al., 2002).

Short and long term effects of infant pain. Painful invasive procedures may cause major physiological disturbances and many have long-term cumulative effects. The pain

that premature infants experience is associated with increased heart rate, blood pressure, and oxygen consumption, all of which cause marked fluctuation in intracranial pressure, possibly leading to intraventricular hemorrhage (IVH) and periventricular leukomalacia (Anand, 1998b; Scanlon, 1991). Pain triggers increased stress hormone secretion which impairs growth and tissue repair (Modi & Glover, 1998; Mooncey et al., 1997; Morelius, Theodorsson, & Nelson, 2005). Pain is also neurotoxic to hippocampal formation, and may have specific adverse effects on cognition, memory and behavior (Anand & Scalzo, 2000; Holsti et al., 2005). Early painful experiences in infancy can lead to prolonged structural and functional alteration in pain pathways that can last into adult life (Fitzgerald & Beggs, 2001), permanently altering normal or common responses to pain.

Interventions for infant pain. Interventions to relieve pain in prematures exist, but each has limitations (see Chapter Two). Pharmacologic interventions are often not used because doctors and nurses persist in the belief that they are unnecessary or dangerous for preterm infants (Dodds, 2003; Franck, 1997). One study showed that morphine does not provide adequate analgesia for acute procedural pain among preterm infants (Carbajal et al., 2005). Non-pharmacological or noninvasive techniques such as sweet-tasting substances, breast milk, non-nutritive sucking (pacifier), sugar coated pacifiers, swaddling, rocking, facilitated tucking, and KC have been shown to be effective in soothing infants undergoing painful procedures (Corff, Seideman, Venkataraman, Lutes, & Yates, 1995; Gray et al., 2002; Gray et al., 2000; Greenberg, 2002; Johnston et al., 2003; Johnston, Stremler, Stevens, & Horton, 1997; Kostandy, 2005; Ludington-Hoe et al., 2005; Mitchell & Waltman, 2003; Rush et al., 2005; Upadhyay et al., 2004). However, only sucrose (Stevens & Ohlsson, 2000) and non-nutritive sucking on pacifiers

(Pinelli, Symington, & Ciliska, 2002), both of which are effective in reducing the crying time in response to heel stick in premature infants, have received adequate attention. Even these two methods are not practiced routinely because questions still remain about implementation. The optimal dose, intensity, frequency and duration of these treatments have not been clarified (Stevens, Yamada, & Ohlsson, 2001), nor have side effects been clearly identified. Mothers of infants in the NICU are unhappy with pain management and wish to participate in comforting their infants (Franck, Cox, Allen, & Winter, 2004).

In summary, the problem being investigated is that existing methods to blunt premature infant pain responses are ineffective, under-used, and have been inadequately studied (Anand, 2001; Carbajal et al., 2005; Franck, 2002; Franck & Gilbert, 2002; Franck & Lefrak, 2001), resulting in persistence of harmful behavioral, physiological and hormonal reactions (Anand, 2000; Grunau, 2000) as infants are persistently exposed to far too much pain that is preventable and treatable (Franck, 2002; Porter & Anand, 1998). According to clinical (Anand, 1998a, 2000a, 2000b, 2001; Anand & Scalzo, 2000), ethical (Franck, 1997, 2001), and policy statements (AHCPR, 1992; American Academy of Pediatrics & American Pain Society, 2001; American Academy of Pediatrics & Canadian Paediatric Society, 2000; Joint Commission on Accreditation of Healthcare Organization, 2001; National Association of Neonatal Nurses, 2000), finding optimal non-invasive and non-pharmacological techniques to reduce neonatal, especially the premature infant pain is an important topic and challenge for neonatal caregivers.

Kangaroo Care (Skin-to-Skin Contact)

KC, or skin-to-skin contact, is the upright prone positioning of the diaper-clad infant skin-to-skin, chest-to-chest between maternal breasts. KC was developed and

initiated in Bogota, Colombia in the late 1970s and was first reported in 1983 by pediatricians Rey and Martinez (Ludington, Anderson, Swinth, Thompson, & Hadeed, 1994). Rey and Martinez advocated using mothers' bodies to correct overcrowding, cross-infection, high mortality and cold stress for LBW infants, resembling the way marsupials mother their young (Anderson, 1989b). Mothers' bodies can be used to resemble marsupial caregiving because infants are placed between breasts in the pouch-like valley between the mammary mounds. During 1980s and 1990s, the KC method spread to industrialized countries, where parents and infants used the KC position for part of the day to promote bonding and attachment (Affonso, Bosque, Wahlberg, & Brady, 1993; Anderson, 1989b; Charpak, Ruiz-Pelaez, Figueroa de, & Charpak, 2001; Delval, 1998; Feldman, Weller, Sirota, & Eidelman, 2003; Johnston et al., 2003; Ludington-Hoe, 2003; Ludington-Hoe & Swinth, 1996; Tessier et al., 1998). In 1996, the International Network of KC (INK) was established at the Workshop on Kangaroo Mother Care for Low Birthweight Infants in Italy (Cattaneo, Davanzo, Bergman, & Charpak, 1998). An explicit and comprehensive definition of KC was developed. KC was defined as early, prolonged and continuous skin-to-skin contact between a mother and her newborn low-birth-weight infant, both in hospital and after early discharge, until at least the 40th week of post-conceptual age, with exclusive breastfeeding and proper follow-up (Cattaneo, Davanzo, Bergman et al., 1998).

KC has been studied in depth since 1983, and most studies have shown that KC has a major, positive impact on preterm infants and their parents. More than 400 English and foreign language reports (Ludington, 2004) have demonstrated that KC is an

effective intervention to improve physiological and behavioral outcomes in preterm and full-term neonates in the first few days, as well as in the later years of life.

Physiological effects of KC. KC has a stabilizing effect on the respiratory and circulatory systems and improves physiological functions. During contact, infants have more stable heart rate and decreased apnea and bradycardia (Clifford & Barnsteiner, 2001; Ludington-Hoe et al., 2004; Ludington-Hoe, Hadeed, & Anderson, 1991; Mazurek et al., 1999; Messmer et al., 1997), improved oxygenation (Acolet, Sleath, & Whitelaw, 1989; Fohe, Kropf, & Avenarius, 2000; Gazzolo, Masetti, & Meli, 2000; Hadeed, Ludington, & Siegal, 1995; Ludington-Hoe, Thompson, Swinth, Hadeed, & Anderson, 1994; Messmer et al., 1997), stable body temperature (J. Bauer, Sontheimer, Fischer, & Linderkamp, 1996; K. Bauer et al., 1997; Chiu, Anderson, & Burkhammer, 2005; Chwo et al., 2002; Fohe et al., 2000; Ibe et al., 2004; Ludington, Morgan, & Cong, 2003; Ludington-Hoe et al., 2004; Ludington-Hoe et al., 1991; Ludington-Hoe et al., 2000; Mazurek et al., 1999), improved weight gain (Lima, Quintero-Romero, & Cattaneo, 2000; McMaster & Vince, 2000; Ramanathan, Paul, Deorari, Taneja, & George, 2001), and no increase in infections (Charpak, Ruiz-Pelaez, & Charpak, 1994; Charpak, Ruiz-Pelaez, Figueroa de, & Charpak, 1997; Charpak et al., 2001; Conde-Agudelo, Diaz-Rossello, & Belizan, 2003). KC infants have been discharged earlier from the hospital, suggesting a positive KC effect on physiological maturation (Brown & Heermann, 1997; Conde-Agudelo et al., 2003; Ludington-Hoe et al., 1999; McMaster & Vince, 2000; Ramanathan et al., 2001; Van Rooyen, Pullen, Pattinson, & Delport, 2002; Wahlberg, Affonso, & Persson, 1992; Whitelaw & Liestol, 1994; Worku & Kassie, 2005). KC (8 – 24 hours per day) was also associated with greater head growth, even after controlling for

head circumference at birth (Charpak et al., 2001; Charpak, Ruiz-Pelaez, & Figueroa, 2005; Rojas et al., 2003). Some studies showed negative effects on heart rate and respiratory rate which might be related to heat stress caused by increasing infant's body temperature during KC (Bohnhorst, Gill, Dordelmann, Peter, & Poets, 2004; Bohnhorst, Heyne, Peter, & Poets, 2001).

Behavioral effects of KC. KC affects behavioral state in the neonatal period. KC infants spend increasing time in quiet sleep (Chwo et al., 2002; Feldman & Eidelman, 2003; Lai et al., 2005; Ludington, 1990; Ludington-Hoe et al., 1999; Ludington-Hoe et al., 1994; Messmer et al., 1997) and alert wakefulness states (Chwo et al., 2002; Feldman & Eidelman, 2003), while reducing transitory states and active sleep (Chwo et al., 2002; Feldman & Eidelman, 2003). Improved organization of sleep-wake cyclicity has also been found (Feldman & Eidelman, 2003; Feldman, Weller, Sirota, & Eidelman, 2002). Infants in KC position cry less than infants in incubators (Chwo et al., 2002; Gray et al., 2000; Ludington-Hoe, Hashemi, Argote, Medellin, & Rey, 1992; Ludington-Hoe et al., 2005; Mazurek et al., 1999; Michelsson, Christensson, Rothganger, & Winberg, 1996; Whitelaw, Heisterkamp, Sleath, Acolet, & Richards, 1988) and spectrographic cry analysis has shown that KC infant cries are less distressful (Michelsson et al., 1996). KC also improves breastfeeding (Dombrowski, Anderson, Santori, & Burkhammer, 2001; Mizuno, Mizuno, Shinohara, & Noda, 2004; Shiau & Andersen, 1997). Improved motor and neurobehavioral development have also been reported as a result of KC in randomized control trials (Feldman, Eidelman, Sirota, & Weller, 2002; Ohgi et al., 2002; Tessier et al., 1998).

Long-term developmental effects: KC has positive effects on the parent-infant relationship. KC increases intimacy and attachment between infant and parent (Affonso et al., 1993; Feldman, Eidelman et al., 2002; Feldman et al., 2003; Roller, 2005; Sontheimer, Fischer, & Buch, 2004; Tessier et al., 1998). For parents, KC appears to provide them with a satisfying role in the care of their small infants (K. Bauer, Uhrig, & Versmold, 1999; Neu, 2004), and many feel more confident in caring for their infants (Affonso et al., 1989; Feldman et al., 2003; Roller, 2005; Tessier et al., 1998).

KC in the NICU has a significant positive impact on infants' physiological, emotional, and cognitive behavior over the first year of life. At three months age, KC infants had higher thresholds to negative emotionality and more efficient arousal modulation while attending to increasingly complex stimuli (Feldman, Weller et al., 2002; Feldman et al., 2003). At six months age, infants had more positive mood (Ohgi et al., 2002), had longer duration of and shorter latencies to mother-infant shared attention and sustained exploration in a toy session (Feldman, Weller et al., 2002), and scored higher on the Bayley Mental Developmental Index and the Psychomotor Developmental Index (Feldman, Eidelman et al., 2002). At 12 months age, KC infants had significantly higher Bayley Mental Developmental Index scores, suggesting that KC has favorable effects on mental and psychomotor development and the effects extend over the first year of life (Ohgi et al., 2002). Another study showed that at 12 months age, KC (24 hours/day until 37-38 weeks PCA) infants had higher IQ (Tessier et al., 2003). The more premature the infant (30-32 weeks) and the sicker, and for those with diagnosed abnormal or doubtful neurological development at 6 months age, the higher the significance in IQ difference (Tessier et al., 2003). The considerable economic value of KC is confirmed as

an important advantage of KC. At least in developing countries, KC costs 50% less than conventional care (Cattaneo, Davanzo, Worku et al., 1998; Lima et al., 2000).

Cochrane reviews. In a Cochrane review of clinical trials on full-term infant outcomes of KC (Anderson, Moore, Hepworth, & Bergman, 2003), early KC (during the first 24 hours post-birth) appeared to have clinical benefit, especially regarding breastfeeding and infant crying outcomes, but has no apparent short or long-term negative effects. In a Cochrane review with preterm infants, KC was associated with a reduction in nosocomial infections, severe illness, and respiratory disease at six months follow-up (Conde-Agudelo et al., 2003), but concluded that there is insufficient evidence to recommend its routine use in the LBW infants. In a review of the effect of KC on successful breastfeeding, Carfoot and colleagues (2003) reported that methodological flaws with the included studies prohibited firm conclusions being reached with regard to the effect of KC on "successful" breastfeeding, and they highlighted the need for further primary research to assess the effect of KC on breastfeeding experience.

In summary, KC with preterm infants has been shown to improve or maintain cardiorespiratory stability, oxygen and energy expenditure, behavioral and cognitive maturation and development, and parent-infant bonding. According to Cochrane reviews, KC seems to reduce severe infant morbidity. Because of these promising outcomes, KC is considered as a safe, effective, and inexpensive intervention in preterm infants. However, well-designed randomized controlled trials of KC are still needed.

Several reports have shown that KC has a powerful effect on reducing pain responses during heel stick in full-term infants (Gray et al., 2002; Gray et al., 2000) and preterm infants (Johnston et al., 2003; Ludington-Hoe et al., 2005). Further studies are

needed to provide enough evidence for application in the NICU to allow more parental involvement in comforting their infants during pain. Gaps this project will fill: (1) there is not enough research on whether KC effectively reduces pain in preterm infants; and (2) no study has tested heart rate variability (HRV) as an indicator of pain in response to KC.

Conceptual Framework

The conceptual framework for this study incorporates Levine's Conservation Model (M. E. Levine, 1967, 1969), the gate control theory of pain (Melzack & Wall, 1965), and the neuromatrix theory of pain (Melzack, 2001). These theories have been chosen because they prescribe and explain how Kangaroo Care is an energy conservation activity and a pain management strategy that can relieve infant procedural pain and help the infant adapt to a painful experience.

Levine's Conservation Model

Levine's Principle of Conservation of Energy is one of the nursing models that has directed the study. The unique focus of the Conservation Model is the conservation or "keeping together" of the person's wholeness, wellness or health. Conservation describes the way complex systems are able to continue to function even when severely challenged. The systems sustain themselves, not only in the face of immediate disruptive threats, but in such a way as to assure the vitality of future responses, work that is accomplished in the most economic way possible (M. E. Levine, 1990). A basic assumption of the Conservation Model is that nursing interventions are conservation or guardian activities that defend and protect wholeness, which is the universal target and major guideline of the nursing practice (M. E. Levine, 1973, 1991). The source of threats to the person's wholeness or integrity is environmental challenge (M. E. Levine, 1973,

1996), for example, repeated heel sticks in preterm infants. The nurse's responsibility is to conserve the patient's energy, and structural, personal, and social integrity.

The Principle of Conservation of Energy refers to balancing energy output and energy input to avoid excessive fatigue and promoting adequate rest, nutrition, and exercise, which is a natural law found everywhere in the universe for all animate and inanimate entities. The sources of energy available to the person are finite, and conservation of energy assures that energy is spent carefully with essential priorities served first (M. E. Levine, 1991). For newborn infants, especially preterm infants, the priority for the energy supply is growth and maturation (Ludington, 1990). However, painful procedures are environmental challenges for preterm infants in NICU and they disturb the energy balance. Pain responses include crying, facial activity, body movement, increased heart and respiratory rates, and hormonal and metabolic changes – all of which are costly energy expenses. KC may be a conservation strategy reducing pain responses, and in turn, reducing energy expenditure, conserving the infant's limited available energy sources and supporting growth and development (Ludington, 1990).

Gate Control Theory of Pain

Early theories, such as the specificity theory (Descarte, 1664/1972), intensity theory (Darwin, 1794; Urb, 1874), pattern theory (Goldschneider, 1886; Livigston, 1943) and affect theory (Marshall, 1894; (cited in McCready et al., 1991; Melzack & Wall, 1970; Stevens & Johnston, 1993) have contributed to the global understanding of pain. However, none has been adequate in providing a general theory of pain to describe all types of pain or pain experience. In an attempt to compensate for the limitations of these theories and to provide a more general framework for pain, Melzack and Wall introduced

the gate control theory (GCT) (Melzack & Wall, 1965). The GCT suggested that at the spinal cord level there is a "gate", and pain is determined by the interactions among three systems: (1) the cells of the substantia gelatinosa (SG) in the dorsal horn, which function as a gate control mechanism influenced by the relative amount of activity in large-diameter and small-diameter fibers; (2) the dorsal column fibers as a central control trigger which activate selective brain processes and then through descending fibers modulate the gate control system; and (3) the central transmission (T) cells in the dorsal horn modulated by the gate mechanism, which activate neural mechanisms that are responsible for perception and response to pain. Therefore, the dorsal horns are not merely passive transmission stations but sites at which dynamic activities occur. The dynamic activities are inhibition, excitation and modulation. The GCT provides a framework for examining the interaction between local and distant excitatory and inhibitory systems in the dorsal horn and brain (Dickenson, 2002).

Tissue damage, such as incurred by heel stick in infants, activates distinct receptors called nociceptors, which are at the terminations of free nerve endings that respond to intense stimuli. Impulses in small diameter nerve fibers (myelinated A-delta and unmyelinated C fibers) tend to open the gate (facilitate transmission of pain), and impulses traveling along large fibers (Aβ fibers) tend to close it (depress transmission of pain) (Kandel, Schwartz, & Jessell, 2000; Melzack & Wall, 1965). The output of the T cells is determined by the afferent nociceptive impulses, the afferent modulator acting on the gating mechanism, and by impulses descending from the brain. Therefore, it is postulated that KC, by providing full body contact and other sensory stimulation concurrently with the heel stick, may activate a non-nociceptive tactile nerve impulse

(large nerve fibers), closing the gate in the dorsal horn and inhibiting nociceptive transmission, and thus, reducing infant pain experience and responses.

Neuromatrix Theory of Pain

Melzack posited that the gate control theory did not incorporate the long-term central nervous system changes in response to noxious input, nor did it include other external factors that may modulate the experience of pain (Loeser & Melzack, 1999). A new theory – the neuromatrix theory of pain, developed by Melzack, is based on the GCT (1965) and has been recently supported by considerable updated evidence from neuroscience and functional imaging studies (Derbyshire, 2000). Functional imaging techniques have demonstrated that there is no single pain center. Instead, multiple central regions are involved during the experience of pain, including the anterior cingulate cortex, insular cortex, thalamus, and the sensorimotor cortex (Derbyshire, 2000; Melzack, 2001). The neuromatrix theory proposes that pain is a multidimensional output produced by a widely distributed neural network – the "*body-self neuromatrix*" in the brain. The neuromatrix theory is composed of four concepts: (1) The *neuromatrix* is a large, widespread network of neurons that consists of loops between the thalamus and cortex as well as between the cortex and limbic system, whose spatial distribution and synaptic links are initially determined genetically and are later sculpted by sensory inputs (Melzack, 1999b). The neuromatrix can be labeled as neuromodules specialized to process information related to major sensory events such as injury and other stimulation; (2) The *neurosignature*, which is a continuous outflow from the neuromatrix; (3) The *sentient neural hub,* which converts or transduces the outflow of neurosignature into a continually changing stream of awareness; and (4) Neurosignature patterns may also

activate neural networks to produce the *pattern of movement* to bring about the desired goal, such as the infant retracting his/her foot from the heel stick (Melzack, 2001).

Melzack posited that the neurosignature of pain experience is determined by the synaptic architecture of the neuromatrix, which is produced by genetic and sensory influences, and modulated by sensory inputs (Melzack, 1999a). The neuromatrix theory presents the concept of pain as a multidimensional experience produced by multiple influences, not just an unpleasant sensory and emotional experience. Pain is produced by the output of a widely distributed neural network **in the brain** rather than directly by sensory-evoked injury, inflammation, or other tissue pathology. The areas of the brain involved in pain experience and behavior are very extensive, including the limbic system, cognitive processes and the somatosensory, visual, and vestibular mechanisms. The pain response is determined by many factors, such as context, company, competitive stimuli, and meaning (Moseley, 2003). During infant heel stick, providing a cluster of stimuli in KC may modulate the pain stimuli and thus reduce pain responses.

The neuromatrx theory proposes that injury does not merely produce pain, but also disrupts the brain's homeostatic regulation systems, thereby producing "stress" and initiating complex programs to reinstate homeostasis. The disruption of homeostasis by injury activates neural, hormonal, and behavioral programs aimed at resumption of homeostasis. The theory posits that the central nervous system is an active system that filters, selects and modulates inputs – inhibition, excitation, and modulation.

The neuromatrix theory of pain provides a new conceptual framework for examining pain management. During KC, the mother's skin-to-skin contact with her preterm infant provides multi-sensory stimulation (emotional, tactile, proprioceptive,

vestibular, olfactory, auditory, visual, and thermal stimulation) in a unique interactive style. When the infant undergoes a heel stick, KC and its multi-sensory inputs may act on the pain matrix programs and modulate and inhibit pain perception, and contribute to the outflowing neurosignature in such a way that pain responses are minimized. Recent studies have shown that the activation of C tactile afferents by light touch produces a faint sensation of pleasant touch; thus, the multimodal function of tactile stimulation of neurons continues to be explored. Functional magnetic resonance imaging (fMRI) analysis during C fiber stimulation showed activation of the insular region, but not of somatosensory areas (Olausson et al., 2002; Wessberg, Olausson, Fernstrom, & Vallbo, 2003). C tactile fibers, as a system for limbic touch, may underlie emotional, hormonal and affiliative responses to caress-like skin-to-skin contact between individuals.

Relationships between KC and Pain Response

The tactile (touch) component of KC is one of the first senses to develop in the fetus, emerging at about 7.5 weeks gestational age (Garcia & White-Traut, 1993; Liaw, 2000). Thus, touch is often viewed as superior to other non-pharmacologic modalities when intervening to soothe infants (Weiss, 1992b). KC possesses certain qualities of touch including long duration (more than 20 minutes in many studies, e.g., (Gray et al., 2000; Johnston et al., 2003; Mooncey et al., 1997), high intensity, and continual non-phasic contact with many body areas especially those of high innervation (hand and face) (Weiss, 1992b). These unique qualities of KC may enhance neural excitation by stimulating cutaneous, proprioceptive, and pressure receptors, in contrast to short-duration, mild-intensity with little pressure contact and contact with only a few minimal body locations (Weiss, 1992a). Many tactile stimulation studies report that preterm

infants who are touched are comforted and soothed, and physiologically, behaviorally and developmentally stronger (Mathai, Fernandez, Mondkar, & Kanbur, 2001; Modrcin-Talbott, Harrison, Groer, & Younger, 2003; M. N. Nelson et al., 2001). Dieter (1997) and Harrison (2000) suggested that tactile stimulation (systematic gentle human touch) may stimulate peripheral nerves that activate the vagus nerve, thus promoting infant comfort and reducing stress, resulting in positive immediate and long term outcomes. Immediate outcomes are reflected in behavioral and physiological indicators of reduced stress and pain, and include maintenance of HR, RR, and oxygen saturation, decreased behavioral distress cues, and decreased energy expenditure. In addition, KC involves olfactory (maternal odor), auditory (mother's voice and heart beat sound), vestibular (mother's rhythmic respiratory movement), and visual (eye-to-eye contact) stimuli. Infants are apparently familiar with their mothers' odor, voice, respiratory and heart beat rhythms from the uterine environment (R. H. Porter & Winberg, 1999).

The central underlying mechanisms of these maternal effects on reducing infant pain have been shown to be related to blunting sympathetic responses, accelerating the parasympathetic recovery of autonomic activation, and activating the endogenous opioid system (Blass, Shide, Zaw-Mon, & Sorrentino, 1995; Hofer, 1994). From a stress perspective, pain increases the secretion of stress hormones and sympathetic responses. Corticotropin-releasing factor (CRF) from the paraventricular nucleus of the hypothalamus and the central nucleus of the amygdala, is a primary regulator of the HPA axis, and neuroendocrine (catecholamines) and behavioral responses to stress (Francis, Diorio, Plotsky, & Meaney, 2002), and is actively stimulated following pain in preterm infants (Anand, 2000a; Anand & Carr, 1989). Release of central and peripheral

catecholamines, including norepinephrine (NE) and epinephrine (E), in response to pain directly control autonomic activation and lead to rapid changes in HR and heart rate variability as well as blood pressure following stressors such as experiencing pain.

Tactile contact between mother and young cause a significant enhancement of sympatho-adrenal maturation, specifically enhancement of CRF and HPA axis reactivity (Kuhn & Schanberg, 1998). Maternal care (licking and grooming) during the first 10 days dampens the newborn rat pups' HPA responsivity to acute stress, including reduced plasma adrenocorticotropic hormone and corticosterone response, increased gene expression of glucocorticoid receptors in the hippocampus, enhanced glucocorticoid feedback sensitivity, and decreased levels of CRF gene expression in the hypothalamus. The greater the frequency of maternal care during infancy, the lower the HPA response to stress in adulthood (Liu et al., 1997; Meaney et al., 2000). An increase in hippocampal corticosterone receptor gene expression moderates the synthesis and release of CRF and thus restrains ACTH release, and consequently corticosteroid and catecholamine release (Winberg, 1998).

In contrast, prolonged periods of maternal separation have the opposite effect. Postnatal maternal separation increases CRF gene expression, HPA, noradrenaline and serotonin, and behavioral responses to stress (Francis et al., 2002; Francis & Meaney, 1999; Liu, Caldji, Sharma, Plotsky, & Meaney, 2000). In human infants, KC results in a significant fall of both plasma and salivary cortisol (Modi & Glover, 1998). Following KC, infants also showed higher vagal tone between 32 and 37 weeks' GA as compared to controls (Feldman & Eidelman, 2003). The proposed hypothesis that the mother's tactile contact with the infant governs the infant's reaction to stress may be supported.

Mother-infant reunion (physical contact and milk) results in increases of endogenous opiate release in animal pups (Blass et al., 1995; Kalin, Shelton, & Lynn, 1995) and human newborns (Mooncey et al., 1997). Mother-infant physical contact triggers release of beta-endorphins in rat pups (Blass & Ciaramitaro, 1994; Blass et al., 1995; Kalin et al., 1995). Endorphins are one of the family of opioid peptides that bind to the μ opioid receptor (three opioid receptors, μ, δ, κ, which have been found throughout the nervous system) and are critically involved in mediation of the pain signal by blocking the perception of pain (Machelska, 2003). The contribution of somatosensory-induced opioid release includes powerful anti-nociception, alleviation of isolation distress, inducement of a euphoric state in infants, and creation of a positive emotional state in both infant and mother (Kalin et al., 1995; E. E. Nelson & Panksepp, 1998). Generalized mother-infant physical contact also activates the oxytocinergic neuron, and increases oxytocin levels in both blood and cerebrospinal fluid in rats after vibrotactile or thermal stimulation (E. E. Nelson & Panksepp, 1998). The ability of oxytocin to increase affiliative behaviors and induce social preference, as well as potentiate the anti-nociceptive effects of the endogenous opioid system have been reported (Blass et al., 1995; E. E. Nelson & Panksepp, 1998). In human infants, concentrations of beta-endrophin dropped significantly after the KC session, but did not change on the control day, whereas cortisol levels dropped on both days, suggesting an attenuation of the stress response, at least in terms of its opioid components (Mooncey et al., 1997). Attenuation of stress response means a decrease in pain response can be expected.

Based on the positive effects discussed above, KC may be a novel and effective method to blunt pain responses. Some of KC's components, when studied individually,

have been found to reduce the severity of responses, especially crying and motor activity, to stress and pain. KC's components include: (1) Maternal continual non-phasic touch. During gentle human touch, preterm infants had improved oxygen, less motor activity, and less behavioral distress (Harrison, Leeper, & Yoon, 1991; Harrison, Olivet, Cunningham, Bodin, & Hicks, 1996). (2) Prone positioning: Preterm infants when prone were less crying, fewer stress responses, less motor activity, less active sleep and more quiet sleep, and improved respiratory function (Chang, Anderson, Dowling, & Lin, 2002; Chang, Anderson, & Lin, 2002; Grunau, Linhares, Holsti, Oberlander, & Whitfield, 2004; Maynard, Bignall, & Kitchen, 2000; Sahni et al., 2002). (3) Warmth: KC is an effective method of maintaining temperature in preterm infants. Maternal warmth is a soothing stimulation for infants. (4) Containment or swaddling: During KC, containment acts to prevent an increase in behavioral distress and plasma cortisol after a heel stick (Campos, 1989; Fearon, Kisilevsky, Hains, Muir, & Tranmer, 1997; Huang, Tung, Kuo, & Ying-Ju, 2004; Malone, Gunnar, & Fisch, 1985; Rush et al., 2005). (5) Maternal heart sounds: The presence of maternal heart beat, a sound that can be perceived by the infant as he/she lies against the maternal chest, prevents full-term newborns from significantly increasing serum and salivary cortisol after painful stimulation (Kawakami, Takai-Kawakami, Kurihara, Shimizu, & Yanaihara, 1996; Kurihara et al., 1996). (6) Vestibular movement: The vestibular component of carrying, similar to the gentle vestibular stimulation of the mother's chest respiratory movements, produces a comforting effect during painful stimulation (Johnston, Stremler, et. al, 1997). (7) Maternal body odor: The olfactory component, maternal odor presented in KC, is capable of attenuating crying to a distressed infant. (R. H. Porter, Makin, Davis, & Christensen, 1991; R. H. Porter &

Winberg, 1999; Sullivan & Toubas, 1998). (8) Mother's voice: The sound of the mother's voice has soothing effects (Sieratzki & Woll, 2002). Newborns showed a peaceful facial appearance, ceased to cry, did not open their eyes, and hardly moved their extremity after listening to mothers' gentle voice (Fifer & Moon, 1994; Nakajima, 1994).

Thus, KC's proposed action as an analgesic is through multi-sensory stimulation inputs to widespread areas of the brain, activation of the neurochemical system, and modulation of the stress-regulation system involved in pain. The actions alter output of the pain matrix (pain responses), thereby blunting pain responses significantly more than non-KC mediated pain situations in preterm infants (see Figure 1).

Assumptions

The underlying assumptions in the proposed study are:

1. Heel stick is a painful procedure.
2. The experience of pain as observed in this study reflects the infants' true and fully disclosed responses to pain.

Research Hypothesis

Based on the background and theoretical framework presented above, two hypotheses will be tested:

Hypothesis 1. Preterm infants' bio-behavioral pain responses (Premature Infant Pain Profile; PIPP) will be significantly lower for KC heel stick, hereafter called KCH, than for incubator heel stick, hereafter called IH.

Hypothesis 2. Preterm infants will have decreased autonomic pain responses, specifically decreased sympathetic responses, as measured by heart rate variability indices (LF, HF, L/H), to KCH than to IH.

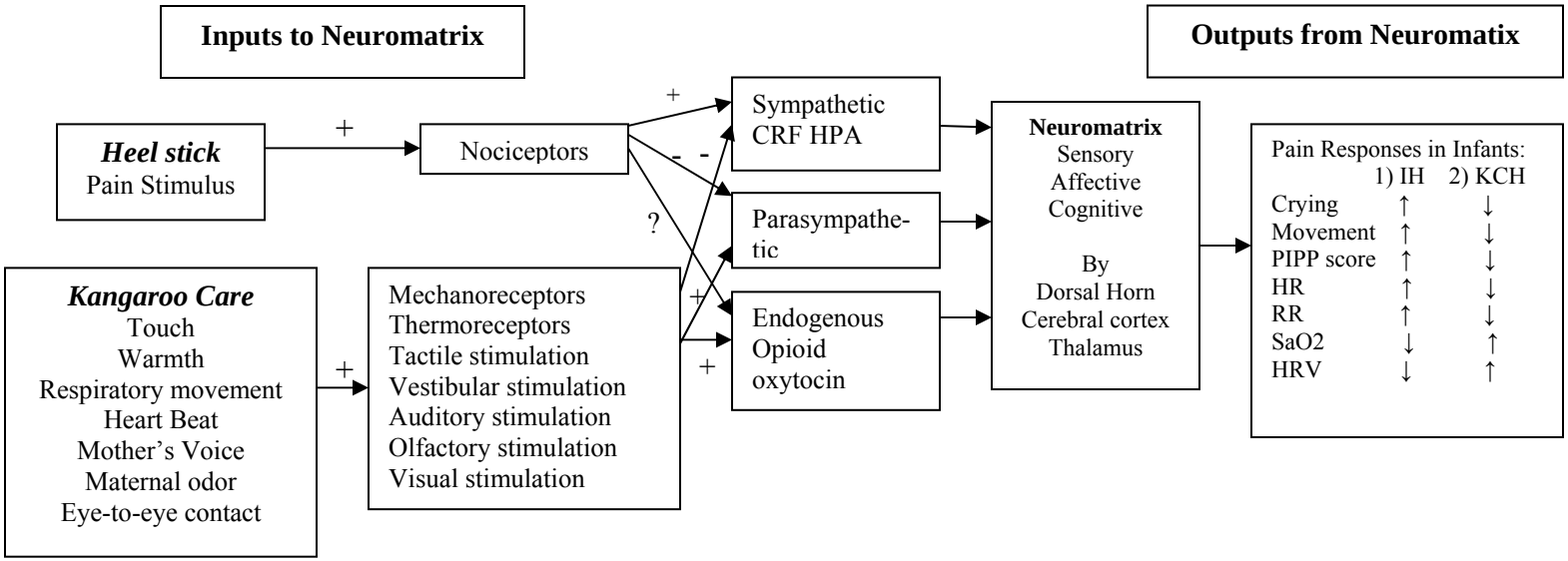


Figure1. Neuromatrix Framework for Kangaroo Care and Infant Pain. IH = incubator heel stick condition; KCH = Kangaroo Care heel stick condition

23

Summary of Chapter One

The background of this study is derived from the existing knowledge base documenting that: 1. premature infants can and do experience pain, 2. heel stick is a common and acknowledged source of pain, 3. premature infants' pain responses are harmful, and 4. existing interventions to modify pain responses remain less than optimal. Previous studies of KC suggest that it may be effective in blunting pain responses. If KC does decrease pain responses, the management of premature infant pain may be promoted by its use, thus filling a gap in the literature relations to an easily employed, side effect-free nonpharmcological pain intervention. This section concluded with the theoretical framework of the study and the study's hypotheses.

CHAPTER TWO

Review of Literature

The purpose of this chapter is to review the conceptualizations of neonatal pain and effectiveness of existing non-pharmarcologic pain treatments in infants. Previous investigations are reviewed to substantiate the hypotheses that KC may be a pain intervention to blunt the pain of heel stick in preterm infants. Theoretical and methodological issues related to pain and its measurements are addressed.

Conceptualizations of Neonatal Pain

Pain is a challenging concept when caring for an adult or a child. The word "pain" is a derivative of the Greek and Latin words for "punishment". Therefore, an early interpretation of pain was that it represented punishment from offended Gods (Homberg, 1988). By the nineteenth century, as science and technology advanced, studies of pain had led to the emergence of a strong theoretic foundation for pain. Along with the development of theoretical frameworks, the concept of pain was evolving. Pain was defined by McCaffrey (1968) as whatever the experiencing person says it is, and existing whenever the person says it does. The definition emphasized that pain is a subjective experience with no objective measures, and that the patient, not clinician, is the authority on the pain and that his or her self-report is the most reliable indicator of pain. The current definition of pain, adopted in 1979 by the International Association for the Study of Pain (IASP), states that pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage (IASP, 1979; Merskey & Bogduk, 1994). The IASP's definition further addresses that pain is always subjective and is a complex personal experience including multiple dimensions such as sensation,

emotion, and cognition that makes an impact on a person's physical and psychosocial functioning. Recent advances in pain measurement and management have benefited from defining pain as a subjective experience, by which clinical health providers can provide pain management according to the patient's self-report of pain (Anand & Craig, 1996).

However, these definitions of pain challenge the understanding of pain in infants who are incapable of self-report, because the current definition of pain relies greatly on the assumption of congruence between feeling pain and reporting pain. Infants cannot report when they are experiencing pain the way older children and adults can. The "gold standard" of self-report generic to the current identification of pain is not appropriate in the infant population. The fact that neonates are incapable of expressing pain verbally contributes to the failure to recognize and aggressively treat pain in infancy (Anand & Craig, 1996; Bellieni, 2005). Some researchers considered that the concept of infant pain can be interchangeable with nociception, signaling system, or distress. However, each of these terms does not comprehensively describe infant pain experience. Each of these synonyms for pain is described below.

Nociception

Nociception may be a more precise term than "pain" to describe the infants perception of painful experiences (Bucher et al., 1995; Stevens & Johnston, 1993). Nociception refers to the neural detection of a noxious stimulus, and the transmission of information about the presence and the quality of that stimulus from the site of stimulation to the brain (McGrath, 1990). For infants who are not capable of describing their pain, nociception permits description and measurement of the response to a noxious stimulus in physiological, biochemical and behavioral terms (Bucher et al., 1995).

However, nociception only addresses the sensory (neurophysical) component of pain, not the cognitive responses that are part of pain perception.

Signaling System

Anand and Craig (1996) offered another perspective on infant pain. They view infant pain as an inherent quality in life; the mechanisms for pain appear early in ontogeny to serve as signaling system for tissue damage (Anand & Craig, 1996). The perception of pain does not require prior experience, but the interpretation and meaning of these sensations develop with experience mainly through positive, negative, and contextual associations with pain. The signaling of tissue damage and hunger are obviously the most necessary emotions for survival of newborns, and as such appear earlier than any other adaptational behaviors during development. This signaling includes physiologic responses such as HR and RR changes. Behavioral and emotional responses such as crying, facial expression, body movement, and change of behavioral state are considered valid indicators of pain. However, these pain indicators in the signaling system must be subjectively observed and judged by others, such as health care professionals and parents (Stevens, Gibbins, & Franck, 2000).

Stress or Distress

Stress or distress are other synonyms used to describe infants' painful experiences (Anand, 1998a). Yet definitions of stress (Kawakami et al., 1996; Kawakami et al., 1997) and distress (Bo & Callaghan, 2000; Campos, 1989) are not clear, and their relationship to infant pain has not been delineated clearly. Selye (1974) defined stress as "the nonspecific response of the body to any demand made upon it" (p.27). Stress is also defined as anything that stimulates adrenocorticotropic hormone (ACTH) secretion (S.

Levine, 2000). Selye (1974) emphasized that stress is not always distress, as distress is always unpleasant (such as hunger and pain), but the general concept of stress also includes the pleasant experiences of joy, fulfillment, and self-expression. Consequently, using the concept of stress to explain infant pain is not appropriate either. Indeed, both pain and stress encompass complex biological, psychological and social phenomena that may or may not be distinctly different (Bellieni, 2005). Though 20 minutes of KC has been found to decrease hormonal stress response (Modi & Glover, 1998; Mooncey et al., 1997; Morelius et al., 2005) and does so better than massage (Gitau, Modi, Giannakoulopoulos, Bond, & Glover, 2002), stress responses are not a focus of this investigation. For the purpose of this study, pain is the concept that is generic to the investigation of "pain" and will not be used interchangeably with "stress" or "distress".

In summary, current pain definitions do not encompass newborn infants, and, thus, they are inadequate. In most infant pain studies, investigators have not provided conceptual or theoretical definitions of infant pain, preferring to solely identify pain referents (McIntosh, 1997). In this study, the definition of pain developed by the IASP will be used as the conceptual definition of pain. The heel stick that is commonly experienced in preterm infants will be the pain stimulus. The infantile forms of self-report of unpleasantness based on the communication level appropriate to this developmental stage are the behavioral and physical alterations caused by pain and will be included in the operational definition of pain (Anand & Craig, 1996).

Mechanism of Infant Pain

The traditional view that neonates were relatively insensitive to pain was refuted more than a decade ago (Anand & Hickey, 1987). In recent years, although broad

similarities between the experience of pain in children and adults have become increasingly evident, essential differences exist between the pain experienced by newborns, infants, children and adults (Anand & Carr, 1989). The differences reflect the unique biologic and behavioral characteristics in different stages of development. The mechanism of neonatal infant pain is discussed below.

Developmental aspects of the mechanism of pain. That premature infants can perceive pain is now well established because peripheral and central structures necessary for nociception are present and functional by 12-16 weeks gestation (Wolf, 1999). Thus, the preterm infant can feel and respond to pain. The cortical capacity to interpret painful stimuli functions as early as 20 weeks of gestation and the cortisol response to pain results in behavioral, autonomic, and hormonal responses (Anand, 1998b; Anand & Craig, 1996). By 23 weeks gestation, the human fetus and preterm infants can mount hormonal stress responses to painful stimuli (Giannakoulopoulos, Sepulveda, Kourtis, Glover, & Fisk, 1994) and receive some analgesia through the endogenous endorphin system (Gibbins & Stevens, 2001b). By 30 weeks gestation, pain transmission is known to occur even prior to complete myelinization of the pain pathways to the brain stem and thalamus (Anand & Hickey, 1992). Therefore, by the end of second trimester, the fetus possess both the anatomical and neurochemical capabilities of pain perception (Anand & Hickey, 1987; Fitzgerald & Beggs, 2001; Kostovic & Rakic, 1990). The nerve pathways associated with pain transmission are functional at term birth, but myelination continues throughout infancy. The density of nerve endings or pain receptors in the skin of newborns is similar to or greater than that in adult skin (Anand, 2000b). Due to immaturity of the nervous system, infants may actually have a pain threshold that is 30-

50% lower than that of adults and a lower pain tolerance than older children (Broome, Rehwaldt, & Fogg, 1998; Fitzgerald, Millard, & MacIntosh, 1988). Therefore, preterm infants are at higher risk than full-term infants for acquired disease, neurologic injury, and adverse developmental outcome as a result of painful experiences during hospitalization (Fitzgerald & Beggs, 2001).

Persistent response - inflammation. Acute pain caused by cellular and blood vessel damage results in an inflammatory process including the release of a "nociceptive soup" or algogenic substances (serotonin, histamine, potassium, hydrogen ions, substances from the arachidonic acid cascade, leukotrenes and prostaglandins, bradykinin, adenosine, substance P, and calcitonin gene related peptides) (Evans, 2001). These biochemical mediators activate or sensitize nociceptors of A delta and C afferent fibers to transmit pain impulses to the spinal cord. Tissue damage in the early neonatal period causes a profound and lasting sprouting response of the local sensory nerve terminals, which results in hyperinnervation of the wounded area that lasts into adulthood (Alvares, Torsney, Beland, Reynolds, & Fitzgerald, 2000). This sprouting response is clearly greatest when the wound occurs at or near birth and declines with age at wounding (Fitzgerald & Beggs, 2001).

The trauma of repeated procedures such as heel stick will result in local hypersensitivity or *hyperalgesia*, which is characterized by an enhanced pain sensation or lowered pain threshold in response to a noxious stimulus. The hyperalgesic effect is greatest at 28 to 33 weeks post-conceptual age (PCA) and is lost by 42 weeks (Andrews & Fitzgerald, 1994, 1999; Fitzgerald, Shaw, & MacIntosh, 1988). The threshold in an area of local tissue damage created by routine heel sticks is 50% lower than the threshold

on the intact heel on the other side, and this lowered pain threshold persists for days and weeks (Fitzgerald, Millard, & McIntosh, 1989). In addition, the inflammation and tenderness in newborns, especially with repeated tissue damage, may extend into adjacent uninjured tissue, causing pain sensations from stimuli (such as touch) that normally do not provoke pain sensation, which is called *allodynia* (pain due to a non-noxious stimulus to normal skin) (Merskey et al., 1979).

Immaturity of pain inhibition in newborns. Descending inhibitory controls are immature at birth and the slow maturation of descending inhibitory pathways is particularly relevant to persistent pain in infants (Fitzgerald & Beggs, 2001). Descending axons from brainstem projection neurons do not extend into the dorsal horn and are not functionally effective at birth possibly due to deficiency of neurotransmitters and delayed maturation of interneurones (Fitzgerald & Beggs, 2001; Wolf, 1999). Additionally, the newborn spinal cord is in a more excitable state than in the adult and the excitable state that leads to lower thresholds for pain sensitization (Fitzgerald & Jennings, 1999). The receptive fields of dorsal horn cells are larger, that is, the fields occupy a relatively larger area of the body surface than they do in adults, increasing the chance of activation by peripheral skin stimulation in infants (Fitzgerald & Jennings, 1999).

Plasticity of neonatal central nervous system. Following inflammation induced by the tissue damage, repetitive A delta and C fiber inputs activate the central neurons in the dorsal horn of the spinal cord and brainstem, resulting in central sensitization, causing them to respond to normal inputs in an exaggerated and extended manner and allow inputs that were previously ineffective to activate the neurons (Woolf & Salter, 2000). Ruda (2000) investigated the impact of neonatal tissue injury and pain on development of

nociceptive neuronal circuitry in animal models, and found that as adults, the animals exhibited spinal neuronal circuits with increased density and segmental distribution of nociceptive primary afferent axons and altered responses to sensory stimulation. Enhanced responsiveness of dorsal horn neurons may translate into a permanently facilitated response to noxious stimulation. Studies on preterm neonates that spent several weeks in the NICU reported dampened (Grunau et al., 2005; Grunau, Whitfield, & Petrie, 1994; Johnston & Stevens, 1996) or heightened behavioral responses (Holsti et al., 2005) and less autonomic responses (Oberlander et al., 2000) to painful procedures.

The neonatal period is a time of great brain plasticity. Accumulating evidence suggests that exposure to repetitive neonatal pain may cause excessive N-methyl-D-aspartate (NMDA) / excitory amino acid activity resulting in excitotoxic damage to developing neurons in supraspinal areas (Anand & Scalzo, 2000). Inadequately treated, prolonged pain and stress may alter neuronal and synaptic organization permanently and result in widespread changes in the immature brain leading to abnormal behaviors in adulthood (Alvares et al., 2000; Pattinson & Fitzgerald, 2004). Clearly experiences of pain will be remembered by the developing nervous system, perhaps for the entire life (Anand, 2000b).

In summary, preterm infants have mature pain perception pathways and are therefore capable of perceiving pain. However, the immaturity of sensory processing in the neonatal nervous system leads to a lower threshold for pain sensitization. Premature infants may be more hypersensitive to nociceptive stimuli than full-term infants. Structural and functional differences in the neonatal central sensory connections enhance and prolong the effects of noxious inputs (Fitzgerald & Beggs, 2001).

Frequency of Pain in Neonates

Infants in the NICU are routinely subjected to various diagnostic, surgical or therapeutic procedures which can result in pain (D. P. Barker & Rutter, 1995; Evans et al., 2005; Johnston, Collinge et al., 1997; Lago et al., 2005; F. L. Porter & Anand, 1998). A broad range of frequencies of painful procedures has been reported. Blood sampling from infants by lancing and squeezing the heel is a routine procedure in maternity and neonatal wards throughout hospitalization (Grunau & Craig, 1987; Lindh, Wiklund, & Hakansson, 1999). Almost every infant undergoes repeated heel puncture to screen for metabolic disorders such as phenylketonuria. Many infants receive repeated heel punctures to monitor blood glucose or hemoglobin (Franck & Gilbert, 2002).

A descriptive study explored the types and frequency of contacts experienced by 16 neonates in NICU and found that neonates were subjected to as many as three invasive procedures per hour (Pohlman & Beardslee, 1987). Barker and Rutter (1995) followed 54 preterm and full-term infants from NICU admission until discharge. The results showed that over 3000 procedures were recorded, and the cumulative number of invasive procedures experienced by each infant within 48 hours of admission to neonatal intensive care unit (NICU) was 60.9. One half (56%) of these procedures were heel sticks, followed by endotracheal suctioning (26%) and intravenous catheter insertions (8%) (D. P. Barker & Rutter, 1995). Infants less than 31 gestational weeks experienced 74% of the procedures and one infant who was 23 weeks gestational age with 560g birth weight underwent 488 procedures during the intensive care unit stay. Repeated heel stick accounted for 55% of the total number of procedures performed on infants during hospitalization (D. P. Barker & Rutter, 1995). Johnston and colleagues (1997)

investigated 239 patients over one week time in 14 Canadian NICUs and found that a total of 2,134 invasive procedures were performed with an average of two per day. Some infants had up to eight per day, and pain medication was given for only 0.8% of the procedures. Sixty-one percent of the invasive procedures were heel sticks and no analgesia was given specifically for the heel stick procedure (Johnston, Collinge et al., 1997). Porter and Anand (1998) conducted a longitudinal study from admission to discharge in one NICU. One hundred and forty four neonates underwent 7,000 procedures, of which more than 6,000 were heel sticks. Only 3% of the total number of procedures were performed with pharmacologic pain management specific for the procedure, while analgesia was never used for some procedures such as heel sticks and arterial blood sampling. In a prospective randomized crossover trial, Stevens et al. (1999) reported that 122 very low birth weight infants received a mean of 134 painful procedures within the first two weeks of life, and approximately 10% of the youngest and/or sickest infants received over 300 painful procedures.

In summary, neonatal infants in NICU undergo invasive procedures with range of 2 to 30 per day, and younger and sicker infants receive more painful procedures. Repeated heel sticks account for 55% to 86% of the total invasive procedures. However, pharmacologic pain management is given for only 0.8% to 3% of the procedures.

Heel sticks to test pain treatments. Heel stick as a noxious stimulus has been used as a model situation by a number of researchers to investigate the pain response of the newborn (Gray et al., 2002; Gray et al., 2000; Grunau et al., 2004; Johnston et al., 2003; Ludington-Hoe et al., 2005). Studies have found that infants had more pain response, particularly crying, during heel stick than venipuncture for blood sampling, and heel

sticks required more repeated punctures to obtain an adequate sample than venipuncture did (Eriksson, Gradin, & Schollin, 1999; Larsson, Tannfeldt, Lagercrantz, & Olsson, 1998; Ogawa et al., 2005; Shah, Taddio, Bennett, & Speidel, 1997). However, one study reported higher maternal anxiety during venipuncture than heel stick (Shah et al., 1997).

Behavioral Pain Responses

Expression of pain through behavior is the only means by which infants can communicate their pain to observers. Behavioral responses of neonatal pain include vocalization (cry), facial expressions, gross motor movements, and changes in behavioral states and functions (such as sleep-wake changes) during and after each pain stimulus.

Crying. The cry response is common in preterm and term infants (Brown, 1987; Gibbins & Stevens, 2001b). Crying is the primary method of communication between infant and caregiver and it may signal alterations in infant internal state (Fuller, 1991). Cry can be described in terms of its presence or absence of the time perspective (latency to cry, duration of cry), and the amplitude and pitch (high or low; measured as fundamental frequency [F_0]). Infant pain cries are spectrographically distinct in terms of frequency and pitch compared to cries due to other stimuli such as hunger, anger or fear, and fussiness (Fuller, 1991, 1996; Ludington-Hoe, Cong, & Hashemi, 2002; F. L. Porter, Porges, & Marshall, 1988). Changes in the patterns of neonatal cries have also been correlated with the intensity of pain experienced during circumcision and can be accurately differentiated by adult listeners (F. L. Porter, Miller, & Marshall, 1986; Warnock & Sandrin, 2004). Bellieni and colleagues (Bellieni, Sisto, Cordelli, & Buonocore, 2004) found that the stationary character of crying intensity increased with increasing pain in full-term infants undergoing heel stick. The most interesting findings

in this study were when DAN pain scores (Douleur Aiguë du Nouveau-né; a behavioral acute pain rating scale for neonates; ranging from 0 to 10) was more than 8, a stereotyped cry was produced, and the regularity and repetition of which suggested a call for attention help. Pain cries of preterm and neurologic impaired infants are considerably different from all other cries (Anand, Sippell, & Aynsley-Green, 1987).

Some preterm and acutely ill infants may not cry during heel sticks and other painful procedures, which may be due to depleted energy reserves, or they may be unable to cry because of the presence of an endotracheal tube (Johnston, Stevens et al., 1999; Ludington-Hoe et al., 2005). In addition to, or even in the absence of crying, the infant forms a "cry face" that is characteristic of pain. In the absence of crying, this is called a "silent cry". Therefore, an audible cry alone may not be a valid or reliable indicator for pain in preterm or acutely ill infants.

Two major difficulties exist with using cry as a measure of pain. First, many preterm infants do not cry when subjected to a significant noxious stimulus, such as a needle puncture (McGrath, 1990), and second, there is little or no specificity to the cry as a measure of pain (Fuller, 2001). However, the use of heel stick as a pain stimulus greatly increases the probability that the resultant crying is due to pain. A psycho-acoustic analysis of cry is not available in clinical situations.

Facial expressions. The facial expression in response to heel stick is different from that in response to other tactile stimuli such as cleaning the heel (Bozzette, 1993; Warnock & Sandrin, 2004). Facial activity is considered the **most reliable** and **consistent indicator of pain** of all the unidimensional approaches across situations and for both full-term and preterm infants and should be the gold standard of behavioral

responses for pain in neonatal infants (Grunau, Johnston, & Craig, 1990; Stevens & Johnston, 1994). Facial expressions include facial grimacing, brows bulged and furrowed, eye squeezed, nasolabial furrowed, nasal roots broadened and bulged, lips opened and pursed, a square mouth, cupped tongue, quivering chin and agitation (Franck, Greenberg, & Stevens, 2000; Phillips, 1995).

Gross motor responses. Motor responses including vigorous gross body movements (movements of arms, legs and trunks and whole body) and attempts to withdraw from a painful stimulus have been observed in full-term and preterm neonates during different phases of a heel lance procedure (Craig, Whitfield, Grunau, Linton, & Hadjistavropoulos, 1993). The flexion withdrawal reflex (FWR) is a clear distinct withdrawal of the limb that can be evoked by a noxious stimulus to the heel and it has been found to correlate between the severity of a stimulus and the latency, amplitude, and duration of the cutaneous withdrawal reflex in preterm and full-term neonates (Andrews & Fitzgerald, 1999; Holsti et al., 2004). In response to a painful stimulus, very low birth weight or sick infants may be flaccid (Franck, 1986). Changes in body movements have been included in some composite measures of pain.

Sleep-wake responses. Alterations in complex behavior and sleep-wake cycles have been observed following painful procedures such as a circumcision without anesthesia. Painful procedures are followed by prolonged periods of non-rapid-eye-movement sleep, increased wakefulness and irritability, and altered arousal level (Alvares et al., 2000; Grunau et al., 2004; Grunau, Oberlander, Holsti, & Whitfield, 1998; Stevens, Johnston, Petryshen, & Taddio, 1996). Painful procedures may have prolonged effects on the neurologic and psychosocial development of infants as addressed in Chapter 1.

Physiologic and Autonomic Responses

Physiological responses to painful stimuli include increases in heart rate, respiratory rate, intracranial pressure and palmar sweating, and are accompanied by decreases in transcutaneous oxygenation saturation, vagal tone, and peripheral blood flow (Gibbins & Stevens, 2001a). In preterm and full-term neonates undergoing circumcision (Williamson & Williamson, 1983), heel lancing (Field & Goldson, 1984; Holsti et al., 2004; Huang et al., 2004; Johnston, Stevens, Yang, & Horton, 1996) or venipuncture (Ogawa et al., 2005; Van Cleve, Johnson, Andrews, Hawkins, & Newbold, 1995) marked increases in HR and blood pressure occur during and after the procedure. However, some studies report that cardiorespiratory responses are less clear in the preterms (Fitzgerald & McIntosh, 1989). The magnitude of change in HR is related to the intensity and duration of the stimulus and to the individual temperament of the infant (Anand & Hickey, 1987).

Oxygenation saturation. Oxygenation, as measured by transcutaneous monitors or pulse oximetry, decreases in response to pain (Franck, 1989; Huang et al., 2004; Lindh, Wiklund, Sandman, & Hakansson, 1997; Van Cleve et al., 1995). Marked decreases in transcutaneous partial pressure of oxygen occur during circumcision (Brady-Fryer, Wiebe, & Lander, 2004; Maxwell, Yaster, Wetzel, & Niebyl, 1987) and tracheal intubation in awake preterm and full-term neonates (Kelly & Finer, 1984; Tan et al., 2005). Arterial blood pressure and intracranial pressure increased and oxygenation decreased in response to pain stimuli (Kelly & Finer, 1984; Stevens & Johnston, 1991). During episodes of vigorous crying, oxygenation may increase, but oxygen delivery to cerebral tissues may be compromised even though the oxygen content of the blood remains stable (Franck, 1989).

Heart rate variability. Heart rate variability (HRV) is an index of the balance of sympathetic and parasympathetic control on HR (Chatow, Davidson, Reichman, & Akselrod, 1995). HRV is a sensitive index of stress due to pain reactivity (Lindh, Wiklund, & Hakansson, 2000) which alters heart rate. HRV is defined as the cyclic changes or fluctuations in the R-to-R interval that occur with respiration (Cowan, 1995). The fluctuation as reflected in the R-R interval can be analyzed to provide a sensitive, noninvasive measure of autonomic input to the sino-atrial node of the heart. Two approaches to measurement and analysis of HRV exist: time domain and frequency domain analyses. The frequency domain analysis delineates parasympathetic from sympathetic components of autonomic control with power spectral analysis (Cowan, Pike, Burr, Cain, & Narayanan, 1993). The spectral power in the high-frequency band (.15 -1.0 Hz) is related to respiratory sinus arrhythmia and reflects parasympathetic activity, while the low-frequency band (.04 - .10 to .15 Hz) is an index of primarily sympathetic activity with some parasympathetic input (Cowan, 1995).

Heart rate variability response has been used as a measure of reactivity to painful events in term and preterm infants. Lindh and associates have conducted a series of investigations assessing pain by frequency domain analysis of HRV in term and preterm infants. Facial expression and spectral analysis of HRV were investigated in 10 preterm infants 27 - 35 weeks PCA during rest and in response to stimulation with von Frey's hairs and heel lance blood sampling (Lindh et al., 1997). Non-noxious flexor withdrawal by application of von Frey's hairs did not evoke visual signs of pain or influence HRV, but the lancing and squeezing of the heel evoked an increase of mean HR and a reduction in both total HRV (3.6 ± 0.7 log mHz^2 to 2.8 ± 0.6 log mHz^2, $p < .04$) and power in the

low frequency band of the HRV spectrum (3.4 ± 0.7 log mHz^2 to 2.3 ± 0.7 log mHz^2, $p < 0.02$). The results showed that the blood sampling procedure caused a clear stress response, and changes in HRV reflected a reduction of vagal tone and an increase of the sympathetic nervous system (Lindh et al., 1997).

In another study, 23 healthy newborn infants were recruited to assess pain by HRV during heel lance (Lindh et al., 1999). Compared with the baseline, sharp lancing gave rise to minor increases in HR and in the low frequency power of HRV. A clear stress response was provoked when the heel was squeezed for blood sampling, indicated by an increased HR (122 ± 14 to 173 ± 22, $p< .001$) and decreased spectral power in the high frequency band (3.4 ± 0.3 log mHz^2 to 2.8 ± 0.4 log mHz^2, $p< .001$), indicating reduced vagal tone. Squeezing the heel was the most stressful event during the heel stick procedure (Lindh et al., 1999).

Lindh and colleagues (2000) next studied the effect of lidocaine-prilocaine 5% cream (EMLA) on the pain response when venipuncture was performed in 60 3-day-old full-term newborns held by mother or father. The incidence of crying, HR, and HRV were used to characterize the reaction of the infant to the painful procedure. The placebo group showed a significantly higher HR (144 ± 20 to 130 ± 17, $p < .01$), and a decrease in total power (4.10 ± .35 log mHz^2 to 4.31 ± .40 log mHz^2, $p <. 02$) and a decrease in power in the low frequency band when compared with the EMLA group (4.00 ± 0.39 log mHz^2 to 4.23 ± .44 log mHz^2, $p< .01$). Vagal activity was comparatively higher in the EMLA group and the response to the puncture was of shorter duration. Another study was conducted to determine whether use of EMLA and oral glucose decreased pain associated with immunization in 3-month-old infants (Lindh, Wiklund, Blomquist, &

Hakansson, 2003). Forty-five infants randomly received EMLA and glucose and 45 infants received placebo cream and water. HR and HRV were recorded and calculated pre- and post-injection. Mean HR and HRV data did not discriminate the pain responses in the treatment group from the placebo group. However, a biphasic transient heart rate response with a marked deceleration followed by a subsequent acceleration was seen more frequently in the placebo group compared to the treatment group. The data implied that the injection without analgesia influenced vagal tone more than injection with EMLA and glucose (Lindh et al., 2003). The studies demonstrated described above that the relatively small tissue damage from heel lance, venipunture or immunization injection caused sufficient pain to evoke a stress response indicated by HR and HRV measurement.

In a study examining the relationship between behavioral and cardiac autonomic reactivity to acute pain, responses to heel stick were assessed in 136 preterm infants (mean birth weight: 1020g, range: 445 - 1500g) and gestational age at birth was 28 weeks (23 - 32 weeks). Infants were tested at 32 weeks postconceptional age (Morison, Grunau, Oberlander, & Whitfield, 2001). Neonatal Facial Coding System (NFCS), sleep/waking state, and finger splay were measured as behavioral responses, and changes in HR and spectral analysis of HRV were assessed as autonomic responses. Distressful facial activity and state moderately correlated with increased in HR ($r = 0.41 - 0.62$), but did not correlate with change in LF and HF, nor with the LF/HF ratio. The majority displayed concordant reactions of behavioral and physiologic reactivity (Morison et al., 2001).

Oberlander and associates conducted a study to compare biobehavioral responses to acute pain at 4 months corrected age between former extremely low birth weight (ELBW) infants and full-term controls, using NFCS and power spectral analysis of HRV

as pain measures (Oberlander et al., 2000). Twenty-one former ELBW infants (mean birth weight =763g) were compared with 24 full-term infants during baseline, lance and recovery periods of a finger-lance blood collection. LF and HF power decreased significantly from baseline, and increased again in the recovery period in both groups, however, there were no significant differences between groups. The ELBW group had a less intense parasympathetic withdrawal in the lance period and a more sustained sympathetic response during recovery than the control group. Further, in the recovery period, two behavioral patterns – early recovery and late recovery, were apparent among the ELBW group. Results suggested that former ELBW infants seemed less physiologically able to modulate their immediate and recovery response to an acute noxious event than full-term infants, which may be related to the long-term effect of early pain experience or a developmental lag in pain response (Oberlander et al., 2000).

In another study, Oberlander (2002) examined neonatal responses to acute pain (heel lance for phenylketonuria) in infants who had prenatal exposure and prolonged prenatal selective serotonin reuptake inhibitors (SSRIs; used to treat maternal depression). The NFCS and cardiac autonomic reactivity (power spectral analysis of HRV) were compared between 22 infants with SSRI exposure (SE); 16 infants exposed to SSRIs and clonazepam (SE+); and 23 nonexposed infant. Baseline, lance, and recovery periods of a heel lance were observed. Infants exposed to SE and SE+ displayed significantly less facial activity to heel lance than control infants; mean HR increased with lance, but was significantly lower in SE infants during recovery; and SSRI infants had a greater return of parasympathetic cardiac modulation in the recovery period. Prolonged prenatal SSRI exposure appears to be associated with reduced behavioral pain

responses and increased parasympathetic cardiac modulation in recovery following an acute pain stimulation (Oberlander, Grunau, Fitzgerald, Ellwood et al., 2002).

The findings of HRV in response to pain are summarized in Table 1. The heel stick and other painful procedures resulted in increased heart rate. Compared with baseline levels, lancing and squeezing triggered a decreased total HRV, LF, and HF, indicating that the pain procedure causes a marked stress response in the infant.

Table1.

Heart Rate Variability Responses during Painful Procedure

Author/Year	Subjects	Pain Stimuli	Results
Lindh, et al. (1997)	10 Preterm, 1 group 24-33 GA 27-35 wks PCA Baseline 1 min, Von Frey 1 min, rest 3 mins, warming 1 min, stick 1 min	Heel stick	Mean HR ↑ during heel stick Total HRV↓ during heel stick LF↓ during heel stick HF no changes LF/HF ratio no changes
Morison, et al. (2001)	Preterm, 3 groups: 23- 26 wks GA = 48 27 - 29 wks GA = 52 30 - 32 wks GA = 36 Baseline 200 secs, blood collection 200 secs, recovery 200 secs values	Heel stick	Mean HR moderately correlated with facial and state response. LF no correlation to behavioral responses all grps HF no correlation to behavioral responses all grps LF/HF no correlation to behavioral responses in all groups
Oberland, et al. (2000)	4 month old preterms, 2 groups: ELBW = 21 Full-term = 24 Baseline 2.2 mins, stick 2.2 mins, recovery 2.2 mins values	Heel stick	Mean HR ↑ baseline to heel stick. LF ↓ from baseline, ↑ in recovery HF ↓ from baseline, ↑ in recovery LF/HF no changes. No sig. diff. between groups. ELBW grp: less parasympathetic withdrawal during stick; more sympathetic response in recovery.
Oberlander, Grunau, Fitzgerald, & Whitfield. (2002)	Preterms, 2 groups: 32 wks PCA n = 12 neurologically impaired control: n = 12 no impairment baseline 2.2 mins, blood collection 2.2 mins, recovery 2.2 mins values	Heel stick	Mean HR ↑ from baseline to heel stick. LF ↓ from baseline to heel stick. HF↓ from baseline to heel stick LF:HF↓ from baseline to heel stick No group differences Infants with proven brain injury had more tongue protrusion at lance than non-impaired.

Table 1. (continued)

Author/Year	Subjects	Pain Stimuli	Results
Grunau, et al. (2005)	Preterm, 2 groups 22-28 wks GA = 30 29-32 wks GA = 57 Baseline 2.2 mins, Lance 2.2 mins values	Heel stick	HR no correlation to pain nor morphine exposure during heelstick for both groups LF no correlation to pain or morphine exposure during heelstick for both groups HF no correlation to pain or morphine exposure during heelstick
Lindh, et al. (1999)	23 Full-term, 1 group Baseline 5 mins, warming 2 mins, sham heelstick 40 (no actual stick, just the motions and touch), sharp heelstick 40 secs, and squeezing values	Heel stick	Mean HR ↑ during heel lancing and squeezing. Total HRV↑ in heel lancing and ↓ in squeezing LF ↑ in heel lancing and ↓ in squeezing HF ↓ in squeezing
Lindh, et al. (2000)	Full-term, 2 groups: EMLA = 28 Placebo = 28 Baseline 5 min, warming 2 min, stick 80 seconds values	Venipunc-ture	During Venipuncture: HR higher in placebo than EMLA group Total HRV lower in placebo than EMLA group LF lower in placebo than EMLA group
Lindh, et al. (2003)	Full-term, 3 month old 2 groups: EMLA + glucose 60 mins before warming = 45, Placebo cream+water = 45 Baseline 3 mins, treatment administration, injection values	Immuniza-tion	Mean HR ↑ from baseline to injection in both grps Total HRV↑ from baseline to injection in both grps LF ↑ from baseline to injection in both groups HF no change from baseline to injection in both groups No sig. diff. between EMLA-glucose and placebo-water groups. Biphasic transient HR with a deceleration followed by a acceleration was more in placebo.
Oberlander, Grunau, Fitzgerald, & Ellwood, et al. (2002)	Full-term, 3 groups: Prenatal exposure to SSRIs SE = 22 SSRI exposure SE+ = 16 SSRI +clomazepan exposure Control = 23 No exposure Baseline 2.2 mins, lance 2.2 mins, recovery 2.2 mins values	Heel stick	Mean HR ↑ with stick in all groups. LF ↓ with lance and ↑ in recovery. HF↓ with lance and ↑ in recovery LF/HF no significant change. SE and SE+ grps had greater parasympathetic return in recovery than controls

Biochemical responses

Hormonal and metabolic changes can be observed following painful procedures. Documented metabolic responses to pain are increased secretion of catecholamines (increased norepinephrine and epinephrine), glucagon, and corticosteroids (increased cortisol) (Anand & Carr, 1989), and decreased prolactin, insulin, and immune responses (Gibbins & Stevens, 2001). The catabolic state induced by acute pain may be more damaging to infants who have higher metabolic rates and less nutritional reserves than older children and adults.

Anand and Scalzo (2000) have shown that pain in infants is associated with excessive NMDA/excitatory amino acid activation, resulting in excitotoxic damage to developing neurons. The metabolic changes promote behavioral phenotypes characterized by increased anxiety, altered pain sensitivity, stress disorders, and hyperactivity/attention deficit disorder which lead to impaired social skills and patterns of self-destructive behavior. Plasma renin activity increased significantly five minutes after venipuncture in full-term neonates and returned to basal levels 60 minutes thereafter (Anand et al, 1987). When preterm neonates received ventilation therapy, chest physiotherapy and endotracheal suctioning, plasma epinephrine and norepinephrine increased significantly (Anand et al, 1987). In neonates undergoing circumcision without anesthesia, plasma cortisol levels increase markedly during and after the procedure (Anand et al, 1987). In preterm and full-term neonates undergoing surgery with minimal anesthesia, a marked release of cortisol, catecholamines, growth hormone, glucagon, aldosterone and other corticosteroids, as well as suppression of insulin secretions was evident (Anand et al, 1987). Neonatal stress responses were three-to-five times greater

than those in adults, although the duration was shorter, possibly because of the lack of deep anesthesia (Anand et al, 1987). Changes in plasma stress hormone also were correlated with the behavioral states (Anders, Sachar, Kream, Roffwarg, & Hellman, 1970). Reduction in these stress hormone levels is a measure of the effectiveness of pharmacologic intervention (Anand et al., 1987). Biochemical measures are difficult to obtain in the critical care setting (Franck, 1989), but in the future, transcutaneous biochemical measures may provide objective data to support the assessment of pain in infants and to evaluate the effectiveness of the treatment regimen for relieving pain.

Consequences of Infant Pain

The experience of pain is associated with many harmful consequences classified by their short and long term effects. The **short term effects** are adverse physiological sequelae in all major organ systems. Many of these sequelae can be life threatening, such as reduced tidal volume, vital capacity, and oxygen saturation. Increased demands on the cardiovascular system include higher heart rate, decreased HRV and increased blood pressure (Van Cleve, Johnson, Andrews et al., 1995) which can also be life threatening. Hypermetabolism also occurs, creating a neuroendocrine imbalance which includes increased levels of plasma cortisol, catecholamines, beta endorphin, aldosterone, glucagons, and growth hormone (Anand, 1998; Franck, 1991). Increases in these hormones may greatly and rapidly produce reperfusion injury and venous congestion, which can lead to intraventricular hemorrhage (IVH) and /or periventricular leukomalacia (Abdel-Rahman & Rosenberg, 1994). The presence and extent of IVH is an important predictor of severe neurologic injury and early mortality (Wells & Ment, 1995).

Long-term effects of pain are related to the plasticity of the infant's nervous system. Ruda and colleagues (2000) found that nociceptive neuronal circuits are formed during embryonic and postnatal times when painful stimuli are normally absent or limited. When neonatal rats experience persistent hind paw peripheral inflammation (similar to repetitive heelsticks in human infants), these rats' spinal neuronal circuits exhibit increased input, segmental changes in nociceptive primary afferent axons, and altered responses to sensory stimulation as adults (Bhutta et al., 2001). Repetitive or prolonged exposure to pain and stress is believed to similarly permanently alter the human infant's neuronal and synaptic organization (Alvares, Torsney, Beland, Reynolds & Fitzgerald, 2000; Anand, 2000; Anand & Scalzo, 2000; Fitzgerald & Beggs, 2001; Larson, 2001), based on previous reports of abnormal behavior due to exposure to repetitive pain in preterm infants (Anand, 1998a; Johnston & Stevens, 1996). Other long-term consequences of repeated painful procedures are decreased sensitivity to the commonplace pain of childhood (Grunau et al., 1994; Oberlander et al., 2000), higher incidence of somatic complaints, somatization of unspecified origin, and increased excitability and responsiveness of neurons in the spinal cord. Spinal cord response changes may persist for minutes to hours after the original painful stimulus ceases or is blocked (Evans, 2001; Grunau et al., 1994). Inhibited development of self-regulation behaviors (Ryan, Kuhl, & Deci, 1997) and emotional and psychiatric disorders (Aisenstein, 1987) also result overtime. Interventions to alleviate pain are important if negative short and long-term consequences are to be prevented or minimized.

In summary, the characteristics of pain in preterm infants can be synthesized as having the following critical attributes: (1) Infants in NICUs have repeated, unpleasant,

distressful, and uncomfortable experiences; (2) Infants have limited pain modulation mechanisms; (3) Infants have multiple responses to a noxious stimulation including behavioral, physiological, and biochemical responses; (4) Infant pain responses are variable related to developmental factors, severity of the illness, and other subjective and contextual factors specific to the infant; (5) Infant pain is a complex phenomenon which is difficult to measure because infants cannot report that they are having pain or differentiate it from other sensations or stressors; (6) Infant pain is a unique experience that serves as a protective mechanism for self-preservation; and (7) Infant pain has short and long-term harmful effects due to the infant plasticity especially if the pain is not treated adequately.

Non-Pharmacological Pain Interventions

Non-pharmacological interventions for neonatal pain also are known as environmental and behavioral interventions (Stevens & Franck, 2001), and can be used singly or in combination with pharmacological interventions. The environmental strategies include reduction of noxious stimuli and implementation of neurobehaviorally supportive relationship-based care (e.g., family-centered approach). The behavioral strategies include containment or positioning (nesting, swaddling, maintaining flexed position and postural support), non-painful sensory stimulation (touch, massage, rocking, talking, music, intrauterine sounds, and visual stimuli), non-nutritive sucking, sucrose without non-nutritive sucking or with nutritive sucking. The non-pharmacological interventions can reduce neonatal pain indirectly by reducing the total amount of noxious stimuli to which the infant is exposed, and directly, by blocking nociceptive transduction or transmission or by activation of descending pain-modulating systems (Frank &

Lawhon, 2000). Several individual physical and behavioral pain management strategies have been studied, primarily within the context of acute pain from single painful events.

Sucrose/Glucose

The administration of oral sucrose/glucose has been the most frequently studied nonpharmacological intervention for the relief of procedural pain in the neonate. Sucrose is a sweet disaccharide consisting of fructose and glucose. The effects of sucrose are thought to be mediated by endogenous opioid pathways activated by sweet taste receptors at the tip of the tongue, and the effects endure after sucrose is injected (orogustatory effect) (Blass & Watt, 1999; Gibbins & Stevens, 2001a). Sucrose/glucose has been examined alone and in combination with non-nutritive sucking (NNS) and/or other pain interventions for its soothing (Barr et al., 1994) and pain-relieving effects in full-term (Abad et al., 2001; Allen, White, & Walburn, 1996; Bilgen, Ozek, Cebeci, & Ors, 2001; Blass & Hoffmeyer, 1991; Blass & Shah, 1995; Blass & Watt, 1999; Carbajal, Chauvet, Couderc, & Olivier-Martin, 1999; Eriksson et al., 1999; Gibbins et al., 2002; Gormally et al., 2001; Haouari, Wood, Griffiths, & Levene, 1995; Isik, 2000; Ors et al., 1999; Ramenghi, Griffith, Wood, & Levene, 1996; Rushforth & Levene, 1993) and preterm neonates (Abad, Diaz, Domenech, Robayna, & Rico, 1996; Bucher et al., 1995; Carbajal, Lenclen, Gajdos, Jugie, & Paupe, 2002; Gibbins et al., 2002; Johnston, Stremler, & Horton, 1997; Johnston, Stremler, Stevens et al., 1997; Mellah et al., 1999; Mitchell & Waltman, 2003; Overgaard & Knudsen, 1999; Ramenghi, Evans, & Levene, 1999; Ramenghi, Wood, Griffith, & Levene, 1996; Stevens et al., 1999). Several systematic reviews have been undertaken that have established the efficacy of sucrose in decreasing procedural pain in neonates (K. Bauer & Versmold, 2001; Benis, 2002; Franck & Gilbert,

2002; Mitchell & Waltman, 2003; Stevens & Ohlsson, 2000; Stevens et al., 1997; Stevens et al., 2001). Promising results have been observed in studies with both full-term and preterm infants.

Of the randomized clinical trials (RCTs) in full-term infants, researchers found that sucrose (12-70%) significantly reduced pain scores more than water (Bilgen et al., 2001; Blass & Watt, 1999; Gibbins et al., 2002; Gormally et al., 2001; Mellah et al., 1999; Overgaard & Knudsen, 1999; Ramenghi, Wood et al., 1996) and decreased the percentage or mean time spent crying (Abad et al., 1996; Bilgen et al., 2001; Blass, 1997b; Blass & Hoffmeyer, 1991; Blass & Shah, 1995; Blass & Watt, 1999; Gormally et al., 2001; Ors et al., 1999; Ramenghi, Griffith et al., 1996). In preterm infants RCTs found that sucrose (concentration of 24-70%) versus water or no treatment significantly reduced pain responses and pain scores (Bucher et al., 1995; Gibbins et al., 2002; Johnston, Stremler, & Horton, 1997; Johnston, Stremler, Horton, & Friedman, 1999; Mellah et al., 1999; Ramenghi, Wood et al., 1996), and reduced crying by 30 – 39 seconds (Abad et al., 1996; Bucher et al., 1995; Ramenghi, Wood et al., 1996).

Infants undergoing heels stick who received 30% glucose had a significantly reduced pain response and less crying than infants who received 10% glucose, water or no treatment (Eriksson et al., 1999; Skogsdal, Eriksson, & Schollin, 1997). One crossover trial of preterm infants compared 10% glucose with no treatment and found that Preterm Infant Pain Profile (PIPP) scores were significantly reduced during the heel stick (Bellieni et al., 2001). However, another RCT comparing 12% glucose to water found no significant difference on mean crying time (Abad et al., 1996). A reduction in

crying is important as it reduces the likelihood of adverse changes in cerebral blood flow (Anderson, 1989a; Ludington-Hoe et al., 2002).

In studies testing the concentration of sucrose or glucose for heel stick, Haouari found that increasing concentrations of 2 ml sucrose from 12.5%, 25% to 50% produced significantly greater reductions in the duration of crying (Haouari et al., 1995). However, two other studies did not find any difference in crying duration with different sucrose concentrations of 12-25% (Abad et al., 1996) or 25-50% (Ramenghi, Griffith et al., 1996). Results of several RCTs suggested that 30% oral glucose solution significantly alleviated pain compared with breast milk and 10% glucose did so in both full-term (Skogsdal et al., 1997) and preterm infants (Carbajal et al., 2002). However, Isik found that 30% sucrose was superior to 10% and 30% glucose solutions in relieving pain, showing sucrose's primary effect on crying time (Isik, 2000). Johnston and associates also compared a single dose (0.5 ml) of 24% sucrose 2 minutes before heel stick to three doses given 2 minutes prior to, immediately before, and during the procedure in preterm infants (Johnston, Stremler et al., 1999). Infants who received repeated doses had lower pain scores during the recovery period than infants who received single dose.

Meta-analyses (Stevens et al., 1997; Stevens et al., 2001) and reviews (Franck & Gilbert, 2002; Stevens & Franck, 2001) provide evidence that 2 ml of 12 to 24% sucrose given orally by syringe or pacifier 2 minutes before a single painful stimulus was associated with a statistically and clinically significant reduction in pain responses. However, no significant reduction in pain has been found to administering sucrose doses greater than 0.50g (2ml of 24% w/v sucrose), and no differences in crying time between preterm and term infants has been found with sucrose doses more than 0.50g.

However, repeated use of sucrose analgesia may have deleterious effects on infants < 31 weeks PCA. Infants who received repeated doses were at risk for poorer neurobehavioral development and physiologic outcomes (Johnston, Filion et al., 2002). The safety of repeated oral administration of sucrose or glucose needs further investigation. Additional studies are needed to determine the minimal effective dose and the efficacy and side effects of repeated sucrose doses for repeated blood sampling, especially in preterm infants. Studies are also needed to determine the most appropriate age of infants and duration of sucrose analgesia.

Formula/Breast Milk/Breastfeeding

Formula (Similac) has been compared with sucrose in soothing and reducing pain in neonate infants. Blass found that formula markedly reduced infant crying and the calm persisted for 3 minutes after substance delivery (Blass, 1997a). Formula also reduced infant crying during the heel stick blood collection procedure, but not during the post-treatment interval (Blass, 1997b). Blass concluded that formula and some of its components can reduce crying, but not during intense acute stimulation (Blass, 1997b).

Several randomized controlled trials investigated the effectiveness of human milk in reducing pain in newborns undergoing heel sticks. The RCTs found no evidence that human milk was better than water in reducing pain responses or crying in neonates undergoing heel stick. None found a significant effect of human milk on change in HR, duration of crying (Bilgen et al., 2001; Bucher, Baumgartner, Bucher, Seiler, & Fauchere, 2000; Ors et al., 1999; Skogsdal et al., 1997) or proportion of infants not crying (Skogsdal et al., 1997). The orosensorial antinociceptive effect of human milk is not as effective as an analgesic as a 25-30% sucrose solution.

In the investigation of the effect of breastfeeding on reducing pain, Bilgen (2001) found no significant effect of breastfeeding versus water on duration of crying. However, during the study, infants were allowed to suck for only 2 minutes before the heel stick, and not during the heel stick, and mothers were not allowed to swaddle or speak to their babies. In Gray's study, breastfed infants were cradled during breastfeeding and had full body skin-to-skin contact with their mothers during the entire procedure. Gray's study found that crying and grimacing were reduced by 91% and 84% respectively in breastfed infants but not in control infants during heel stick. Heart rate also was substantially reduced by breastfeeding in KC during heel stick (Gray et al., 2002).

Non-Nutritive Sucking/Pacifiers

Non-nutritive sucking (NNS) is the provision of a pacifier in an infant's mouth to promote sucking behaviors without breast milk or formula (Stevens & Franck, 2001). NNS as a strategy to prevent and manage neonatal pain has been well studied in infants, although the underlying mechanism remains unclear (Franck & Lawhon, 1998). Two mechanisms have been proposed: (1) NNS is thought to produce analgesia through nonopioid pathways by stimulation of orotactile receptors that activate the vagal nerve and elevate pain threshold (Barr et al., 1994; Dieter & Emory, 1997; Gibbins & Stevens, 2001a); and (2) Sucking is also hypothesized to trigger the release of serotonin, which may directly or indirectly modulate transmission and processing of nociception (Stevens & Franck, 2001; Zangen, Nakash, & Yadid, 1999).

The effects of pacifiers on heel stick pain in full-term and preterm infants have been tested in several randomized control trials. Pacifiers given two-to-five minutes before heel stick significantly reduced pain responses (Bellieni et al., 2001; Field &

Goldson, 1984; Greenberg, 2002; Stevens et al., 1999) and the percentage of time spent in distress, fussy, or awake state (Corbo et al., 2000; Field & Goldson, 1984). Stevens (1999) compared a pacifier dipped in 24% sucrose to one dipped in water during heel stick in preterm infants. Both treatments significantly reduced pain response indicated by PIPP scores. Bellieni (2001) performed a study to compare NNS plus 10% glucose, multimodal sensory stimulation (tactile, vestibular, gustative, olfactory, auditory and visual stimuli), 10% glucose alone, NNS alone, to no treatment in preterm infants undergoing heel stick. Sensory stimulation and NNS plus glucose had a greater analgesic effect than no treatment, and sensory stimulation was statistically better than NNS plus glucose. The NNS was also more efficacious than sucrose or glucose alone in reducing pain in term infants undergoing venipuncture (Carbajal et al., 1999).

Campos (1989) found that NNS soothed infants more rapidly than swaddling, but there was rebound distress when the pacifier was removed. NNS and rocking during heel stick in term neonates reduced crying, but NNS produced sleep, whereas rocking produced alert state (Campos, 1994). A study testing the effect of NNS, music therapy, and the combination of both on heel stick pain in term infants demonstrated that the combination of NNS and music therapy had the strongest effect on improving neonate's transcutaneous oxygen levels and reducing pain behavior (Bo & Callaghan, 2000).

Gibbins and Stevens (Gibbins & Stevens, 2003), in an investigation of the influence of gestational age (GA) on the efficacy and short-term safety of sucrose and NNS for procedural pain relief, found that the sucrose plus NNS was associated with the lowest pain scores in each GA group (27 – 31 weeks, 32 – 35 weeks, and > 36 weeks). Research on the effects of gestational age on the efficacy and safety of repeated doses of

sucrose is desirable (Gibbins et al., 2002). Based on the results of these research studies and the lack of evidence of any known untoward effect in preterm infants, NNS has generally been implemented as part of the current standard of practice in most NICUs (Stevens & Franck, 2001). The combination of sucrose and NNS is the most efficacious non-pharmacologic intervention for single heel stick pain to date.

Swaddling/Containing /Positioning

Swaddling, containing, and other positioning strategies have been selected as techniques to relieve pain induced stress by delineating the infant's boundaries, maintaining a flexed position, and providing constant stimulation simultaneously to the proprioceptive, thermal and tactile sensory systems (Campos, 1989; Stevens & Franck, 2001). At two weeks of age, full-term infants demonstrated slight decreases in heart rate and crying time after heel stick when swaddled (Campos, 1989). Swaddling and containment significantly reduced pain responses to heel stick, including lower mean HR, shorter crying time, and less behavioral disturbance in preterm newborns (Fearon et al., 1997; Taquino, 1994). However, Fearon (1997) reported that swaddling had no effect on behavior of infants aged <31 weeks PCA.

Prone compared with side or supine positioning was ineffective in reducing pain in preterm infants during heel lance (Grunau et al., 2004; Stevens et al., 1999). However, Grunau and colleagues (2004) found that prone position promoted deep sleep at 32 PCA, which confirmed the finding of the previous study that ventilated preterm infants when prone compared with supine over 2 hours, had less crying, less active sleep, more quiet sleep and fewer startle, tremor, and twitch (Chang, Anderson, & Lin, 2002). Positioning interventions require further investigation in regard to variation in duration and positional

techniques to delineate their usefulness as strategies for pain control.

Holding/Rocking/Multisensory Stimulation

Several behavioral strategies of pain management involve varying amounts of contact with humans, such as holding, rocking, massage, and multisensory stimulation. Contact techniques provide tactile, vestibular, and kinaesthetic stimuli to the infant, and have been reported to modulate behavioral state and decrease stress behaviors (White-Traut, Nelson, Silvestri, Cunningham, & Patel, 1997). However, few studies have specifically evaluated the efficacy of these techniques in preventing, eliminating or reducing pain (Stevens & Franck, 2001). Facilitated tucking (holding the infant's extremities flexed and contained close to his trunk) in preterm infants during heel stick reduced mean heart rate, decreased crying time, and reduced sleep disturbance time (Corff et al., 1995). Holding of the infant by his/her mother or the research assistant during heel stick significantly reduced pain scores and duration of crying in full-term infants (Gormally et al., 2001; Savaser, 2001). Rocking was compared to pacifiers during heelstick in healthy term neonates (Campos, 1994): both interventions reduced crying, and rocking produced alert states while pacifiers predominantly produced sleep states. However, Johnston's study found no significant difference of rocking compared with no rocking on reduction of heel stick pain in preterm infants (Johnston, Stremler, Stevens et al., 1997).

McIntosh and colleagues found that tactile and vocal stimulation presented together slightly decreased physiological distress caused by heelstick (McIntosh, van Veen, & Brameyer, 1994). Bellieni and associates investigated whether multisensory stimulation consisting of tactile (massage), auditory (voice), visual (eye contact),

gustatory (glucose) and olfactory stimuli (perfume) was effective analgesia when compared to oral glucose or NNS alone in full-term (Bellieni et al., 2002) and preterm infants (Bellieni et al., 2001) during heel stick. Mulitsensory stimulation was found to be most effective reducing pain scores and crying duration both in term and preterm infants.

However, one study reported that handling and immobilization had no effect on reducing infant heel stick pain and handled infants even had a higher mean HR, greater behavioral arousal, and displayed more facial activity as compared with nonhandled infants (F. L. Porter, Wolf, & Miller, 1998). The findings suggested that infants who undergo common nursery experiences such as handling and immobilization as part of their routine care may exhibit greater physiological and behavioral reactivity to subsequent painful procedures. Further investigation of holding, rocking, handling and multisensory stimulation as potential sources of analgesia in infants is needed to conduct.

Odor/Familiar Odor/Maternal Odor

The role of olfaction as a soothing tool in full-term infants has been examined in several studies (Goubet, Rattaz, Pierrat, Bullinger, & Lequien, 2003; Kawakami et al., 1997; Sullivan & Toubas, 1998; Varendi, Christensson, Porter, & Winberg, 1998). The effects of odor were based on the newborn's particular sensitivity to maternal odors (R. H. Porter & Winberg, 1999) and/or odors acting as distracters (Goubet et al., 2003). Sullivan and Toubas assessed the infant's responsiveness to presentation of his/her own mother's odor (hospital gown) and other mother's odor compared with clean gown and no gown (Sullivan & Toubas, 1998). The results indicated that crying babies stopped crying when either own mother or other mother odor was presented, and awake babies responded specifically to their own mother's odor by increasing mouthing. Varendi and

associates examined whether the odors of amniotic fluid and mother's breasts influence the crying of the full-term newborn when separated from its mother (Varendi et al., 1998). Infants exposed to amniotic fluid smell cried significantly less than babies in the breast odor and control groups.

When maternal milk odor (familiar order) was presented during a heel stick, infants cried and grimaced significantly less during the recovery phase compared with the heel stick phase, whereas, infants who were presented with an unfamiliar odor or no odor showed no significant changes during recovery. Infants who smelled their mother's milk also exhibited significantly less motor agitation during the heel stick compared with the other groups (Rattaz, Goubet, & Bullinger, 2005). Kawakami and associates assessed the effect of artificial odors (lavender and milk) on behavioral and physiological responses to heel stick (Kawakami et al., 1997). They found that neither the lavender nor the milk group had reduced crying, but had lower cortisol levels than the control group, suggesting that presentation of odor stimuli has an effect on physiological responses to painful procedures. Another recent study examined the effects of a familiar odor (vanillin) during blood draws (heel stick and venipuncture) in preterm newborns (Goubet et al., 2003). Heel sticks were found to elicit more behavioral distress than venipunctures. By comparison, infants presented with a familiar odor either during the heel stick or the venipuncture had a significant decrease in crying and grimacing than infants presented with an unfamiliar or with no odor.

The mechanisms underlying the effectiveness of maternal odors and other odors on soothing and reducing pain are unknown. Possibly that the phenomenon is opioid mediated because the olfactory and gustatory (sucrose reducing pain) systems are highly

interconnected. Animal studies have demonstrated that positive odors decrease and negative odors increase nociceptive behaviors, which represent the opioid-mediated interactions between olfaction and pain (Jahangeer, Mellier, & Caston, 1997). Additionally, the opioid system plays a role in olfactory learning and odor preference in rats (Roth & Sullivan, 2001; Shide & Blass, 1991).

Sound/Music/Maternal voice

Therapeutic effects of sound, music, maternal voice, and heart beat on soothing and reducing pain responses have been examined in infants (Butt & Kisilevsky, 2000; Kawakami et al., 1996; Kurihara et al., 1996; Standley, 1998; Standley & Moore, 1995). Presenting heart beat sounds and white noise to full-term infants during heel stick had a calming effect, including less reactive behavioral responses and less salivary cortisol release than the control group who received neither (Kawakami et al., 1996).

The homogeneity of findings in a meta- analysis suggested that music had statistically significant and clinically important benefits for premature infants in the NICU (Standley, 2002). Music has been demonstrated to modulate both physiological (HR) and behavioral (state of arousal and facial expression of pain) responses of preterm infants more than 31 weeks PCA following a stress-provoking heel stick (Butt & Kisilevsky, 2000). Standley and Moore (1995) used music (lullabies) and mother's voice as an intervention to reduce the risk of complications in oxygenated preterm infants, and found that mother's voice and music could regulate and stabilize the infant's oxygen intake. Mother's voice, which is repeatedly experienced before birth, becomes familiar to the fetus, thus the neonate responds selectively by orienting to it and showing HR decelerations; alien voices produce HR accelerations (Fifer & Moon, 1994; Ockleford,

Vince, Layton, & Reader, 1988). Positive effects of sound, music, and maternal voice on soothing preterm and full-term infants have been found.

Conceptualizations of Kangaroo Care (Skin-to-Skin Contact)

Rey and Martinez originated the term "Kangaroo Care" (Rey & Marinez, 1983). The name KC is derived from its similarities to marsupial caregiving, in which the prematurely born kangaroo is guided into the mother's pouch, where it is kept warm, contained, and breast fed until maturation (Ludington-Hoe et al., 1994). Since 1983, the method has been called as kangaroo baby care, kangaroo care, kangaroo method, kangaroo mother care, kangaroo mother method, mother kangaroo program and skin-to-skin contact. However, the most commonly names in published reports are Kangaroo Care or Skin-to-Skin Contact (SSC). During KC (SSC), a mother holds her diaper-clad preterm infant upright and prone between her breasts and allows self-regulatory breastfeeding as the mother sits or recline in padded rocking chairs that tilt back to about a 60-degree angle (Anderson, 1989b). Rey and Martinez emphasized an essential triad of factors that form the core of KC: mother's love, warmth and breast milk (Martinez, Rey, & Marquette, 1992). KC provides a modified uterine-like environment for preterm and critically ill infants to support them in their adaptation to the new world.

Attributions of KC

Separation of mothers from their neonates at birth has become standard practice in Western countries, despite mounting evidence that separation may have serious harmful effects (Franck, Bernal, & Gale, 2002; Kuhn & Schanberg, 1998). KC involves several critical factors (Figure1) including its being a simulated uterine environment, being natural and being human, closeness and touch, relaxation and calm, nutrition and love,

and mutual caregiving between the mother and the infant. Many of these factors are unique and missing from other intervention strategies designed to counteract separation. Each critical attribute is described below.

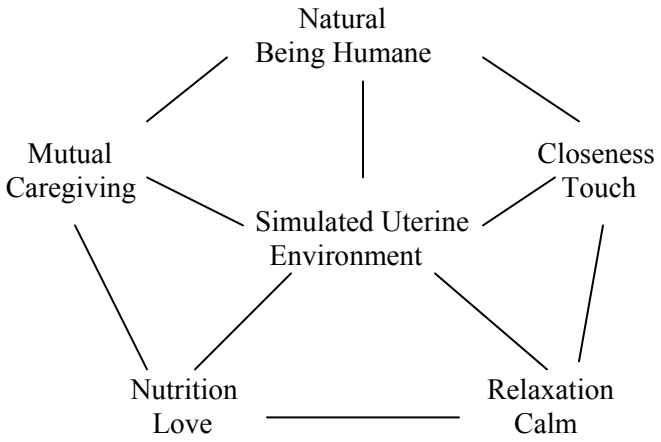


Figure 2. Critical attributes of kangaroo care

A simulated uterine environment. KC is an intervention for modifying the environment so it more closely approximates the world within the womb. The baby is positioned prone, contained, and nested in KC. Anderson (1999) describes the KC environment at the breast as a familiar maternal milieu.

Gale (1998) reported that birth represents a habitat transition, while the basic needs for a baby remain the same. At birth, the environment of the NICU probably comes as quite a shock to the baby as it is full of overwhelming auditory, visual and other noxious stimuli. The preterm infants' developmental needs are not optimally met when they live in the incubator. The uniqueness of KC is that it provides an appropriate balance between the understimulated tactile-propioceptive system and the overwhelmed later-developing sensory modalities (Feldman & Eidelman, 1998). KC can evoke quiescence, decrease arousal and significantly increase the amount of quiet sleep just as

uterine environment does (Ludington-Hoe & Swinth, 1996). Kangaroo Care's simulation of the uterine milieu is a major attribute of the KC concept and practice.

Natural and humane. The first modern neonatologist, Pierre Budin, allowed mothers to help care for their preterm babies. However, in 1896, the baby nursery (or incubator) was established where premature infants were cared-for and taken away from their mothers (Rey & Martinez, 1983). At the beginning of the twentieth century, preterm infants and their mothers were completely separated because of the establishment of isolation techniques in the nursery. The mother could only meet her infant when the infant was discharged from the unit, sometimes as late as 3 months post birth in some institutes. The efficient modern medical technology common today is not considered by all to be a humane method of care for preterm infants (Bergman & Jurisoo, 1994; Charpak, Ruiz, & Figueroa de Calume, 2000); KC is a more humane approach.

KC is based on a deep respect for natural processes (Anderson, Marks, & Wahlberg, 1986). Most mammals use parental body warmth and breastfeeding to ensure their offspring survive (Charpak et al., 2000). The mother protects her baby from a hazardous environment, and the child regards his/her mother as a shield and analgesic. KC brings a set of natural or humane values to the highly technical care of infants, including human sounds, smells, touch, warmth, emotion, and natural feeding directly from the mother on the baby's demand. KC's use is still limited due to concern over potential heat loss in preterm infants, but this concern underrates the mother's ability to keep the infant warm. Numerous studies have demonstrated that infants experience body heat gain (abdominal, axillary, rectal, leg, and toe temperatures) (Ludington-Hoe et al., 2004; Ludington-Hoe et al., 1991; Ludington-Hoe et al., 1992; Ludington-Hoe et al.,

2000; Swinth, Nelson, Hadeed, & Anderson, 2000). KC is a natural experience that also empowers mothers in their role as primary provider of the physical and emotional needs of their fragile infants (Charpak et al., 2000). In addition, KC does not need expensive, sophisticated equipment and KC's simplicity permits it to be applied almost everywhere with every infant and mother. KC contributes to the humanization of neonatal care and to better bonding between mother and baby in all types of populations.

Closeness and touch. KC is a synonym for skin-to-skin contact; SSC is an essential element of KC. KC provides the opportunity for the infant to be near the mother's chest, which is the ideal ecological niche for the newborn (Bergman & Jurisoo, 1994), creating a very special kind of closeness between mother and baby, both physically and emotionally. KC is different from other kinds of touch such as handling, stroking, swaddling, containing, and massaging in many ways. First, these other kinds of touch cannot provide the infant with the whole mother – full body contact and love. Second, hypoxia has been associated with handling during nursing or medical procedures (Long, Philip, & Lucey, 1980; Norris, Campbell, & Brenkert, 1982). Third, stroking results in behavioral distress and decreased transcutaneous oxygen pressure levels in preterm infants 26-30 weeks gestational age (McGehee & Eckerman, 1983; Oehler, 1985). Even with gentle human touch (e.g. a hand resting gently on the infant's thigh), $TcPO_2$ levels decrease in spite of increased respiratory regularity (Harrison et al., 1996). Gentle human touch may have no effect on heart rate or oxygen saturation of preterm infants, even though it is supposed to be soothing to preterm infants (Harrison, Leeper, & Yoon, 1990; Harrison et al., 1996; Harrison et al., 2000; Tribotti, 1990). The benefits of massage to preterm infant developmental outcomes are still unclear, even though Field's

research program provides encouraging results (Field, 2002) and massage can reduce infant stress (Gitau et al., 2002). The cost-effective use of time to administer massage or provide KC should be considered too (Vickers, Ohlsson, Lacy, & Horsley, 2000). Closeness and the gentle human touch of KC are important communication methods between the mother and the baby.

Relaxation and calm. Present medical and nursing care of premature infants is often life-saving, but the highly technical environment may be hazardous to a small, fragile infant. The artificial bright light and noise and intrusive stimuli of the NICU are stressful to preterm infants whose physiological demands are already great (Sims, 1988). Prolonged hospitalization in the NICU creates significant emotional, physical and financial stresses for infants and their families. KC is described as a stressless condition (Ludington-Hoe et al., 1994) which provides a humanized approach to protect infants from noxious stimuli and reintroduces the familiar, soothing stimuli of the other's heart beat, respirations, voice, containment, motion, warmth, and touch.

Relaxation is a condition in which muscles become less tonic, and visible signs of tension disappear (Ludington-Hoe & Swinth, 1996). Infants receiving KC usually are calm and relaxed in terms of quiescence of motor activity and the need for self-consoling behaviors such as sucking or hand-to-mouth maneuvers is eliminated (Ludington-Hoe & Swinth, 1996). Mothers have noticed the quiescence: “My baby is always restless in the incubator. With me, it sleeps quietly and is relaxed” (Stening & Roth, 1993). The database supporting KC’s quiescent effects is extensive. With KC, preterm infants have significantly lower activity level (K. Bauer, Pyper, Sperling, Uhrig, & Versmold, 1998; Ludington, 1990; Ludington-Hoe et al., 1999; Ludington-Hoe et al., 1994; Tornhage,

Stuge, Lindberg, & Serenius, 1999; Wahlberg, Persson, & Affonso, 1990) and have a 2.5 fold quiet regular sleep increased (Chwo et al., 2002; Ludington-Hoe et al., 1992; Ludington-Hoe et al., 1994). Crying is virtual nonexistent (Ludington, 1990) and KC infants at 6 months after discharge cry less than controls (Whitelaw et al., 1988). KC reduces serum cortisol by 66%, β-endorphin by 74% (Mooncey et al., 1997), and salivary cortisol by 60% (Gitau et al., 2002) when given for 20 minutes after an incubator heelstick or after an infant has rested in an incubator.

Mothers also experience relaxation with KC. Mothers commonly express relief and intense pleasure in being able to hold their infant closely. "I felt very calm and relaxed and felt so comforting to have her on my chest." A father reported that "the first time I was all tense and motionless. Later the peacefulness of the child engulfed me." (Ludington-Hoe & Golant, 1993) Results of a study by Ludington and colleagues (1995) demonstrated that the overall stress level was significantly lower in the mothers using KC than in the control mothers on the fifth day of KC, a finding similar to Morelius et al. (2005) data on how maternal stress dropped from the first to the forth KC session.

Nutrition and love. Nutrition and love are essential for the infant's optimal survival, growth, and development. Both contribute to improved physical, psychological, social and emotional developments for preterm infants. Anderson posits that self-regulatory breastfeeding is the crucial element of complete KC and exclusive breastfeeding should be the source of nutrition and protection in the first months of life (Anderson, 1991). Fortunately, KC mothers are more inclined to breastfeed their in (Mikiel-Kostyra, Boltruszko, Mazur, & Zielenska, 2001), produce more milk (Thompson, 1996), lactate longer (Furman, Minich, & Hack, 2002; Mikiel-Kostyra, Mazur, &

Boltruszko, 2002), be breastfeeding at discharge (Anderson, in press; Charpak et al., 2001; Lima et al., 2000; Ramanathan et al., 2001). Thus, KC's role in breastfeeding is well established and KC should be used to promote breastfeeding.

Mutual caregiving process. KC is a reciprocal or mutual caregiving process (Anderson, 1991). Both the physiologic and psychological state in infants and their parents will be affected by KC. Numerous studies have shown that KC improves preterm infants' cardiorespiratory stability (Fischer, Sontheimer, Scheffer, Bauer, & Linderkamp, 1998), prevents body heat loss (Ludington-Hoe et al., 2000), increases quiet sleep (Chwo et al., 2002; Feldman, Weller et al., 2002), reduces pain response (Gray et al., 2002; Gray et al., 2000; Johnston et al., 2003; Ludington-Hoe et al., 2005), and enhances development (Dodd, 2005; Feldman & Eidelman, 2003; Feldman, Eidelman et al., 2002; Feldman, Weller et al., 2002; Feldman et al., 2003; Tessier et al., 2003). The interaction between the infant and the parent can help the preterm baby adapt to external environmental events, supporting and promoting the infant's neurobehavioral organization (Feldman & Eidelman, 2003). An interactionally organized infant is one who can respond to maternal input and presence in such a way as to encourage additional interaction without giving distress cues (Ludington-Hoe & Swinth, 1996). Although it is rare that infants younger than 40 weeks' postconceptional age can demonstrate interactional organization (Gorski, Davison, & Brazelton, 1979), some preterm infants have been observed gazing intently at the parent and maintaining the gaze for exceptional lengths of time during KC (Gale, Franck, & Lund, 1993). Once alertness has been achieved, the infant may be able to focus his or her cognitive skills on attentiveness, processing input from the environment.

KC reduces parents' anxiety by having them interact with their preterm infant. Parents are often anxious and might not be prepared for a sudden premature birth. Mothers of preterm infants suffer feelings of guilt and low self-esteem because they believe the prematurely of the baby is their fault (Davis & Spurr, 1998; Stening & Roth, 1993). Parent's confidence is enhanced and maternal grief process related to premature birth resolved during KC (Affonso et al., 1993). KC mothers comment that they feel more familiar with their babies, can handle them without the fear of dropping or hurting them, feel more at ease about positioning them to breastfeed, and can be even more excited about the infant coming home (Affonso et al., 1993). Fathers comment that much of their worry regarding their ability to handle their babies dissipates with KC (Parker & Anderson, 2002). In summary, KC empowers the parents to learn about their infant's state, behavioral cues, positioning, comfort, and improves their parenting skills. With KC experience, maternal comments reflect an awareness of infant behavior: "This is the first time I have heard him burp") (Ludington-Hoe & Golant, 1993 p9).

KC Provides Multi-sensory Stimulation to Modulate Pain Perception

Mother's physical contact with her preterm infant through direct skin-to-skin care provides tactile, olfactory, auditory, thermal, and proprioceptive stimulation in a unique interactive style. Previous investigations have noted in both animals and human infants that interventions providing separate components of the maternal proximity constellation, such as maternal holding, or odor, or voice, and or rocking modulated pain responses.

Maternal proximity and social stimulation. Infants of many mammalian species experience isolation as a stressful event. Rat pups separated from their mothers show diminished growth, increased apoptosis, heightened stress reactivity, delayed prefrontal

brain growth, and disturbed orientation (Anand & Scalzo, 2000). A large body of literature elaborates on the effects of maternal care-giving on decreased HPA-axis responses (Francis & Meaney, 1999; Liu, Caldji et al., 2000; Liu et al., 1997; Plotsky, Thrivikraman, & Meaney, 1993), enhanced neuronal survival and synaptogenesis in the hippocampus (Kuhn & Schanberg, 1998; Rojas et al., 2003; Schanberg, Evoniuk, & Kuhn, 1984), and improved performance in cognition tests, including animal studies that clearly show some of the underlying mechanisms (Hofer, 1994; Meaney, 2001). The findings provide evidence for the importance of parental care as a mediator of the effects of environmental adversity on neural development (Meaney, 2001). The results have helped to formulate the mechanism of KC on providing neural developmental benefits in preterm infants (Bhutta & Anand, 2002).

Tactile stimulation. Touch and proprioception are the primary senses in the newborn period (Feldman & Eidelman, 2003). However, much of the tactile stimulation that is experienced by preterm infants is intrusive, painful, and aversive to learning (Harrison et al., 2000). Small preterm infants often become hypoxic following tactile stimulation associated with medical or nursing procedures, parents' visits, or just plain tactile stimulation (Harrison et al., 1990; Harrison et al., 2000; Oehler, 1985). Caregivers in NICU limit the amount of comforting touch to preterm infants because of concerns about the potentially negative effects of tactile stimulation, including hypoxia, crying, behavioral agitation, and increased levels of intracranial pressure (Harrison & Woods, 1991). However, recent studies have documented that gentle human touch has a soothing effect as evidenced by decreased levels of active sleep, motor activity, and behavioral distress (Harrison et al., 1990; Harrison et al., 1996). Dieter and Emory (1997) have

suggested that tactile stimulation may activate the vagus nerve by stimulating peripheral nerves, thereby, promoting infant comfort and reducing stress, in addition to increasing the release of gastrointestinal hormones and enhancing weight gain; resulting in positive immediate and longer term outcomes. KC provides a continual, non-phasic tactile and proprioceptive stimulus, thereby modulating infants' stress and pain responses (Gitau et al., 2002; McCain, Ludington-Hoe, Swinth, & Hadeed, 2005).

Positioning stimulation. Positioning and handling the preterm infant in a flexed posture mimics intrauterine conditions, supports the development of flexor tone prior to term, and provides the infant with a sense of containment that allows better self-organization (Aucott, Donohue, Atkins, & Allen, 2002). Ventilated preterm infants during the first week post-birth who were in a prone position as compared with supine or side-lying had fewer episodes of oxygen desaturation, improved respiratory function, less crying, less active sleep, more quiet sleep states, and fewer stress responses (startle, tremor, and twitch) (Chang, Anderson, Dowling et al., 2002; Chang, Anderson, & Lin, 2002; Maynard et al., 2000). Thus, prone position may help conserve energy and assist infants' extrauterine adaptation.

Auditory stimulation. Infants know their mothers' voice and heart beat from the uterine environment (DeCasper & Fifer, 1980; DeCasper & Prescott, 1984). A review of studies of mother-infant interaction showed that the sound of the mother's voice has soothing effects (Sieratzki & Woll, 2002). Both the newborn and fetus show heart rate decelerations in response to mother's speech sounds (Fifer & Moon, 1994). Early experience with mother's voice may have both acute and enduring effects on the developing brain, as well as on later social and emotional development (Fifer & Moon,

1994). Newborns shown a peaceful Buddha-like facial appearance ceased to cry, did not open their eyes, and hardly moved either extremity after listening to their mothers' gentle voice (Fifer & Moon, 1994; Nakajima, 1994). Hearing the recorded maternal heartbeat also reduces behavioural responses to pain and the level of cortisol and dehydroepiandrosterone (DHEA), while the recorded sound of the Japanese drum with the same rhythm showed no significant effect (Kurihara et al., 1996). The mother's voice and heart beat can be perceived by the infant as he/she lies against her chest during KC, suggesting the potential for soothing effects on infant response to pain.

Vestibular stimulation. Rhythmic motions of maternal breathing provide vestibular stimulation to preterm infants. Rhythmic stimulation has been provided by oscillating or rocking mattresses, waterbeds, and a breathing teddy bear that is placed in isolettes (Aucott et al., 2002). Vestibular stimulation facilitates quiet sleep (Thoman, Ingersoll, & Acebo, 1991). Infants experienced decreased HR, longer durations of quiet sleep, fewer active awake and fussy states, and fewer state changes and awakenings while on a waterbed, resulting in reduced energy expenditure (Deiriggi, 1990; Deiriggi & Miles, 1995). Rocking is thought to mimic maternal walking, and mimic movements experienced by the infant in uterus. Rocking by the mother has been reported to decrease pain response in full-terms (Campos, 1994).

Olfactory stimulation. Infants apparently know their mothers through olfactory memory of amniotic fluid, which is similar to breast milk in odour (R. H. Porter & Winberg, 1999; Varendi, Porter, & Winberg, 1996). Amniotic fluid odor and maternal breast odor elicit positive responses by human infants such as head orienting, and moving towards and reaching for the odor, and crying significantly less than infants in the control

groups who were separated from their mothers (Varendi et al., 1998; Varendi & Porter, 2001). These studies provide evidence of the human infant's fine olfactory discrimination capacity, and indicate that maternal odors presented during KC may play an important role in the mediation of infant behavior and in reduction of pain responses.

In summary, the use of KC as a source of multiple sensory stimuli may help it as a pain response reducer. Based on the evidence presented above, the combination of tactile, auditory, vestibular, olfactory, and positioning stimulation should decrease pain response. The effects of multisensorial stimulation have been documented as being statistically better than a single sense as pain treatment in infants (Bellieni et al., 2001)

Effects of KC on Modulating Pain Responses

Kangaroo Care, through multiple-sensory stimulation, may also provide analgesia when infants undergo painful stimulations. Four studies were found in the literature investigating the effects of KC on reducing procedural pain (heels stick and venipucture).

Gray and colleagues (2000) conducted a randomized controlled trial that compared healthy term neonates, 15 of whom were given KC for 10-15 minutes before heel stick and remained in KC for the heel stick, and 15 who were swaddled in their crib during a heel stick for routine blood sampling. HR, crying time and facial grimacing (using the three actions recorded in the PIPP) were recorded for a two minute baseline, throughout the 155-159 second blood draw, and for a three minute recovery period. Crying was reduced by 82%, grimacing by 64%, and heart rate increased 8-10 bpm in the KC group as compared to 36-38 bpm in the swaddled group. Similar findings occurred when pain responses were measured during a heel stick conducted when the infant was breastfeeding in the KC position (Gray et al., 2002).

Johnston and colleagues (2003) reported the recent completion of a cross-over study of 74 healthy preterm infants (mean GA = 33.6 $\pm$ 1.1 wks; mean birth weight = 2033 $\pm$ 406 gms) who received 30 minutes of KC prior to and during a heel stick and later remained in an incubator prior to and during another heel stick within four days of the first heel stick. The order of KC or incubator care was randomly determined. Mean PIPP score was calculated for each 30 second period for two minutes following completion of the heel stick. No significant effects for site of heel stick, order of condition, or severity of illness were found. Pain scores were significantly lower in the KC condition at 30 seconds (PIPP = 10.1 KC, 11.6 incubator), 60 seconds (PIPP = 10.7 KC, 12.9 incubator), and 90 seconds (PIPP = 10.3 KC, 12.1 incubator) post-heel stick, but not at 120 seconds (10.7 KC vs 10.07 incubator). Johnston's study was tightly controlled as all infants were tested within 10 days of hospitalization, but no measure of the number of previous invasive procedures was taken, and only behavioral responses were reported. The effect of KC on physiologic and biochemical responses to pain was not studied, and is a contribution this dissertation will make.

Ludington-Hoe and associates (2005), in a study of 24 preterm infants given three hours of KC or incubator care in a randomized cross-over design, found less crying during KC heel stick (5.0 seconds for KC heel stick vs 43.0 seconds for incubator heel stick) and three infants did not cry at all during the KC heel stick. Infants also slept more during KC than in the warmer. Mean rise in HR from baseline to heel stick was less in the KC condition (3 - 13 beats/min) than in the incubator condition (8 - 23 beats/min). The authors concluded that KC positioning before and during heel stick is a simple and inexpensive analgesic intervention to ameliorate pain in stable premature infants.

The clinical trials described above support the probability that KC reduces pain responses, including crying, grimacing, change of heart rate, and serum and salivary cortisol in full-term and preterm infants undergoing procedural pain. Based solely on Gray's report, Stevens and Franck (2001) stated that further investigation of KC as a potential source of analgesia in neonates is most certainly warranted, a position supported by others (Franck & Gilbert, 2002; Johnston, Stevens et al., 2002). In addition to the four RCTs reviewed, studies showing the soothing effect of KC regarding behavioral, physiological, and biomedical pain response further support the proposed hypotheses.

Modulating Behavioral Pain Responses

Crying. In a recent Cochrane review, a large between group (KC versus control) difference in infant crying was found (OR 21.89, 95% CI 0.36 to 1.11) (Anderson et al., 2003). Several studies have demonstrated that full-term and preterm infants have less crying during KC (Chwo et al., 2002; Gray et al., 2000; Mazurek et al., 1999; Whitelaw et al., 1988), and spectrographic cry analysis has shown that KC infant crying was less distressful (Michelsson et al., 1996). Mazurek and associates reported that full-term infants in KC cried 3 times less than infants wrapped and kept separate from their mom, and less than infants wrapped in blanket and held by mom (Mazurek et al., 1999). Full-term infants in a cot cried 10 times more than KC infants, and the spectrographic analysis showed that the discomfort cry was elicited mainly by separation from the mother (Michelsson et al., 1996). During 60 to 90 minutes KC, infants were observed to have no crying or to cry no more than one minute (Christensson, Cabrera, Christensson, Uvnas-Moberg, & Winberg, 1995; 1992).

During KC with preterm infants, crying is virtually non-existent (de Leeuw, Colin, Dunnebier, & Mirmiran, 1991; Ludington, 1990), suggesting that KC is a comforting experience, supported by the increased number of alpha wave periods during KC on EEG (Ludington, Scher, Morgan, & Johnson, 2003). Preterm infants during 60 minutes of KC had less crying compared to control infants (2% vs. 6%, p=.000) (Chwo et al., 2002). When fathers gave KC for 2 hours in preterm infants' first 24 hours of life, fussiness, crying and hard crying occurred infrequently (Ludington-Hoe et al., 1992). Preterm infants of 25-to-36 wks gestational age who were given unlimited access to KC during their NICU stay, cried less at 6 months of age than control infants who did not receive KC (Whitelaw et al., 1988).

An infant's cry is a stress behavior and one of the most potent distress signals the infant offers (Ludington-Hoe et al., 2002). In premature infants, crying clearly occurs in response to pain. Because KC is a very effective method for preventing crying based on the results from prior studies, KC could be a promising strategy to minimize the crying response to pain.

Sleep and Behavioral States: Several studies have shown that one-to-three hours spent in KC results in increased frequency of quiet sleep, longer durations of quiet sleep, and more alert wakefulness, reduced transitory states, reduced active sleep, and improved organized sleep-week cyclicity during and after KC (Chwo et al., 2002; Feldman & Eidelman, 2003; Feldman, Weller et al., 2002; Gale et al., 1993; Gale & VandenBerg, 1998; Ludington, 1990; Ludington, Morgan et al., 2003; Ludington-Hoe et al., 1992; Ludington-Hoe et al., 1994; Messmer et al., 1997). A term newborn in KC is quiet, but if separated and put in a cot, the newborn cries – a cry which has qualities in common with

the "distress call' observed in animal species (Christensson et al., 1995). Within seconds of restored contact with mother, the infant becomes quiet. Being in a calm and comforted state during KC is suggested by the autonomic stability observed during KC (Bosque, Brady, Affonso, & Wahlberg, 1995; Ludington, 1990). Full-term infants have increased sleep time and reduced awake time during KC (Karlsson, 1996).

In preterm infants, many studies have shown that KC has both short- and long-term effects on infants' sleep-wake patterns. The average duration of quiet sleep bouts increases during KC, and is twice as long in KC as in comparable pre-KC and post-KC periods (Ludington, 1990; Ludington-Hoe & Swinth, 1996; Ludington-Hoe et al., 1994). During paternal KC, preterm infants spend 45% to 65% of time in quiet regular sleep, and active and quiet irregular sleep followed by very active sleep and awakeness were barely present (Hosseini, Hashemi, & Ludington, 1992; Ludington-Hoe et al., 1992). KC infants had longer rest/deeper sleep period than pre- and post- KC in incubator (K. Bauer et al., 1998; Messmer et al., 1997). During 60 minutes of KC, preterm infants had more quiet sleep compared to control infants (62% vs. 22%, p=.000) (Chwo et al., 2002). Feldman and Eidelman (2003) found that one hour of KC per day for at least 14 consecutive days starting when preterm infants were 31-33 weeks PCA resulted in increased quiet sleep, decreased active sleep, and accelerated self-regulation by 37 weeks PCA when compared to 35 preterms who did not receive KC.

Thus, another relationship between KC and pain responses exists. The state relationship dimension of neurobehavioral organization involves the preterm infant's ability to make smooth transitions between sleep, quiet, and awake phases, and to maintain the most desirable state of quiet sleep (Ludington-Hoe & Swinth, 1996). Infants

in quiet sleep show the least facial reaction and longest latency to cry in comparison with those in quiet awake state (Craig & Grunau, 1993; Grunau & Craig, 1987). State is such an influential contextual factor for pain that it is included in the scoring of the PIPP (Stevens, Johnston, & Horton, 1994). Early pain studies reveal that the behavioral state of the infant at the time of the painful procedure is correlated with changes in plasma cortisol. In quiet and sleep states, plasma cortisol increases are less than when the infants are active or awake (Anders, Sachar, Kream, Roffwarg, & Hellman, 1970; Tennes & Carter, 1973). Because of the unique effects of KC on the integration of self-regulation and stimulation (Feldman & Eidelman, 1998), and because quiet sleep is associated with diminishing pain responsivity (Franck & Lawhon, 1998; Stevens & Johnston, 1994; Stevens et al., 1994), it use of KC as a method to decrease the physiological response to pain appears logical.

Modulating physiologic and autonomic pain responses

HR, RR, and oxygenation saturation: Many studies reported that during KC, infants have more stable heart rate, decreased apnea and bradycardia (Clifford & Barnsteiner, 2001; Ludington-Hoe et al., 1991; Ludington-Hoe & Swinth, 1996; Mazurek et al., 1999; Messmer et al., 1997), and improved oxygen saturation (Cleary, Spinner, Gibson, & Greenspan, 1997; Fohe et al., 2000; Gazzolo et al., 2000; Hadeed et al., 1995; Ludington-Hoe et al., 1994; Messmer et al., 1997). Studies have also shown that oxygen saturation rises and respiratory support can be reduced for ventilated babies during KC (Drosten-Brooks, 1993; Fohe et al., 2000). Some studies have shown that there is no significantly difference in frequency or duration of apnea, bradycardia, and HR during KC as compared to pre-KC and control group periods in an incubator (Affonso et al.,

1993; Bosque et al., 1995; de Leeuw et al., 1991; Ludington-Hoe et al., 1994; Wahlberg et al., 1992). A Cochrane review found no statistical difference in HR between KC and controls among full-term KC studies (Anderson et al., 2003), but a meta-analysis of KC with preterm infants showed a heart rate increase of 10 beats per minute due to warming (Dorsey & Ludington-Hoe, 1998). KC's effect on HR during or after heel stick has not yet been studied.

Autonomic maturation: KC may promote preterm infants' autonomic maturation. Preterm infants who received one hour of KC per day for at least 14 consecutive days starting when infants were 31-33 weeks PCA had more rapid maturation (greater gains) of vagal tone between 32 and 37 weeks PCA, when compared to preterms who did not receive KC (Feldman & Eidelman, 2003).

Modulating biochemical responses

A RCT comparing complete KC (early discharge and 24 h/day KC with unlimited breastfeeding) to traditional care for preterm infants on the level of 17alpha-hydroxy-progesterone (17-OHP), thyroxine-stimulating hormone (TSH) and thyroxine (T4) at 1-5 days post birth, at 2 weeks later, and at calculated term (41 weeks GA) (Weller et al., 2002) found that KC does not impair the hypothalamic-pituitary-thyroid axis and adrenal function, at least in healthy preterm infants (Weller et al., 2002). A Cochrane analysis showed that blood glucose and base excess were also significantly higher in KC infants (Anderson et al., 2003).

When preterm infants undergo painful procedures, maternal KC might significantly reduce circulating β-endorphin (mean change 74%) and cortisol (mean change 66% - 78%) (Modi & Glover, 1998; Mooncey et al., 1997) and salivary cortisol

(mean change 60%) (Gitau et al., 2002), and there was no evidence of any adverse effect. Untreated pain in infants can lead to changes in plasma stress hormones and a state of hypermetabolism and catabolism (Anand, 1998a). The results of KC studies suggested that heel stick pain responses may be diminished because KC blunts HPA axis activity prior to heel stick.

In summary, KC is believed to be an effective method to blunt pain responses because of its underlying components – mainly its multi-sensory stimulation effects, which, when studied individually, have been proven to reduce the severity of responses to stress or pain. Several studies have also demonstrated that KC directly or indirectly blunts pain responses in full-term and preterm infants. However, most of these studies were not RCTs and did not control for infant state, environmental conditions, demographic characteristics of the subjects, and circadian rhythmicity factors in the design. The studies have also been limited by small sample sizes. KC has been given for 20 minutes-to-several hours at each session and for one day-to-consecutive weeks at a time. The effect of various durations and frequencies of KC on reducing infant pain responses needs further investigation. The present study addressed the following methodological inadequacies and gaps in the literature: (1) this study used a randomized cross-over design to effectively control for threats to internal validity; (2) infant behavioral state was measure and analyzed to control for its influence on pain responses; (3) infants' demographic characteristics (e.g., gestational age and postnatal age), severity of illness, and the number of previous pain experiences were monitored; (4) sample size was adequate to test the hypotheses; and (5) HRV indices were used as indicators of pain in response to KC.

Confounding Variables – Factors That Influencing Preterm Infant Pain Response

Gestational Age and Postnatal Age

Preterm neonates may be more sensitive to pain than more mature infants, because preterms show lower tactile thresholds than term infants and demonstrate additional decreases in the pain threshold after exposure to painful stimuli (Fitzgerald & Beggs, 2001). This altered excitability spreads to multiple levels of the spinal cord and may cause non-noxious stimuli (handling, physical examination, and other nursing procedures) to be perceived as noxious stimuli and activate systemic physiologic responses to stress (Grunau, Oberlander, Whitfield, Fitzgerald, & Lee, 2001; Stevens & Johnston, 1994). An overall decrease in HR and increase in HRV (especially increased parasympathetic modulation) with increasing PCA in LBW infants have been demonstrated in 31-38 weeks PCA infants (Sahni et al., 2000). The influence of gestational age and postnatal age on pain responses was controlled by selecting infants with 30 – 32 weeks of gestational age and less than 14 days of postnatal age.

Previous Experiences of Pain and Inflammation

Early pain exposure at a very low gestational age may alter the autonomic substrate, resulting in infants who are in a perpetual state of stress. The number of painful procedures a preterm infant has undergone is the greatest predictor of decreased behavioral response to pain (Als et al., 1994). Grunau and colleagues' study (2001) demonstrated that the most significant factors associated with altered behavioral and autonomic pain reactivity at 32 weeks' PCA were a greater number of previous invasive procedures since birth and gestational age (GA) at birth, both of which were related to a dampened response. Preterm newborns who were younger, asleep, and had undergone a

painful event more recently were less likely to demonstrate behavioral and physiologic indicators of pain (Johnston, Stevens et al., 1999). Morison (2003) reported that preterm infants with more prior pain exposure, lower Apgar, and lower GA at birth displayed more motor stress cues but less facial activity post-lance. Cote (1991) proposed that when preterm neonates are confronted with disturbing repetitive painful stimuli they may "shut down" by going into a deep sleep, and their extremities appear tightened and flexed, eye blinks are absent, and pulse is rapid. However, full-term infants who had diabetic mothers and were exposed to repeated heel sticks in the first 24-to-36 hours of life learned to anticipate pain and exhibited more intense pain responses during venipuncture than normal infants (Taddio et al., 2002). Early damage, such as localized inflammation, can result in prolonged structural and functional alterations in pain pathways that can last into adult life (Torsney & Fitzgerald, 2003; Woolf & Salter, 2000). The influence of previous pain experiences on pain responses was controlled by permuted block randomization and by recording the number of previous pain procedures the preterm infant experienced and including the number as a covariate in the statistical test.

Gender

In Guinsburg and colleagues' study (2000), a significant gender difference was noted for both preterm and full-term newborn infants in the NFCS. Recently born female neonates of all gestational ages expressed more facial features of pain than male infants, which may be related to differences in pain processing and/or pain expression among genders. The influence of the infant's gender on pain responses was controlled by permuted block randomization. The infant's gender was also recorded and analyzed to determine its influence on pain responses and the need for statistical control.

Severity of Illness

Severity of illness affects the cry response to pain, not facial action or physiologic arousal in response to pain (Stevens & Johnston, 1994). Comparing parenchymal brain injury (PBI) preterm infants with controls, both groups of infants mounted similar responses to heel stick with no difference in facial or HRV responses. The only statistically significant difference between groups was that infants with PBI had more tongue protrusion at lance (Oberlander, Grunau, Fitzgerald, & Whitfield, 2002). The score of severity of illness was recorded and analyzed as a covariate to control its influence on pain responses.

Behavioral State (Sleeping/Waking state)

Several reports have demonstrated that a full-term or premature infant in a sleep state will show less response to pain than an infant in an awake state (Grunau & Craig, 1987; Stevens & Johnston, 1994). Over 31-38 weeks PCA, HR decreases during quiet sleep, while multiple aspects of HRV increase during both quiet and active sleep in LBW infants (Sahni et al., 2000). The infant behavioral state was recorded and analyzed as a covariate to control its influence on pain responses.

Characteristics of the Painful Stimulus

Neonatal infants can have differential responses to procedural pain (heel stick, venipucture, suctioning) or operative/postoperative pain (circumcision), and non-invasive or invasive pain (Grunau et al., 1990). Newborn infants show increased magnitude of physiologic and behavioral responses to increasingly invasive procedures, and even very prematurely born infants respond to pain and differentiate stimulus intensity (F. L. Porter et al., 1999). The duration, origin and location of the painful stimulus (Fuller, 2001) and

the context within which the painful stimulation occurs, such as environment (Modrcin-McCarthy, McCue, & Walker, 1997) and concurrent sounds (Kawakami et al., 1996), can influence infant pain responses. In the present study the painful stimulus was controlled by using only heel stick with a standardized procedure by one "NICU-only" phlebotomist.

CHAPTER THREE

Methods

Design

A prospective cross-over design was used with random assignment to either the KC heel stick (KCH) condition first or the incubator heel stick (IH) condition first. Group A received IH on Day 1 and KCH on Day 2; Group B received KCH on Day 1 and IH on Day 2 (Table 2). Random assignment to groups according to the two different sequences of treatments was accomplished by a permuted block design. Permuted block design ensured the highest possible equivalence among subjects exposed to different conditions (Chow & Liu, 1998).

Carry-over effect from one condition to the next is a concern with any cross-over design. Previous research has shown that physiological and behavioral state effects of KC disappear within three hours of KC's cessation (Bohnhorst et al., 2001; Bosque et al., 1995; de Leeuw et al., 1991; Ludington-Hoe, Ferreira, & Goldstein, 1998; Ludington-Hoe et al., 2000). Modi & Glover (1998) reported that blunting effects of 20 minutes of KC on plasma and salivary cortisol were not sustained a day later. The findings suggest that 24 hours is a sufficient wash-out period, strengthening the rigor of the cross-over design. The study heel stick procedure included four phases: Baseline (20 minutes), Heel Warming (5 minutes), Heel Stick (15 seconds) and squeeze (30 seconds to 2-3 minutes), and Recovery (20 minutes). Measuring pain responses during heel stick was described in the section of data collection procedure (p 97).

Table 2.

Crossover Design

	Day One	Washout Period	Day Two
Group A	Incubator heel stick (IH) condition	24 hours in routine care	KCH condition
Group B	Kangaroo Care heel stick (KCH) condition		IH condition

Setting

The study was conducted in a level II, 13-bed NICU in Richland, WA. that was served by two neonatologists and two certified neonatal nurse practitioners. The NICU had 225 admissions per year, 64% females and 36% males (64% were non-Hispanic whites, 36% Hispanic, 7% Asian). Eligible subject availability in this unit ranged from 1-7 infants/month with the average being 3-4 infants/month based on a 3-month review. Noise and light levels were kept appropriate for developmental care. Next to each incubator there was adequate room for a recliner chair for the mother to sit in during KC. Privacy was maintained with a curtain drawn around the infant's incubator and the recliner. Routine care to minimize heel stick pain in this unit was nesting; all infants were nested. No other pain interventions were routinely used for heel stick.

Sampling and Subjects

Male and female healthy preterm infants were selected from NICU using convenience sampling. *Infant inclusion criteria* were (1) 30-to-32 weeks gestational age and 2-to-9 days postnatal age to partially control for previous pain experiences (Johnston & Stevens, 1996); (2) cared-for in an incubator; (3) either NPO or on bolus feeding to control for feeding effects on HRV (Veerappan et al., 2000); and (4) whose mothers were

English speaking. *Infant exclusion criteria* were (1) known congenital anomalies; (2) severe periventricular/intraventricular hemorrhage (≥ Grade III); (3) history of minor or major surgery; (4) use of sedation or vasopressors or analgesics to control for the effect of sedative medication on pain responses; (5) drug exposure history to control for the drug effects on pain responses (Oberlander et al., 2005; Oberlander, Grunau, Fitzgerald, Ellwood et al., 2002); and (6) any signs of tissue breakdown or inflammation/necrosis of either heel, because tissue damage increases pain responsiveness (Ruda et al., 2000). Tissue breakdown was measured by the Neonatal Skin Condition Score (Appendix A) developed by Lund and Osborne (2004). Infants with a score of more than 6 were excluded from the study.

Sample size. The sample size was calculated using results from Johnston's (2003) effect size of .55 on the scores of Premature Infant Pain Profile (PIPP), KCH: M = 10.7, SD = 4.39; IH: M = 12.9, SD = 3.51, at 60 seconds post heel stick. For the primary hypothesis (the effect of KC on PIPP pain scores), using a two-sided ANOVA with α = .05, and power = .80, a total of 26 subjects (mother-infant dyads) were needed for analysis in this cross-over study (Cohen, 1988). To account for possible attrition, 5 additional subjects were enrolled for a total of 31 subjects. Sources of loss were due to equipment failure, maternal withdrawal, and maternal illness. Maternal attrition may occur because of discomfort about being with their infant during a painful procedure. No study was found that measured HRV as an outcome of KC in relation to preterm infant pain responses. Based on Lindh's finding (1997) of an increase in low frequency power with M = .30, SD = .35 log mHz^2 in the response of preterm infants to heel stick compared to baseline, 18 subjects were needed to test the hypothesis of the effect of KC

on HRV, with $\alpha = .05$ and power = .80. Thus, complete data sets from 26 infants were planned to be sufficient to test the HRV hypothesis as well.

Randomization Procedure

A permuted block procedure was employed to assign the subjects to two groups representing different treatment sequences (Group A: IH on Day 1 and KCH on Day 2; Group B: KCH on Day 1 and IH on Day 2). The permuted block randomization, with a block size of 4 patients, was used to prevent treatment sequence effects and possible covariate imbalance. A statistician helped the investigator generate a list of randomization codes using the SAS® procedure PLAN. The list of random codes consisted of the subject's number and the treatment assignment. Infants were assigned to groups according to the random codes.

Experimental Intervention

In Kangaroo Care, the diaper-clad infant was held skin-to-skin, prone, and upright between maternal breasts. The infant was covered across the back with a blanket folded in fourths and with the mother's cover gown. Mother and infant were seated in a La Fuma recliner at an incline of 30 - 40°. Mothers were encouraged to keep their hands clasped behind the infant's back and allow their infants to sleep.

In the routine care condition, the diaper-clad infant was placed in the incubator prone and nested at a 30 to 40 degree incline to resemble KC as much as possible. For both groups, routine care by staff was given during the 24-hour washout period between Day 1 and Day 2. Infants were either NPO or on bolus feeding (naso-gastric tube, bottle feeding, or breastfeeding) every 3 hours. If the infant was on bolus feeding, he/she was fed just before the study and was not fed during the study.

Heel Stick

Though venipuncture has been shown to be more effective for obtaining blood samples and less painful than heel sticks in newborn infants (Ogawa et al., 2005; Shah & Ohlsson, 2001; Shah et al., 1997), the heel stick was chosen as the pain stimulus for the following reasons: (1) Heel stick is the most common tissue damaging procedure for prematures; (2) Heel stick can be standardized better across times and investigators; (3) Heel stick has been used in many other studies (Johnston et al., 2003; Lindh et al., 1999; Ludington-Hoe et al., 2005; Taddio et al., 2002), making cautious comparisons across studies possible.

Heel sticks were done for blood samples in another study (Ludington-Hoe, 2005) and some heel sticks were conducted along with blood collection ordered by physicians. One "NICU-only" phlebotomist who followed the National Association of Neonatal Nurses (1995) standardized procedure using the Tenderfoot^{TM} spring-loaded heel incision device conducted the heel sticks for infants receiving 80-minute protocol. Another phlebotomist did the heel sticks for all other subjects using same procedure and lancet.

Demographic Variables and Instruments

The infant's demographic variables were recorded on a form developed by the investigator (Demographic Questionnaire, Appendix B) that included the infant hospital number, date of birth, infant's postconceptional age, postnatal age, gender, race, ethnic background, birth type, birth weight, weight at the time of testing, feeding conditions, APGAR scores (at birth, 1-minute and 5-minute after birth), severity of clinical condition and pre-study KC experience. The mother's demographic data included age, race, ethnic background, occupation status, yearly income, educational level, and marital status. The

investigator obtained the information by checking the infant's medical record and interviewing the mother.

Dependent Variables and Instrumentation

The major dependent variables were infant behavioral, physiological, and autonomic responses to heel stick pain. Both behavioral and physiological responses were measured by the Preterm Infant Pain Profile (PIPP). The autonomic nervous system responses to pain were measured by heart rate variability indices.

Premature Infant Pain Profile. The Premature Infant Pain Profile (PIPP, Appendix C) is a multidimensional pain scale specifically developed to assess acute pain in preterm neonates (Stevens, Johnston, Petryshen, & Taddio, 1996). The PIPP includes 7 objective pain indicators (4 behavioral, 2 physiologic, and 1 contextual) and each indicator is evaluated on a 4-point likert scale (0, 1, 2, 3). The behavioral indicators include the magnitude of changes of three facial actions (brow bulge, eye squeeze, and nasolabial furrow) with 0 = none and 3 = maximum, and the behavioral state with 0 = active/awake, 1 = quiet/awake, 2 = active/sleep, and 3 = quiet/sleep. The physiological indicators are HR and $SaO_{2,}$ with 0-to-3 indicating the amount of change from baseline values. The contextual indicator on the PIPP is gestational age. The younger sleeping infants, who are reported to have less sustainable responses than older alert infants, will score higher and are not penalized for less robust behavioral and physiological responses (Stevens, 1996). The scores obtained for the seven indicators are summed for a Total Pain Score with a possible Total Score of 21 for preterm infants. A Total Score of 6 or less generally indicates "minimal or no pain", a Total Score greater than 6 to 12 indicates "mild pain", whereas a Total Score greater than 12 indicates "moderate to severe pain".

The PIPP Total Scores for each phase of the heel stick procedure were calculated. Behavioral data was analyzed from videotapes; physiological data was from the pulse oximeter system; and the infant's gestational age from the infant's medical record. A video camera recorder was set up and focused in on the infant's face to record facial actions. The videotapes were later reviewed and scored by the investigator and one other certified PIPP scorer.

Construct validity of an instrument links the measure to its theoretical meaning and reflects the degree to which the instrument accurately measures the phenomenon of interest. The behavioral and physiological indicators in the PIPP were derived from a review of the literature, clinical observation, and the investigators' conceptualization of pain as a multidimensional phenomenon (Stevens et al., 1996). Initial construct validity was established by subjecting four existing data sets to a secondary data analysis (Stevens et al., 1996). Two extreme situations, a pain (heel stick) and a nonpain (handling) situation were used to test the construct validity of PIPP in preterm infants of 32-34 weeks GA. The mean Total PIPP Score for the pain situation was 12.9 ± 3.4 and for the nonpain situation was 6.0 ± 2.7. The scores were significantly different (paired $t = 12.24$, $p < 0.001$, and Mann-Whitney $U = 765.5$, $p < 0.001$) and suggested that the PIPP was accurately discriminating between the pain and nonpain situation (Stevens et al., 1996). Construct validity was also supported in a real vs. a sham heel stick procedure in preterm infants of 28 – 30 weeks GA. The scores were significantly different ($t = 2.4$, $p < 0.02$, and Mann-Whitney $U = 132.0$, $p < 0.016$) between the real heel stick situation (10.3 ± 4.5) and the sham situation (6.3 ± 3.2) (Stevens et al., 1996). Ballantyne and colleagues (1999) validated the PIPP in the clinical setting and in real time using three events

(baseline, nonpain, and pain) in four GA groups of neonates (< 28 weeks, 28-31 weeks + 6 days, 32-35 weeks + 6 days, and > 36 weeks). Repeated measures analysis yielded a statistically significant main effect for event which differentiated pain from nonpain and from baseline ($F = 48$, $p < 0.001$), supporting construct validity.

The internal consistency of the PIPP was evaluated initially by Stevens and colleagues (1996). Correlation coefficients for individual items ranged from 0.59 to 0.76. The standardized item alpha for the six items (excluding GA) was 0.71. Inter-rater reliability analysis of individual event (baseline, pain, and nonpain) scores of the PIPP yielded reliability coefficients of 0.93 - 0.96 and intra-rater reliability coefficient analyses for individual events were 0.94 - 0.98 (Ballantyne et al., 1999).

The Masimo SET Radical pulse oximeter (Masimo Corp., Irvine, CA) was used to measure HR and SaO_2. Masimo system had adaptive filters to work in real time to ensure accurate reading through all patient conditions (patient motion or movement, low perfusion, and intense ambient light), and was used with a Low Noise Optical Probe neonatal size transducer (LNOP Neo) across the infant's foot. Numeric values for mean calculations of HR and SaO_2 were stored in the pulse oximeter. Each data point was a unit of analysis.

Synchrony between the videotape and pulse oximeter data needed to be established during data collection and coding. First, to ensure that the numeric values of HR and SaO_2 were collected in real time and recorded concurrently with facial actions, the video camera was focused on the time of day displayed on the Masimo oximeter for approximately 10 seconds at the beginning of data collection, and then it was focused on the infant's face. Second, the research nurse or the phlebotomist called out on video the

specific study phase, e.g. Heel Warming or Heel Stick, that was beginning. Third, the investigator and the PIPP coder documented the video time and pulse oximeter time on the PIPP coding chart and compared these two times. No correction was needed if the difference in the time was less than 30 seconds. If the difference in the time was more than 30 seconds, the pulse oximeter time was corrected according to the video time. The investigator and coder recorded the real time for each study phase by the time when the research nurse or phlebotomist called out the phases on the video.

Double coding by two coders was used. The two PIPP scores for the same subject were compared. If the score differed between the two scorers, the scorers went back together and discussed the observations until consensus was achieved yielding intra-rater reliability of 95% between the two coders in this investigation.

Heart rate variability. HRV is the beat-to-beat variation of the heart rate as measured by a time series power analysis. HRV has not been studied as an outcome measure of the effect of KC on infant pain. The frequency domain of HRV was measured in this study. Analysis of the frequency domain of HRV, for example, spectral analysis, is a tool for assessment of sympatho-vagal balance. The interplay between the components of the autonomic nervous system may be more precisely described by spectral analysis in comparison with calculations of mean and variance of the HR (time domain variables) for assessment of HRV (Lindh et al., 1997). Under resting conditions, the electrocardiogram (ECG) of healthy individuals exhibits periodic variation in R-R intervals. This rhythmic phenomenon, known as respiratory sinus arrhythmia (RSA), fluctuates with the phase of respiration – cardio-acceleration during inspiration, and cardio-deceleration during expiration. The RSA is predominantly mediated by

respiratory gating of parasympathetic efferent activity to the heart: vagal efferent traffic to the sinus node occurs primarily in phase with expiration and is absent or attenuated during inspiration. These rate changes are too brief to be detected by pulse or stethoscope, but can be measured from the cardio-respiratory monitor by creating and analyzing the beat-to-beat (R-to-R wave) variation via a time series power analysis that yields low frequency (LF), high frequency (HF), and the LF/HF ratio data.

The R-R intervals were measured using the ANS-R1000 (Ansar, Inc., Philadelphia, PA), a non-invasive signal monitor, which was an accessory connecting to the infant cardio-respiratory monitor that captured ECG and respiratory data on-line. The ANS-R1000 consisted of a data acquisition card installed on a PC, a disk containing the software program, and a PC with key-board and monitor. The ECG was converted by the computer to a digital value. A time series curve of the R-R intervals was generated and represented on a tachogram. The time between R waves was used as the heart beat interval marker; the inspiration and expiration excursions of the respirations were used as the cycle marker. Following smoothing of the curve, the computer re-sampled at regular intervals over the same period using a Fast Fourier Transform (FFT). The FFT is suitable for recordings as short as 10-15 minutes in length, and correlated well with the autoregressive model which is particularly suitable for brief measuring periods (Cowan et al., 1993). The HRV data from each strip were digitized as the software program ran.

Spectral analysis of the transformed data generated two components of clinical interest: a low frequency (LF, 0.04 to 0.15 Hz) component (which is mediated by both sympathetic and parasympathetic parts of the autonomic system), and a high frequency (HF, 0.15 - 1.0 Hz) component (which is identified with vagal tone and is determined by

the respiratory frequency). The ratio of the low-to-high frequency spectra (LF/HF) is also measured and is an index of sympathetic-parasympathetic balance. Movement and artifact were eliminated by comparing amplitude (height) of the current R-wave with the amplitude for the last acceptable R-wave. Waves more than 38% greater than the previous wave were automatically eliminated.

The LF, HF, LH/HF and mean HR for each 128-second segment (521 points) were displayed on the screen of the monitor. When data collection was complete, the data were printed out using a Hewlett-Packard Laser printer.

The equipment was checked and calibrated by the biomedical engineer at the beginning of the study. The ANS-R1000 was auto calibrated each time when it was turned on. Dr. Gail McCain, who is the owner and an expert in HRV in infants, trained the investigator and data collectors on use of the equipment and on printing out the data.

Potentially Confounding Variables

Infant's behavioral state. The infant's behavioral state influences HRV and PIPP in full-term infants, but the effects of state on preterm HRV at rest and during painful procedures are still uncertain (Eiselt et al., 1993; Stevens et al., 1994). None the less, the Anderson Behavioral State Scoring System (ABSS; Appendix D) was used to measure behavioral state. The ABSS was adapted from a scale by Parmelee and Stern and was designed to measure the behavioral state of preterm infants (Anderson et al., 1990). The ABSS has 12 categories of behavioral state: 1 = very quiet sleep, 2 = quiet sleep, 3 = restless sleep, 4 = very restless sleep, 5 = drowsy, 6 = alert inactive, 7 = quiet awake, 8 = restless awake, 9 = very restless awake, 10 = fussing, 11 = crying, and 12 = hard crying. An infant was observed for 30 seconds, and the number of the highest behavioral state

observed was recorded with one exception: the desirable but relatively rare state of alert inactivity (ABSS = 6) was recorded when it occurred, even if a higher numbered state also occurred during the same 30-second assessment period because alert inactivity state is the optimal state in infants (Gill, Behnke, Conlon, McNeely, & Anderson, 1988). The research nurse observed and coded behavioral state for 30-second every minute throughout each phase (Baseline, Heel Warming, Heel Stick, and Recovery). An experienced research nurse who had previous ABSS experience trained the investigator and two other research nurses before the study began. Inter-rater reliability was initially 95% for the investigator, research nurses, and the experienced nurse, and 95% inter-rater reliability was maintained throughout the study by retraining after every 5th subject.

Content validity of the ABSS was established with a panel of neonatal nurse clinicians/researchers, and a developmental pediatrician (McCain, 1992). Convergent validity means that evidence from different sources measured with different instruments all indicate a similar meaning of the construct. The convergent validity of the ABSS was established with heart rate (an index of energy expenditure) during pre-KC ($r = 0.73$, $p = 0.007$), KC ($r = 0.76$, $p = 0.004$) and post-KC ($r = 0.65$, $p = 0.01$) periods (Ludington, 1990). Concurrent validity of the ABSS was established with the Brazelton Neonatal Behavioral Assessment Scale in 24 infants in 1994 (Anderson, 1995). Inter-rater reliability of previous studies was at a level of .71 across seven observers (Gill et al., 1988) and .95 between two observers over a 3-day period (McCain, 1992).

Severity of illness. Severity of illness can affect the infant's ability to mount a response to pain (Grunau et al., 2001; Johnston, Stevens et al., 1999; Morison et al., 2003). Severity of illness was measured by the Score for Neonatal Acute Physiology

Version II (SNAP-II; Appendix E) (Richardson, Corcoran, Escobar, & Lee, 2001). SNAP-II is a simplified neonatal illness severity score that measures six physiologic variables during the first 12 hours of life (mean blood pressure, lowest temperature, PO_2 / FiO_2 ratio, lowest pH, seizures and urine output). Total scores range from 0 (normal) to 115 (life-threatening) points. Data for SNAP-II scoring was obtained from the infant's medical record after the infant was recruited.

The SNAP-II was derived from the well-validated 34 elements of the Score for Neonatal Acute Physiology (SNAP). Criterion validity compares the correlation of a new score with the existing score that measures the same phenomena (Carmines & Zeller, 1979). In a 30-site study, involving 25,429 newborns, criterion validity was established by correlation coefficients between SNAP and SNAP-II ($r = 0.91$) in all birth weight infants. Goodness of fit testing (Hosmer-Lemeshow test) was used to assess deviations between observed and expected values of neonatal illness severity and mortality using the SNAP-II. The P value for goodness of fit was .90, indicating extremely good fit (Richardson et al., 2001). The SNAP-II is a simple, accurate, and robust instrument to measure illness severity for infants undergoing newborn intensive care.

Number of previous invasive procedures. The number of previous invasive procedures was found to positively correlate with the infant's response to pain (Grunau et al., 2001; Morison et al., 2003). The number of previous invasive procedures was obtained daily from nurses' checklist or nurses' notes from the time of admission to the NICU until the day of data collection. A checklist of invasive procedures was created as used in previous studies (Johnston, Collinge et al., 1997; Simons et al., 2003) and included heel stick, venipuncture, arterial puncture, nasogastric tube insertion, intubation,

suctioning, extubation, lumber puncture, insertion umbilical line, urinary bladder catheterization, and other miscellaneous invasive procedures.

Infant body temperature. Infant body temperature influences HR and HRV (Kagan, 1982) and was considered as a covariate. The infant skin temperature was detected and displayed on the control panel of the incubator. A skin probe was connected to the incubator and taped on the infant's abdomen, which is part of standard care. Temperature was recorded each minute to the Data Collection Chart (Appendix F) by the observers from Baseline to end of Recovery phase.

Procedure of Data Collection

Design of Protocol and Manual

A Manual of Study Protocol (Appendix G) was prepared. Clear definitions of entry and diagnostic criteria and methodology were included in the written manual so all investigators could apply them in a consistent manner throughout the study. Descriptions of manipulation procedures and study equipment (HRV equipment and video camera) were also included in the manual.

Training and Pretesting

Training sessions for observers were conducted to promote standardization of procedures. Training content included the use of equipment, setting up the mother and infant in the KC position, and scoring and interpreting PIPP score. Pretesting of the HRV equipment and video camera was conducted before data collection began.

Recruitment Procedure

The research nurse made daily rounds of the NICU to identify subjects approaching 30-to-32 weeks PCA and conferred with the Chief of Neonatology regarding

the appropriateness of each selected infant for recruitment. The research nurse obtained consent from the infant's physician and then telephoned the baby's mother, explained the study, and obtained oral informed consent. The mother was then met by the research nurse or the principal investigator when she next came in to visit the baby. The research nurse explained the study and showed a picture of a mother-baby dyad experiencing KC. The mother was given a chance to have her questions about the study answered. Those who consented to participate were given a copy of the written consent form. After written informed consent had been obtained from the mother, the infant was assigned to one of the two study groups by permuted block randomization. The mother was then informed of her group assignment (testing sequence).

Data Collection Procedure

The infant's and mother's demographic data, infant's severity of illness (SNAP-II score), and the number of previous invasive procedures were obtained from the patient's medical record and an interview with the mother. Data collection on the infant's responses to pain in KCH or IH conditions was conducted on two consecutive mornings. For the first 18 subjects, the study was conducted using an 80-minute protocol (infants in KC or incubator for 80 minutes before the Heel Warming phase). For the last 10 subjects, a 30-minute protocol was used (infants in KC or incubator for 30 minutes before the Heel Warming phase). The 80-min protocol was the original design and a 30-min protocol was implemented after completion of the 80-min protocol cohort to accommodate ethical concerns generated by data from the first 18 subjects in the 80-min protocol.

The 80-minute protocol. The 80-min protocol was so named because the treatment or control condition lasted 80 minutes (Figure 3). Sixty minutes occurred

before data collection began, and then the next 20 minutes were spent collecting Baseline phase data. For the **KCH condition**, the infant was transferred by the research nurse from the incubator into KC using the standing transfer technique (Gale, Franck, & Lund, 1993) at 8:30 a.m., after the naso-gastric or bottle feeding. The mother moved to the La Fuma chair and after she was seated, the chair was reclined. KC began at 8:30 a.m. and continued for a full 60 minutes before data collection began. Data collection started at 9:30 a.m. and continued until 10:20 a.m. Data collection included four study phases: (1) Baseline (20 minutes), (2) Heel Warming with a warm pack (5 minutes), (3) Heel Stick (15 seconds) and blood collection (and possible further squeezes, 0.5-to-5 minutes), and (4) Recovery (20 minutes). For heel warming, the infant's foot was retracted from beneath the blanket as the infant remained in KC. The heel stick was conducted on the retracted foot when all needed blood (0.5 ml) had been collected, a bandaid was placed on the lancet site and the foot was placed beneath the blanket. KC continued for the Recovery phase. Thus, PIPP, HRV, behavioral state, and infant body temperature data were collected once a full 60 min of KC or incubator care had been completed and continued until 20 min after the heel stick was completed. At the end of data collection, the nurse returned the infant to the incubator using the standing transfer technique.

For the **IH Condition**, the infant was fed and, wearing only a diaper, was placed prone and nested in his/her incubator. The infant remained in this position for all phases, especially for Heel Warming and Heel Stick. At the end of data collection the staff nurse was asked to position the infant as she wished. Data collection procedures were the same as in the KCH condition, except that the heel stick was conducted with the infant in the incubator rather than in KC and the foot did not have to be moved for warming and stick.

The 30-minute protocol. The project director at the data collection site reported that infants who received the 80-min protocol in the KCH condition seemed to be irritated when awakened from the deep sleep they had been in for at least 60 minutes by heel warming manipulations. Some infants cried more during KCH than IH leading the research team to question continuance of the study. The project director's clinical observations were supported by a recent report (Ludington-Hoe, Scher, & Johnson, In press) that found infants in KC go into a deep sleep and that the deep sleep is uninterrupted by spontaneous arousals. When in the intensified deep sleep of KC, awakening may be very disruptive; causing the increased crying observed upon heel warming that was seen in KC infants, but not in incubator infants when heel warming manipulations began. The project director also related that the study phlebotomist had difficulty collecting blood in the KC position and would do it no more. Because crying is physiologically detrimental (Ludington-Hoe et al., 2002), and the ethical reasons were compelling, a new phlebotomist and a new 30-min protocol were used for the last 10 subjects. Thirty minutes of KC had already been shown to be effective in reducing infant pain (Johnston et al., 2003; Kostandy, 2005) and minimizing crying. A 30-min protocol also made comparisons the data reported here with previously published data possible.

The procedure in the 30-min protocol was similar to the 80-min protocol, except that the study started at 9:20 a.m. and data collection started at 9:30 a.m. for both KCH and IH (Figure 3). The infant was held by the mother in the KC position (KCH) or in the incubator (IH) for 10 min before data collection, followed by a 20 min Baseline for a total of 30 min of KC or incubator care before Heel Warming. Data collection continued for Heel Warming (5 min), Heel Stick (0.5 - 3 min), and Recovery (20 min).

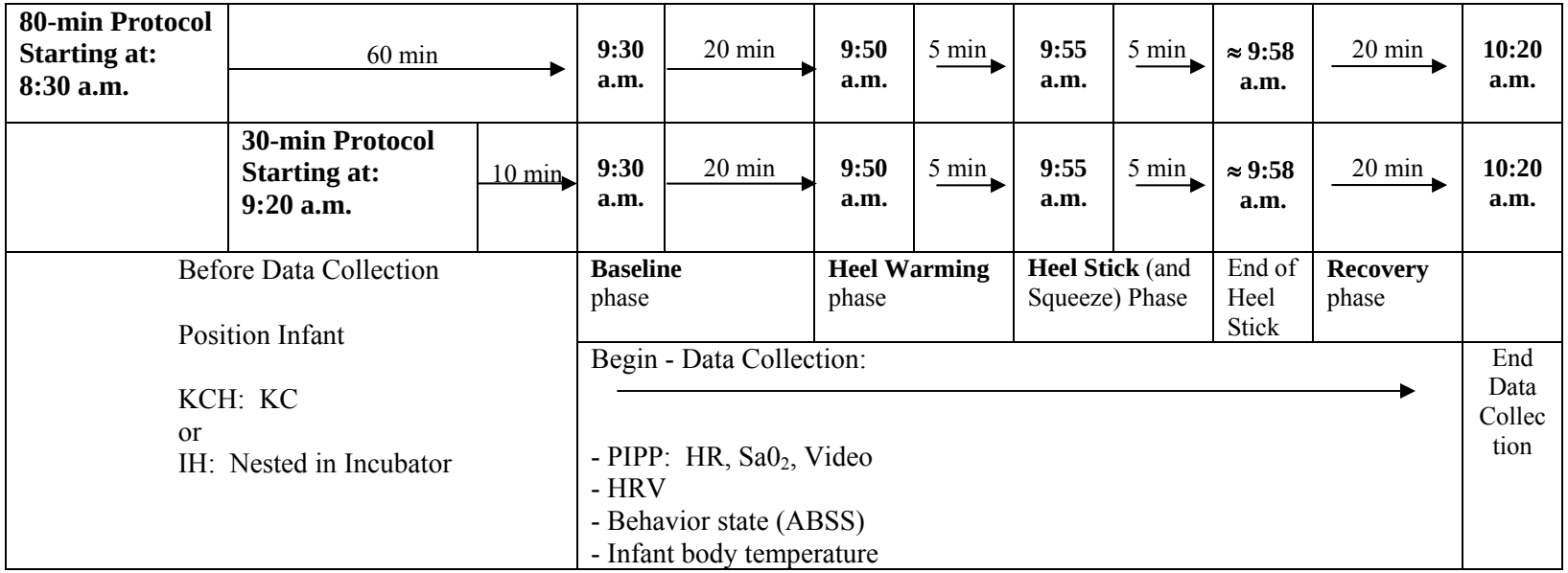


Figure 3. Data collection time line.

100

Protection of Human Subjects

The study protocol was reviewed and approved by the Institutional Review Board for Human Subjects of the Frances Payne Bolton School of Nursing, Case Western Reserve University, University Hospitals of Cleveland, and Kadlec Medical Center. Before each infant's participation in the study, oral and written informed consent (Appendix H) was obtained from his/her mother.

Benefits and Risks. No direct medical benefit to the infant and his/her mother for participating in the study existed. Subjects might benefit from participation in the study by investigator attention and by the opportunity to help to contribute to the development of nursing knowledge about neonatal pain. Mothers may have learned more about their infants' behavior during the study. Infants and their mothers may have benefited further by enhanced bonding and attachment as frequently occurs with KC.

The infant's skin could experience a temporary drop in temperature when he/she is moved from the incubator onto the mother's chest. The research nurse used a blanket to cover the infant and quickly transferred the infant to the KC position to lessen this risk. When the mother was sitting without movement for one and half hours, an increase in the risk of clot formation in her legs existed. The mother was instructed to keep her legs elevated and supported and to slightly shift her position at any time she desired while holding her infant.

Confidentiality. The records of this study were kept private. They were kept in a locked file and any report to be published will not include any information making identification of a participant possible. Access to research records was limited to the researchers. Only the study ID was used on the videotape as identification, and the infant

and mother's names were not identified. The video tapes will be kept for research purposes and then they will be destroyed by a professional company. The list linking participant ID numbers and names was kept in a locked file cabinet and will be shredded seven years after data collection is completed, as mandated by the federal government.

Data Management and Analysis

Data Coding and Entry

During the study design stage, the codebook was developed and written coding instructions were added to the data collection forms to minimize coding errors. Data coding included three parts: (1) General information of infants and mothers were coded on the demographic and medical data sheets (Appendix B). (2) Infant behavioral states (ABSS scores) and abdominal temperature were recorded and coded on the data collection chart (Appendix F). (3) For coding the PIPP score, HR and SaO_2 values were derived from the pulse oximeter with times that matched the facial actions on the videotape. The changes from baseline were calculated by the investigator. The facial actions were coded from the videotape. The Total PIPP Score was calculated for each 30 second interval (Appendix C). (4) The HRV data were printed out from the HRV equipment. The data were entered into an SPSS 13.0 (SPSS Inc., Chicago, IL) data file.

Data Cleaning

Data cleaning began during data collection so that systematic errors in data collection, coding, or entry could be corrected before a large amount of data was collected (Roberts, Anthony, Madigan, & Chen, 1997). Measures of frequency, central tendency and dispersion, and minimum and maximum values were used to identify errors

and outliers. Means and standard deviations were used to compare values to the known normal range (e.g., postconceptual age and body weight).

Incomplete and irretrievably missing data can arise, for example, from the equipment malfunction, from carelessness in completing study forms, or from inability of participants to provide necessary information. Missing data leads to loss of statistical power and bias. Missing data were deleted. Assumptions of ANOVA were tested.

Testing the Assumptions

Basic assumptions including normality, linearity, homoscedasticity, multicollinearity, and singularity were tested. The data were tested for the meeting assumptions of the ANOVA statistical test. The assumptions for ANOVA include (1) the dependent variable should be a continuous variable (interval or ratio level of data) that is normally distributed; (2) the groups should have equal variance or meet the homogeneity of variance requirement, and (3) the cells compared in the ANOVA should be equal (Munro, 2005). The assumption of compound symmetry also must be met for repeated measures analysis of variance (RM-ANOVA). The assumptions of equality and homogeneity of variance for ANOVA analyses were met as tested by Levene's Test of Equality of Error Variances and the assumption of compound symmetry for RM-ANOVA was met as tested by Mauchly's Test of Sphericity. Because the HRV data were not normally distributed, logarithm transformation was applied before HRV analyses were conducted.

Data Safety and Monitoring

The data files were locked and the confidentiality of data was monitored by the investigator. Inter-rater reliability was tested by reestablished after every 5th participant.

A worksheet was made for the data collectors to indicate adverse events, inter-rater reliability results, recruitment, refusals, withdrawals, and missing tests.

Date Analysis

SPSS version 13.0 software package was used in data analysis. Descriptive statistics, including frequency, percentage, mean, standard deviation and range were used to describe infant and maternal characteristics. Infant behavioral states were described as percentage of time in each state. Means of PIPP scores and Geometric means of HRV indices were calculated for each of the four phases (Baseline, Heel Warming, Heel Stick, and Recovery) and for each of the two conditions (KCH and IH). The geometric mean [$GM = (a_1 \cdot \ldots \cdot a_N)^{1/N}$] was used instead of the arithmetic mean [$M = (a_1 + \ldots + a_N)/N$] for calculating HRV indices, because the GM results in an average rate of change (McCain et al., 2005). The data were also explored by graphic tools (e.g. line chart) for PIPP and HRV indices over conditions and phases.

To test Hypothesis 1, the treatment effect of KC on pain scores (PIPP) was tested using repeated-measures analysis of variance (RM-ANOVA), with condition (KCH vs. IH) as the repeated factor. To test Hypothesis 2, the Generalized Estimating Equations (GEE) model was used to analyze KC effect (KCH vs. IH) and sequence effect (Group A vs. Group B) using SAS 8.0 (Cary, NC). The phase effects were also tested using RM-ANOVA with phase as the repeated factor in both KCH and IH conditions.

CHAPTER FOUR

Results

The results are reported in five sections: the demographic and medical characteristics of infants and mothers in the final sample; tests for confounding variables; descriptive analyses of dependent variables; hypotheses testing; and additional findings related to heart rate, tachycardia, bradycardia, oxygen saturation, and desaturation.

Sample Size

Thirty-five eligible mother-preterm infant dyads were approached during a 17-month period between March, 2004 and August, 2005 in a level II, NICU in Richland, Washington. Four mothers refused to participate in the study because they either were not interested in the study (n = 2), felt they would be afraid to hold the baby during the heel stick (n = 1), or were concerned about the possibility of the baby experiencing an extra heel stick (n = 1). Three mothers withdrew from the study after consent and randomization (maternal illness = 1, not realizing that consent was for a heel stick not needed for medical care = 1, and not wanting to be present during a heel stick = 1). One mother withdrew after finishing one day of data collection and gave no apparent reason. The final sample consisted of 28 preterm infants and their mothers. Those who provided no data were not include in the analysis. Fourteen mother-infant dyads were randomized to group A (incubator heel stick [IH] on day 1 and KC heel stick [KCH] on day 2); 14 subjects were randomized to group B (KCH on day 1 and IH on day 2). The first 18 subjects were tested in an 80-min protocol and the last 10 subjects in a 30-min protocol. Figure 4 shows the flow diagram of enrollment, intervention allocation, follow-up, and data analysis as required of RCTs (Campbell, Elbourne, & Altman, 2004).

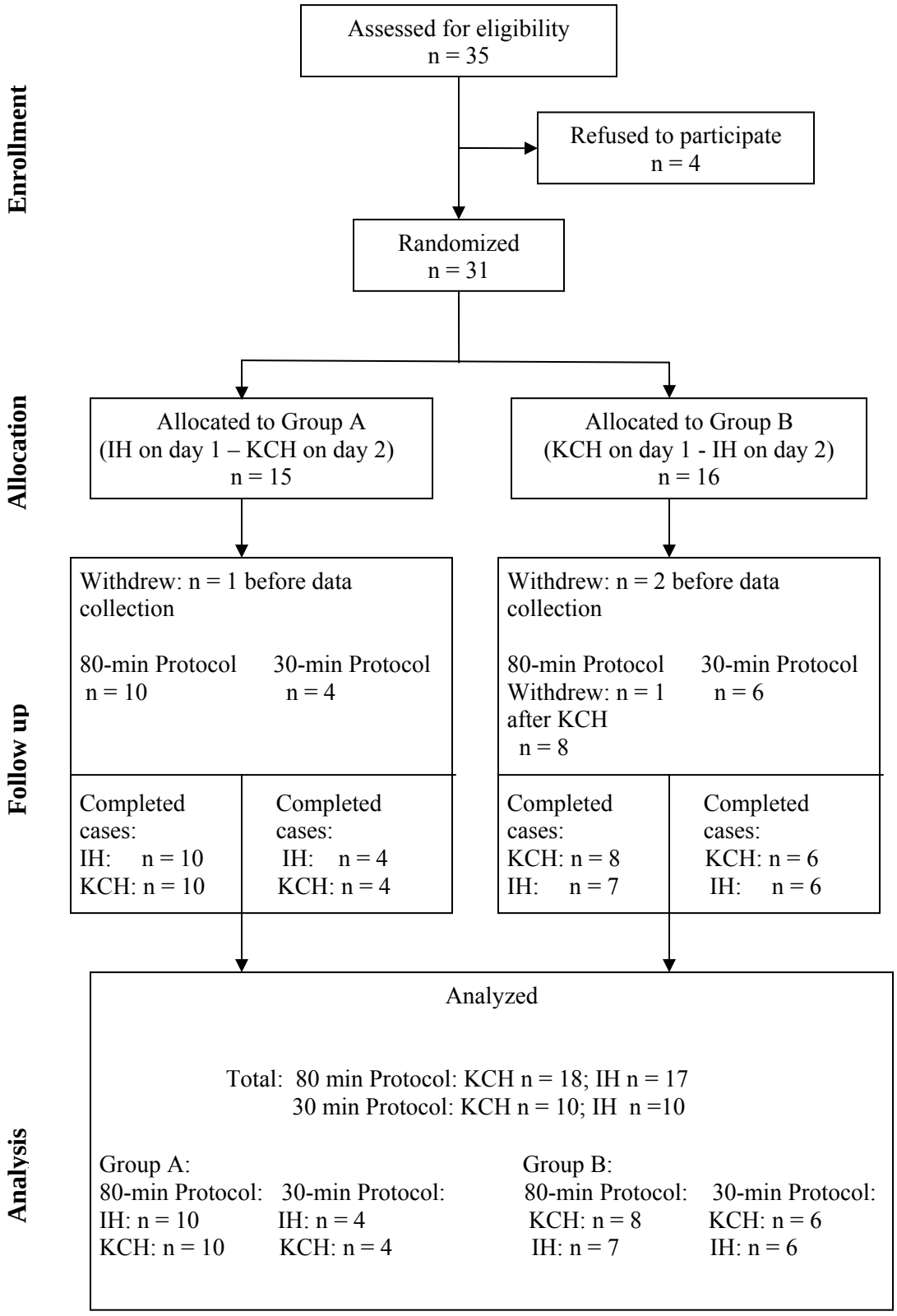


Figure 4. Flow diagram showing number of subjects during enrollment, intervention allocation, follow-up, and data analysis. KCH = Kangaroo Care Heel stick condition; IH = Incubator Heel stick condition.

Heart rate variability (HRV) data were obtained from only the first 14 of the 18 subjects who received the 80-min protocol and were considered as a subgroup. Only 14 subjects provided HRV data because the HRV machine became unavailable thereafter.

Demographic and Medical Characteristics of the Infants and Mothers

The majority of infants were 32 weeks GA (54%), male (61%), white (57%), Cesarean section birth (71%), had no intraventricular hemorrhage (IVH) (96%), mixed feeding type (46%), breast milk feeding (46%), and had no pre-study KC experience (57%) (Table 3). Mean postnatal age upon entry to the study was 6 ± 2 days. Mean birth weight was 1,707 ± 306 g and mean weight on the first day of study was 1,641 ± 302 g. Mean Apgar score at 1 minute was 7 ± 2; at 5 minutes was 8 ± 1. The average severity of illness (SNAP II) score was 7 ± 8. Therefore, the infants studied here were relatively healthy low birth weight infants as they were 32 weeks and had high Apgar scores. Healthy LBW infants are the majority of the infants in the NICU today.

By the first day of study, the mean number of previous painful procedures was 26 ± 7 with a wide range of 11 to 40 (Table 4). Previous painful procedures were heel sticks, intubation, arterial punctures, lumbar punctures, chest tube insertion, urinary catheterization, vitamin K injections, and placement of umbilical lines, percutaneous venous catheters, and/or peripheral intravenous lines. Heel stick was the most frequently performed procedure (mean of 14 out of the 26; 54%).

Mothers were 27.36 ± 5.75 years old. The majority of mothers were married (61%), White (57%), and had completed high school (54%) (Table 4).

Differences in demographic and medical characteristics between group A and B were assessed using Chi square for categorical variables and independent-samples t-test

for continuous variables. Kangaroo Care is a standard option offered to mothers in this unit. Having pre-study KC experience was the only characteristic significantly different between the two sequences of experimental conditions: Group A (14%) and group B (64%), $\chi^2 = 8.43$, $p = .004$. No significant differences were found in other demographic and medical characteristics in infants and their mothers between group A and group B. Because all subjects served as their own controls, demographic and medical data are presented for all subjects according to protocol and as one group.

Infants' and their mothers' demographic and medical characteristics were similar between the 80-min and 30-min protocol subgroups. Pre-study KC experience was the only characteristic significantly different between the 80-min protocol subgroup (17%) and the 30-min protocol subgroup (80%), $\chi^2 = 10.14$, $p = .001$. Otherwise, no significant differences between these two subgroups in demographic and medical characteristics were found. Within the 80-min and 30-min protocol subgroups, no differences between the group A and group B were found in demographic and medical characteristics.

Demographic and Medical Characteristics of the HRV Subgroup

A subgroup of 14 infants (eight male and six female) provided HRV data using the ANSAR system. Heart rate variability data from other subjects was not obtained because the ANSAR system became unavailable after the 14th subject. In the HRV subgroup, seven subjects were in group A and seven were in group B. No statistical differences between group A and group B were found on demographic and medical characteristics and no difference was HRV subgroup from the rest of the sample. The HRV data were missing for one subject in the KCH condition and for four subjects in the IH condition due to staff forgetting to save and/or print out data.

Table 3.

Frequency and Percentage of Infants' Demographic and Medical Characteristics for the 80-min Protocol, 30-min Protocol, and Heart Rate Variability Subgroups, and the Total Sample

Characteristics	80-min Protocol Subgroup (n = 18)		30-min Protocol Subgroup (n = 10)		HRV Subgroup (n = 14)		Total Sample (N = 28)	
	n	%	n	%	n	%	n	%
Gestational age								
30 weeks	5	27.78	2	20.00	5	35.71	7	25.00
31 weeks	4	22.22	2	20.00	3	21.42	6	21.43
32 weeks	9	50.00	6	60.00	6	42.86	15	53.57
Gender								
Male	12	66.67	5	50.00	8	57.14	17	60.71
Female	6	33.33	5	50.00	6	42.86	11	39.29
Race								
White	18	100.00	9	90.00	14	100.00	27	96.43
American Indian	0	0.00	1	10.00	0	0.00	1	3.57
Hispanic background								
Yes	9	50.00	2	20.00	7	50.00	11	39.29
No	9	50.00	8	80.00	7	50.00	17	60.71
Birth type								
Vaginal	6	33.33	2	20.00	5	35.71	8	28.57
Cesarean section	12	66.67	8	80.00	9	64.29	20	71.43
IVH presence								
None	17	94.44	10	100.00	13	92.86	27	96.43
Grade I	1	5.56	0	0.0	1	7.14	1	3.57
Feeding type								
Bottle	9	50.00	0	0.00	8	57.14	9	32.14
Gavage	0	0.00	5	50.00	0	0.00	5	17.86
Breast	0	0.00	1	10.00	0	0.09	1	3.57
Mixed	9	50.00	4	40.00	6	42.86	13	46.43
Milk type								
Breast milk	5	27.78	8	80.00	4	28.57	13	46.43
Formula	1	5.56	0	0.00	0	0.00	1	3.57
Mixed	1	5.56	2	20.00	0	0.00	3	10.71
Missing [a]	11	61.11	0	0.00	10	71.42	11	39.29
Pre-study KC experience [b]								
Yes	3	16.67	8	80.00	3	21.43	11	39.29
No	14	77.78	2	20.00	10	71.42	16	57.14
Missing	1	5.56	0	0.00	1	7.14	1	3.57

Note: IVH = intraventricular hemorrhage. [a] Missing answers for milk type: the question was added in the data collection chart after the first 11 subjects were recruited. [b] Pre-study KC experience was significantly different ($p < .05$) between the 80-min protocol subgroup and the 30-min protocol subgroups. Otherwise, no significant differences between subgroups and the total sample were found for the above variables.

Table 4.

Mean, Standard Deviation and Range of Infants' Medical Characteristics for the 80-minute Protocol, 30-minute Protocol, and Heart Rate Variability Subgroups, and the Total Sample

Characteristics	80-min Protocol Subgroup (n = 18)			30-min Protocol Subgroup (n = 10)			HRV Subgroup (n = 14)			Total Sample (N = 28)		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Postnatal age (days)	5	1	3 - 8	6	2	4 - 9	6	1	4 - 8	6	2	30 - 9
Birth weight (g)	1779	277	1295 - 2241	1577	327	714 - 1844	1770	294	1295 - 2241	1707	306	714 - 2241
Weight on Day 1 of study (g)	1720	287	1210 - 2197	1504	292	745 - 1815	1697	303	1210 - 2197	1641	302	745 - 2197
1 minute APGAR score	7	2	1 - 9	7	1	5 - 9	6.79	2.12	1 - 9	7	2	1 - 9
5 minutes APGAR score	8	1	6 - 9	8	1	6 - 9	8.29	0.91	2 - 9	8	1	6 - 9
SNAP II score	6	7	0 - 23	8	8	0 - 30	5	7	0 - 23	7	8	0 - 30
Number of previous pain experiences	28	7	16 - 40	22	6	11 -27	29	7	20 -40	26	7	11-40
Heel skin condition score on KCH	4	1	3 - 5	4	1	3 - 6	4	1	3 - 5	4	1	3 - 6
Heel skin condition score on IH	4	1	3 - 4	4	1	3 - 5	4	1	3 - 4	4	1	3 - 5

Note. SNAP II = Score for Neonatal Acute Physiology Version II, KCH = kangaroo care heel stick condition; IH = incubator heel stick condition. No differences were found between subgroups and the total sample using t-tests.

110

Table 5

Frequency and Percentage of Mothers' Demographic Characteristics for the 80-minute Protocol, 30-minute Protocol, and Heart Rate Variability Subgroups, and Total Sample

Characteristics	80 min Protocol Subgroup (n = 18)		30 min Protocol Subgroup (n = 10)		HRV Subgroup (n = 14)		Total Sample (N = 28)	
	n	%	n	%	n	%	n	%
Marital status								
Married	10	55.56	7	70.00	8	57.14	17	60.71
Single	7	38.89	2	20.00	5	35.71	9	32.14
Divorced	0	0.00	1	10.00	0	0.00	1	3.57
Separated	1	5.56	0	0.00	1	7.14	1	3.57
Race								
White	18	100.00	9	90.00	14	100.00	27	96.43
American Indian	0	0.00	1	10.00	0	0.00	1	3.57
Hispanic background								
Yes	9	50.00	2	20.00	7	50.00	11	39.29
No	9	50.00	8	80.00	7	50.00	17	60.71
Employment								
Full time	3	16.67	3	30.00	2	14.29	6	21.43
Part time	3	16.67	3	30.00	1	7.14	6	21.43
Homemaker	1	5.56	2	20.00	1	7.14	3	10.71
Student	0	0.00	2	20.00	0	0.00	2	7.14
Missing [a]	11	61.11	0	0.00	10	71.43	11	39.29
Yearly income								
Under $5000	1	5.56	1	10.00	1	7.14	2	7.14
$10,000 - $19,999	1	5.56	0	0.00	1	7.14	2	7.14
$30,000 - $39,999	2	11.11	1	10.00	1	7.14	3	10.71
$40,000 - $49,999	1	5.56	2	20.00	1	7.14	3	10.71
$50,000 - $59,999	0	0.00	1	10.00	0	0.00	1	3.57
$60,000 - $69,999	0	0.00	3	30.00	0	0.00	3	10.71
Unknown	2	11.11	2	20.00	0	0.00	4	14.28
Missing [a]	11	61.11	0	0.00	10	71.43	11	39.29
Education								
Completed grad sch	1	5.56	1	10.00	1	7.14	2	7.14
Completed college	0	0.00	4	40.00	0	0.00	4	14.28
Some college	1	5.56	2	20.00	0	0.00	3	10.71
Completed high sch	3	16.67	3	30.00	1	7.14	6	21.43
Some high school	1	5.56	0	0.00	1	7.14	1	3.57
Junior high school	1	5.56	0	0.00	1	7.14	1	3.57
Missing [a]	11	61.11	0	0.00	10	71.43	11	39.29
Mothers' age (M±SD)	27.44 ± 5.59		27.20 ± 6.34		27.36 ± 5.75		28.86 ± 5.35	

Note. Completed grad sch = Completed graduate school, Completed college = Completed 4 years of college, Some college = Some college (≥ 1 year and < 4 years), Completed high sch = Completed high school. [a] Missing answers: questions were added in the data collection chart after the first 11 subjects were recruited.

Tests for Potentially Confounding Variables

Severity of illness, number of previous invasive procedures, heel skin conditions, infant body temperature, duration of heel stick procedure, and infant behavioral state were considered as potentially confounding variables that could affect infant pain responses. The paired-samples t test was used to compare each of these confounding variables between KCH and IH condition in the subgroups and in the Total Sample. The independent-samples t test was used to compare the confounding variable between the subgroups in KCH or IH condition. No differences were found between KCH and IH and the three subgroups on these variables. Infants' body temperature, duration of heel stick, and behavioral states were considered separately by testing differences between KCH and IH conditions and between the 80-min protocol subgroup (the HRV subgroup is part of the 80-min protocol subgroup) and the 30-min protocol subgroup.

Infant abdominal temperature. The investigator or research nurses recorded infant abdominal skin temperature each minute by reading the control panel of the incubator. Mean infant abdominal temperature (36.52°C - 36.86°C) remained in the thermal neural zone for all subjects in both the KCH and IH conditions and across the four study phases (Table 6). No significant differences were found in mean abdominal temperature between KCH and IH conditions during any of the four study phases and between the 80-min protocol and 30-min protocol subgroups.

Duration of heel stick. The number of minutes actually required to complete each heel stick was recorded (Table 7). In the 80-min protocol subgroup, with phlebotomist #1, the duration of the heel stick procedure in the KCH condition (4.24 ± 1.14 minutes) was significantly longer than the duration in the IH condition (3.47 ± 1.18 minutes), t(16)

= 2.75, p = .014. The phlebotomist reported that it was difficult for her to collect the blood sample in the KC position. After completing the first 18 subjects who received the 80-min protocol, phlebotomist #1 was replaced by phlebotomist #2 and a 30-min protocol. No difference in heel stick duration was found between the KCH and IH conditions conducted by phlebotomist #2 who also performed all heel sticks in the 30-minute protocol subgroup.

Infant behavioral state. The Anderson Behavioral State Scoring System (ABSS) was used to measure infant state over one 30-second assessment each minute during the four study phases. One state (the highest) was designated for each state assessment. Percent time in each state was calculated and compared between KCH and IH, and between the 80-min protocol and 30-min protocol subgroups (Table 8). During the Baseline phase, infants were in Quiet Sleep 59% (80-min protocol) to 65% (30-min protocol) of the time in KCH and 49% (80-min protocol) to 60% (30-min protocol) in IH. During the Heel Stick phase, infants cried 79% (80-min protocol) to 55% (30-min protocol) of the time in KCH and 51% (80-min protocol) to 66% (30-min protocol) in IH. During the Recovery phase, infants were predominantly in Quiet Sleep, 51% (80-min protocol) to 74% (30-min protocol) of the time in KCH and 51% (80-min protocol) to 63% (30-min protocol) in IH.

Comparing KCH and IH, infants receiving the 80-minute protocol with phlebotomist #1 cried more in KCH than IH during the Heel Stick, $t(17) = 2.78$, $p = .013$, and Recovery, $t(17) = 2.58$, $p = .02$. Infants receiving the 30-minute protocol had less Active Sleep in KCH than in IH during the Baseline, $t(9) = 3.29$, $p = .009$ and Recovery, $t(9) = 3.44$, $p = .007$.

Comparing the 80-min protocol infants with the 30-min protocol infants in the KCH condition, 80-min protocol infants had more Active Sleep than 30-min protocol infants during Baseline, $t(25) = 3.46$, $p = .002$, Heel Warming, $t(25) = 2.98$, $p = .006$, and Recovery, $t(25) = 2.72$, $p = .012$, and had less Quiet Sleep during Heel Warming, $t(25) = 2.35$, $p = .027$. No differences were found between the 80-min and 30-min protocol subgroups in the IH condition.

Table 6

Mean and Standard Deviation of Infant Abdominal Skin Temperature (°C) During the Four Study Phases in the 80-minute Protocol and 30-minute Protocol Subgroups and the Total Sample

Phases	80-min Protocol Subgroup (n = 18)				30-min Protocol Subgroup (n = 10)				Total Sample (n = 28)			
	KCH		IH		KCH		IH		KCH		IH	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
Baseline	36.76	0.25	36.64	0.34	36.52	0.07	36.63	0.23	36.67	0.23	36.64	0.23
Heel Warming	36.77	0.27	36.62	0.39	36.56	0.15	36.63	0.28	36.68	0.25	36.63	0.25
Heel Stick	36.79	0.31	36.56	0.43	36.57	0.16	36.52	0.41	36.70	0.27	36.55	0.27
Recovery	36.86	0.29	36.67	0.32	36.52	0.26	36.63	0.28	36.72	0.32	36.66	0.32

Note. KCH = Kangaroo Care Heel stick condition; IH = Incubator Heel stick condition. No significant differences in infant body temperature were between KCH and IH conditions in two subgroups and in the Total Sample

Table 7

Mean and Standard Deviation of Duration (Minutes) of Heel Stick Procedure between the Two Phlebotomists (n = 28)

Condition	KCH		IH		t	p
	Mean	SD	Mean	SD		
Phlebotomist #1 – 80-min Subgroup (n = 18)	4.24	1.14	3.47	1.18	2.75	**.014***
Phlebotomist #2 – 30-min Subgroup (n = 10)	4.10	1.60	4.70	2.83	0.63	.546
Total	4.19	1.30	3.93	2.00	0.64	.528

Note. KCH = Kangaroo Care Heel stick condition; IH = Incubator Heel stick condition. * $p < .05$

115

Table 8.

Mean Percent Time Spent in Each Behavioral State (ABSS) During the Four Study Phases in the 80-min and 30-min Protocol Subgroups and in the Total Sample

	80-min Protocol Subgroup (n = 18)				30-min Protocol Subgroup (n = 10)				Total Sample (N = 28)			
	KCH		IH		KCH		IH		KCH		IH	
Phase & State	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Baseline												
Quiet Sleep [a]	59.31	28.18	49.35	32.63	65.37	28.82	60.33	17.80	61.48	28.03	53.27	28.37
Active Sleep [b]	24.13	16.70	25.74	23.91	**4.62****	8.02	**17.82***	10.43	17.16	16.96	22.91	20.28
Drowsy [c]	6.05	13.23	7.67	11.14	24.45	27.09	17.27	16.54	12.62	20.87	11.10	13.83
Alert Inactivity [d]	1.11	4.71	0.29	1.24	1.09	2.53	0.34	1.08	1.10	4.02	0.31	1.16
Awake [e]	2.78	10.60	0.00	0.00	5.38	7.26	2.04	4.61	3.71	9.48	0.73	2.84
Fussy/Cry [f]	1.06	2.08	0.28	1.18	1.43	2.47	2.15	5.46	1.19	2.19	0.95	3.41
Heel Warming												
Quiet Sleep [a]	53.62	34.37	59.90	31.09	81.20	18.18	65.48	31.39	63.47	32.17	6189	30.73
Active Sleep [b]	27.76	22.82	17.69	14.52	5.53	7.31	19.40	18.34	19.82	21.52	18.30	15.67
Drowsy [c]	3.81	11.26	3.33	10.29	8.86	20.09	7.62	13.12	5.61	14.85	4.86	11.33
Alert Inactivity [d]	2.04	5.96	0.00	0.00	0.00	0.00	0.00	0.00	1.31	4.83	0.00	0.00
Awake [e]	1.11	4.71	0.00	0.00	1.00	3.16	0.00	0.00	1.07	4.16	1.19	6.29
Fussy/Cry [f]	6.11	13.35	2.41	5.60	3.43	7.35	5.51	9.30	5.15	11.48	3.52	7.13
Heel Stick												
Quiet Sleep [a]	7.10	12.57	7.78	18.89	30.36	30.94	27.73	36.22	15.41	23.40	14.90	27.51
Active Sleep [b]	8.02	12.64	22.31	34.29	14.53	31.60	6.25	10.62	10.35	21.06	16.58	28.97
Drowsy [c]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Alert Inactivity [d]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Awake [e]	0.00	0.00	1.85	7.85	0.00	0.00	0.00	0.00	0.00	0.00	1.19	6.29
Fussy/Cry [f]	**79.34***	29.18	**51.39***	46.11	55.12	38.29	66.02	39.94	70.69	34.12	56.61	43.83
Recovery												
Quiet Sleep [a]	51.01	26.62	50.60	33.58	74.01	36.09	63.63	22.61	59.23	31.72	55.26	30.34
Active Sleep [b]	24.36	19.43	18.92	17.78	**6.37****	9.99	**21.15***	14.02	17.94	18.66	19.72	16.30
Drowsy [c]	13.31	14.22	11.79	17.70	14.99	30.62	13.71	14.78	13.91	20.99	12.47	16.46
Alert Inactivity [d]	0.27	1.13	0.88	3.72	0.00	0.00	0.00	0.00	0.17	0.91	0.56	2.99
Awake [e]	0.81	2.47	1.14	3.83	0.00	0.00	0.48	1.52	0.52	2.00	0.91	3.18
Fussy/Cry [f]	**4.69***	7.57	**0.29***	1.24	4.63	11.02	1.05	3.33	**4.67***	8.75	**0.56***	2.19

Note. ABSS = Anderson Behavioral State Scoring System; [a] ABSS = 1 or 2 (Quiet Sleep); [b] ABSS = 3 or 4 (Active Sleep); [c] ABSS = 5 (Drowsy); [d] ABSS = 6 (Alert Inactivity); [e] ABSS = 7, 8 or 9 (Awake); [f] ABSS = 10, 11, or 12 (Fussy/Cry). KCH = Kangaroo Care Heel stick condition; IH = Incubator Heel stick condition. Comparison of KCH and IH by Paired t-test results: * < .05, ** < .01.

116

Description of Dependent Variables

Premature Infant Pain Profile (PIPP) Scores

The Total PIPP Scores including four behavioral (behavioral state, brow bulge, eye squeeze, and nasolabial furrow), two physiologic (HR and SaO_2), and one contextual (gestation age) factors, were recorded and calculated every 30 seconds during each phase. The behavioral data were analyzed from video tapes, physiological data from the pulse oximeter, and the infant's gestational age from the infant's medical record based on the Ballard Exam. A Total PIPP Score of 6 or less indicates "minimal or no pain," while greater than 6 to 12 indicates "mild pain," and greater than 12 indicates "moderate to severe pain" (Johnston et al., 2003; Stevens & Gibbins, 2002).

Throughout the Baseline and Heel Warming phases, infants had no PIPP scores that indicated pain expression in both the KCH and the IH conditions. Mean PIPP scores during Heel Stick and Recovery phases are reported in Tables 9 and 10 respectively. **During Heel Stick**, for the 80-min protocol subgroup, PIPP pain scores were similar between the KCH (range of means = 13.63 to 17.50) and IH (range of means = 13.25 to 18.00) conditions from 0.5 minute to the 5.5 minutes (11 time points) post stick and both KCH and IH infants had "moderate to severe pain." For the 30-minute protocol subgroup, PIPP pain scores were consistently lower in KCH (range of means = 4.00 to 10.60; one time point of "minimal or no pain" and 10 points of "mild pain") than IH (range of means = 9.75 to 14.33; 3 time points of "mild pain" and 8 time points of "moderate to severe pain"). In the 30-min protocol subgroup, PIPP scores lower in KCH than IH; differences were 3.01, 2.81, 2.84, 1.39, 2.64, 2.43, 5.40, 3.73, 0.42, 5.66 and 10.00 points from the 0.5 minute to the 5.5 minute respectively (11 time points) after the

stick. Because a difference of two points in PIPP scores is considered clinically important in infants (Johnston et al., 2003; Stevens & Gibbins, 2002), infants in the KCH condition had clinically significantly less pain than infants in the IH condition at nine of the 11 time points. No difference was found after the stick at the times points 2.0 and 4.5 minutes. Infants had 4.31% to 71.43% (average of 27% for 11 time points) less pain in KCH than in IH. The average effect size of the 11 time points was 0.84 (large effect) (Cohen, 1988) showing that KC had a strong effect on reducing pain during Heel Stick phase.

During the **Recovery phase**, in the 80-min protocol subgroup, infants in KCH had "mild pain" from the 0.5 minute to 3.0 minutes and "minimal to no pain" from 3.5 to 5.0 minutes, while infants in IH had "moderate to severe pain" at the 0.5 minute, "mild pain" from 1.0 to 2.0 minutes, and "minimal or no pain" from 2.5 to 5.0 minutes. In the 80-min protocol subgroup, PIPP scores in the KCH condition appeared to reach the recovery point (PIPP $\leq$ 6) more slowly (requiring 3.5 minutes) than in the IH condition (requiring 2.5 minutes). In the 30-min protocol subgroup, during the Recovery phase, infants in KCH had "mild pain" from 0.5 to 1.0 minute and "minimal or no pain" from 1.5 to 5.0 minutes, while infants in IH had "mild pain" from 0.5 minute to 1.5 minutes and "minimal or no pain" from 2.0 to 5.0 minutes. The PIPP scores in the KCH condition returned to a score $\leq$ 6.0 more quickly by 1.5 minutes in Recovery while IH condition scores recovered later at 2.0 minutes. The PIPP scores in KCH were clinically lower than in IH at 0.5 minutes (1.9 points, 21% different) and at 1.5 minutes (1.9 points, 25% different) and the effect sizes were medium (0.59) and large to medium (0.68) respectively at these two time points during Recovery in the 30-min protocol subgroup.

Table 9.

Mean and Standard Deviation of PIPP Scores During Heel Stick Phase for the 80-min Protocol and 30-min Protocol Subgroups and the Total Sample

Time points [a]	80-min Protocol Subgroup (n = 18)						30-min Protocol Subgroup (n = 10)						Total Sample (N = 28)					
	KCH			IH			KCH			IH			KCH			IH		
	n	M	SD	n	M	SD	n	M	SD	n	M	SD	n	M	SD	n	M	SD
0.5 min	16	13.63	2.73	16	13.25	3.24	10	**10.10**†	3.81	9	**13.11**†	4.26	26	12.27	3.57	25	13.20	3.55
1.0 min	16	15.06	1.34	16	14.94	3.12	10	**10.30**†	3.09	9	**13.11**†	5.01	26	13.23	3.18	25	14.28	3.90
1.5 min	16	15.50	1.71	16	15.06	3.04	10	**10.60**†	3.53	9	**13.44**†	5.43	26	13.62	3.49	25	14.48	4.03
2.0 min	15	15.93	2.40	15	15.67	2.38	10	10.50	4.14	9	11.89	5.11	25	13.76	4.15	24	14.25	4.00
2.5 min	14	15.93	2.84	11	16.09	0.83	10	**8.80**†	3.58	9	**11.44**†	5.79	24	12.96	4.74	20	14.00	4.48
3.0 min	12	16.42	1.38	8	15.25	1.45	7	**10.43**†	4.47	7	**12.86**†	4.85	19	14.21	4.08	15	14.13	3.62
3.5 min	8	16.38	1.67	6	14.17	2.86	5	**8.60**†	4.56	8	**14.00**†	5.73	13	13.38	4.91	14	14.07	4.57
4.0 min	6	16.67	1.21	3	15.33	1.16	3	**9.67**†	4.04	5	**13.40**†	5.68	9	14.33	4.15	8	14.13	4.45
4.5 min	4	15.50	3.70	2	15.50	1.41	3	9.33	3.79	4	9.75	5.19	7	12.86	4.74	6	11.50	4.49
5.0 min	2	17.50	0.71	1	18.00		3	**8.67**†	4.51	3	**14.33**†	2.89	5	12.20	5.81	4	15.25	2.99
5.5 min	2	17.00	1.41	0			1	**4.00**†		2	**14.00**†	4.24	3	12.67	7.57	2	14.00	4.24

Note. [a] time points in minutes after the heel stick; KCH = Kangaroo Care Heel stick condition; IH = Incubator Heel stick condition. PIPP $\leq$ 6 = "minimal or no pain"; PIPP > 6 to 12 = "mild pain"; PIPP > 12 = "moderate to severe pain". † Infants in the KCH condition had clinically significantly less pain (difference of PIPP $\geq$ 2 points) than infants in the IH condition after the stick.

119

Table 10.

Mean and Standard Deviation of PIPP Scores During Recovery Phase for the 80-min Protocol and 30-min Protocol Subgroups and the Total Sample

Time points [a]	30-min Protocol Subgroup (n = 10)						30-min Protocol Subgroup (n = 10)						Total Sample (N = 28)					
	KCH			IH			KCH			IH			KCH			IH		
	n	M	SD	n	M	SD	n	M	SD	n	M	SD	n	M	SD	n	M	SD
0.5 min	17	10.94	3.85	16	12.19	3.80	10	**7.30**[†]	1.95	10	**9.20**[†]	4.44	27	9.59	3.69	26	11.04	4.24
1.0 min	17	8.88	4.08	16	9.56	3.12	10	6.30	1.95	10	7.70	4.06	27	7.93	3.63	26	8.85	3.55
1.5 min	17	7.59	3.62	16	8.94	3.11	10	**5.80**[b†]	1.69	10	**7.70**[†]	3.89	27	6.93	3.14	26	8.19	3.49
2.0 min	17	7.29	3.27	16	7.00	1.46	10	5.00	1.33	9	**5.33**[b]	1.50	27	6.44	2.91	24	6.38	1.66
2.5 min	17	7.24	3.25	16	**5.88**[b]	1.26	10	5.20	1.40	10	5.90	2.23	27	6.48	2.86	26	**5.88**[b]	1.66
3.0 min	17	6.35	2.26	16	5.88	1.20	10	5.00	1.33	10	5.80	1.87	27	**5.85**[b]	2.05	26	5.85	1.46
3.5 min	17	**5.35**[b]	1.97	16	5.50	1.16	10	4.90	1.37	10	5.70	2.71	27	5.19	1.76	26	5.58	1.86
4.0 min	17	4.76	1.79	16	4.94	1.06	10	4.80	1.03	10	6.20	3.65	27	4.78	1.53	26	5.42	2.42
4.5 min	17	4.35	1.41	16	4.63	0.96	10	4.50	1.08	10	6.00	3.77	27	4.41	1.28	26	5.15	2.48
5.0 min	17	4.47	1.46	16	4.88	1.63	10	4.60	1.08	10	5.30	1.34	27	4.52	1.31	26	5.04	1.51

Note. [a] time points in minutes after Heel Stick phase completed; KCH = Kangaroo Care Heel stick condition; IH = Incubator Heel stick condition. [b] PIPP score returned to "minimal or no pain". PIPP $\leq$ 6 = "minimal or no pain"; PIPP > 6 to 12 = "mild pain"; PIPP > 12 = "moderate to severe pain". [†] Infants in the KCH condition had clinically significantly less pain (difference of PIPP $\geq$ 2 points) than infants in the IH condition.

120

Heart Rate Variability (HRV) Indices

Heart rate (R-to-R intervals) and heart rate variability indices were recorded using the ANS-R1000 system (Ansar, Inc., Philadelphia, PA) on 14 infants who received 80-min protocol. Description of the mean and standard deviation of HR and the geometric mean of HRV indices (after automatic elimination of movement and artifact data) during the four study phases in the KCH and IH conditions are reported in Table 11. Mean HR in KCH was 6.0 beats/min (Baseline), 2.0 beats/min (Heel Warming), 6.0 beats/min (Heel Stick) and 8.0 beats/min (Recovery) less than in IH. Mean Low frequency power (LF, predominate sympathetic activity) was 123% (Baseline), 184% (Heel Warming), 71% (Heel Stick), and 384% (Recovery) higher in KCH than in IH during the four phases. Mean high frequency power (HF, parasympathetic activity) was 43% (Baseline), 284% (Heel Warming), 106% (Heel Stick), and 3,878% (Recovery) higher in KCH than in IH during the four phases. The mean LF/HF ratio (balance of sympathetic-parasympathetic activity) was 43% lower during Baseline and 28% lower during Recovery in KCH than in IH; and was 21% higher during Heel Warming and 122% higher during Heel Stick in KCH than in IH. In summary, a trend has been shown that LF and HF were higher in the KCH condition than IH condition across all four study phases indicating increased HRV.

Table 11.

Mean Heart Rate and Geometric Means of Low Frequency Power, High Frequency Power, and Low Frequency/High Frequency Ratio During the Four Study Phases

	KCH (n = 13)		IH (n = 10)	
	M	SD	M	SD
HR (beats/min)				
Baseline	146.46	9.36	152.07	12.83
Heel Warming	152.10	11.65	153.71	11.91
Heel Stick	158.64	13.58	164.87	14.48
Recovery	146.42	14.17	153.86	14.12
LF (ms^2)				
Baseline	6.30	10.38	2.83	2.65
Heel Warming	19.44	54.37	6.84	6.38
Heel Stick	30.05	41.26	17.62	24.55
Recovery	15.16	29.85	3.13	2.46
HF (ms^2)				
Baseline	2.05	3.77	1.38	3.10
Heel Warming	22.91	70.57	5.96	8.62
Heel Stick	48.50	71.04	23.52	35.96
Recovery	19.89	54.42	0.50	0.52
LF/HF ratio				
Baseline	6.32	4.56	11.18	9.00
Heel Warming	5.12	5.71	4.23	4.90
Heel Stick	3.89	6.69	1.75	1.84
Recovery	7.61	5.26	10.60	8.63

Note. KCH = Kangaroo Care Heel stick condition; IH = Incubator Heel stick condition; LF = Low Frequency power (predominately sympathetic activity); HF = High Frequency power (sympathetic activity); LF/HF ratio = Low Frequency and High Frequency power ratio (balance of sympathetic-parasympathetic activity). Lower LF/HF ratio indicates a better balance

Hypotheses Testing

PIPP scores and HRV indices were compared between KCH and IH conditions during the four study phases (Baseline, Heel Warming, Heel Stick, and Recovery) to test for KC effects. Repeated-measures analysis of covariance (RM-ANCOVA for PIPP) and generalized estimating equations model (GEE for HRV) were used. Comparisons were also run for dependent variables across the four study phases to test for phase effects using RM-ANOVA.

Hypothesis 1. Preterm infants' bio-behavioral pain responses (Premature Infant Pain Profile; [PIPP]) will be significantly lower for KC heel stick (KCH) than for incubator heel stick (IH). A repeated-measures analysis of covariance, with experimental condition (KCH vs. IH) as the repeated factor, order of conditions (Group A vs. Group B) as the between-subject factor, and previous painful experience and pre-study KC experience as covariates, was conducted for each of the five 30-second periods following heel stick through 2.5 minutes. Two and half minutes was chosen because it represents the time frame during which most (n = 20/28 or 71%) of the heel stick procedures had been completed.

Hypothesis one was supported by the results from the data in the Total Sample and in the 30-min protocol subgroup. Pain score (PIPP) was significantly lower in KCH (M = 12.27 ± 3.57) than IH (M = 13.20 ± 3.55) at 0.5 minute post stick during the Heel Stick phase, $F(1, 20) = 6.11$, $p = .023$, but not at 1.0, 1.5, 2.0, and 2.5 minutes in the Total Sample. During Recovery in the 30-min protocol subgroup, infants in the KCH condition had less pain than infants in the IH condition at 2.0 minutes, KCH M = 5.00 ± 1.33, IH M = 5.33 ± 1.50, $F(1, 5) = 17.72$, $p = .008$, at 3.5 minutes, KCH M = 4.90 ± 1.37, IH M =

5.70 ± 2.71, F(1, 6) = 8.83, p = .025, at 4.0 minutes, KCH M = 4.80 ± 1.03, IH M = 6.20 ± 3.65, F(1, 6) = 89.84, p = .000, and at 4.5 minutes, KCH M = 4.50 ± 1.08, IH M = 6.00 ± 3.77, F(1, 6) = 20.93, p = .004. In the Total Sample, KCH PIPP scores were less than IH scores at 4.0 minutes, KCH M = 4.78 ± 1.53, IH M = 5.42 ± 2.42, F(1, 22) = 6.98, p = .015 and at 4.5 minutes, KCH M = 4.41 ± 1.28, IH M = 5.15 ± 2.48, F(1, 22) = 5.63, p = .027 (Table 12, Figure 5 and 6). No significant differences in PIPP scores were found between KCH and IH during Heel Stick in the 80-min protocol and 30-min protocol subgroups, and during Recovery in the 80-min protocol subgroup. Covariates, previous painful experience and pre-study KC experience were not significantly related to PIPP scores in the analysis.

Table 12.

Comparison of PIPP between KCH and IH (Testing KC Effects) Using Repeated-Measures Analysis of Covariance during Phases of Heel Stick and Recovery in the 80-min Protocol and 30-min Protocol Subgroups and the Total Sample

	80-min Protocol Subgroup (n = 18)			30-min Protocol Subgroup (n = 10)			Total Sample (N = 28)		
Phase and Time	F	df	p	F	df	p	F	df	p
Heel Stick Phase									
0.5 min	2.346	1 (11)	.154	0.107	1 (5)	.757	6.107	1 (20)	**.023***
1.0 min	0.098	1 (11)	.760	0.240	1 (5)	.645	2.918	1 (20)	.103
1.5 min	0.020	1 (11)	.890	0.011	1 (5)	.919	3.797	1 (20)	.066
2.0 min	0.007	1 (10)	.936	4.407	1 (5)	.090	0.195	1 (19)	.664
2.5 min	0.641	1 (6)	.454	5.528	1 (5)	.065	0.090	1 (15)	.768
Recovery Phase									
0.5 min	0.061	1 (12)	.809	2.136	1 (6)	.194	1.056	1 (22)	.315
1.0 min	0.316	1 (12)	.584	1.233	1 (6)	.309	0.063	1 (22)	.804
1.5 min	0.283	1 (12)	.605	0.004	1 (6)	.953	0.156	1 (22)	.697
2.0 min	0.007	1 (11)	.936	17.717	1 (5)	**.008****	0.247	1 (22)	.625
2.5 min	0.253	1 (12)	.624	1.435	1 (6)	.276	0.466	1 (22)	.502
3.0 min	0.040	1 (12)	.846	4.398	1 (6)	.081	1.687	1 (22)	.207
3.5 min	0.483	1 (12)	.500	8.833	1 (6)	**.025***	4.060	1 (22)	.056
4.0 min	0.611	1 (12)	.449	89.839	1 (6)	**.000*****	6.982	1 (22)	**.015***
4.5 min	0.126	1 (12)	.729	20.926	1 (6)	**.004****	5.629	1 (22)	**.027***
5.0 min	0.227	1 (12)	.643	5.948	1 (6)	.051	1.300	1 (22)	.266

Note. PIPP = Premature Infant Pain Profile; KCH = Kangaroo Care Heel stick condition; IH = Incubator Heel stick condition.
* $p < .05$, ** $p < .01$, *** $p < .001$

125

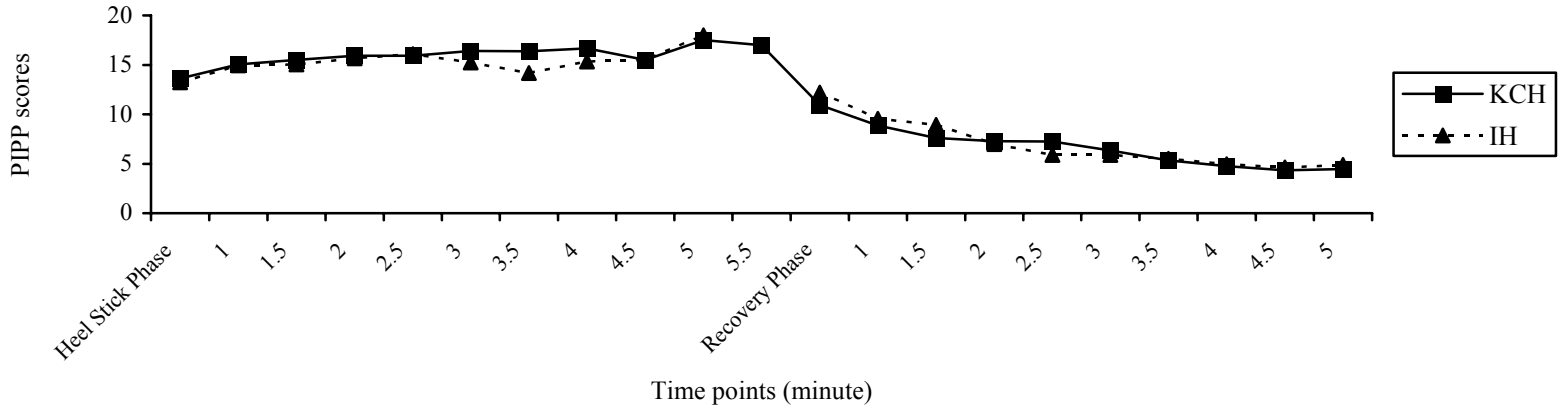


Figure 5. PIPP scores during Heel Stick and Recovery phases in the 80-min protocol subgroup in the KCH and IH conditions. KCH = Kangaroo Care Heel stick condition; IH = Incubator Heel stick condition.

126

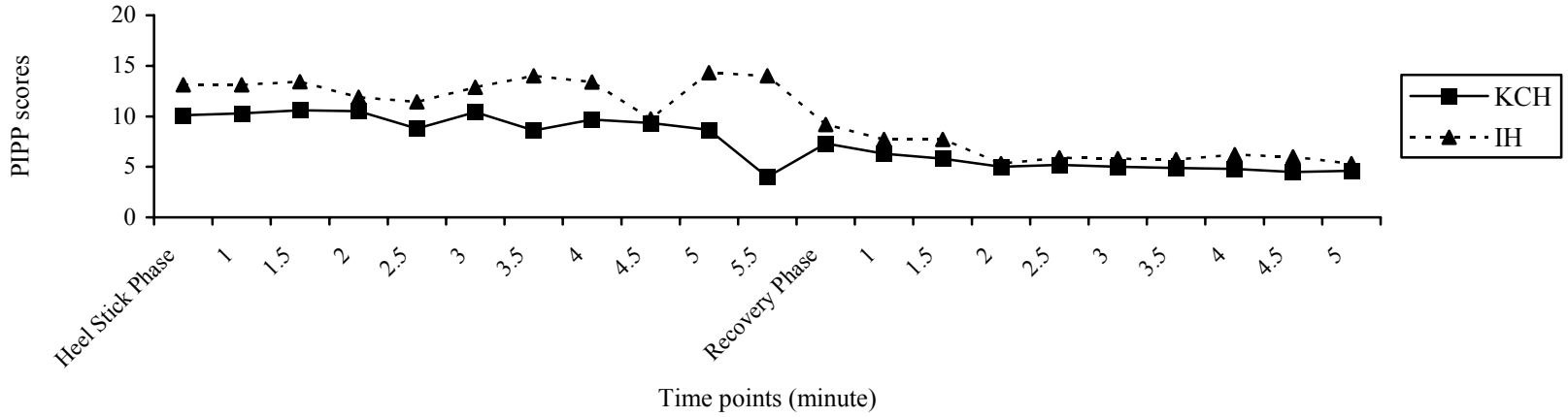


Figure 6. PIPP scores during Heel Stick and Recovery phases in the 30-min protocol subgroup in the KCH and IH conditions. KCH = Kangaroo Care Heel stick condition; IH = Incubator Heel stick condition.

Hypothesis 2. Preterm infants during KCH as compared to IH, will have decreased autonomic pain responses (specifically decreased sympathetic responses) as measured by heart rate variability indices (LF, HF, LF/HF).

Though the mean HR showed little difference between the KCH and IH conditions, the repeated measures ANOVA indicated significant differences between the conditions (KC treatment effects) and between the phases. When testing the treatment effects of KC, general estimated equation (GEE) model analysis showed HR was significantly lower in the KCH than in the IH condition during Baseline phase, $Z(1, 19) = 2.20$, $p = .027$ and Heel Stick phase, $Z(1, 16) = 2.43$, $p = .015$. Heart rate was higher during Heel Stick than Baseline, Heel Warming, and Recovery in both KCH, $F(3, 12) = 4.43$, $p = .010$ and IH, $F(3, 9) = 12.16$, $p < .001$. Heart rate returned to Baseline value during Recovery under both KCH and IH conditions.

The geometric mean was used to calculate HRV indices because it results in an average rate of change and represents variation in heart rate over time (McCain et al., 2005). GEE model analysis for treatment effects (Table 13, Figure 7) showed: (1) LF (sympathetic activity) was higher in KCH than IH during Baseline, $Z(1, 19) = 2.59$, $p = .010$ and Heel Stick, $Z(1, 17) = 3.43$, $p = .001$; (2) HF (parasympathetic activity) was higher in KCH than IH during Baseline, $Z(1, 19) = 2.00$, $p = .045$; and (3) LF/HF ratio (balance of sympathetic-parasympathetic activity) was lower in KCH than IH during Recovery, $Z(1, 18) = 3.59$, $p < .001$. The result of higher LF in KCH than IH during Heel Stick phase did support hypothesis two.

When comparing HRV indices across phases (Table 14, Figure 7), LF was higher during Heel Stick than the other phases in both KCH condition, $F(3, 10) = 4.06$, $p = .016$

and IH condition, $F(3, 9) = 4.75$, $p = .009$; HF was higher during Heel Stick than other phases in both KCH condition, $F(3, 10) = 6.18$, $p = .002$ and IH condition $F(3, 9) = 4.30$, $p < .001$; and LF/HF ratio was lower during Heel Sick than other phases in both KCH, $F(3, 10) = 6.07$, $p = .002$ and IH, $F(3, 9) = 11.63$, $p < .001$. HRV indices returned to Baseline values during Recovery in the IH conditions but remained elevated in the KCH condition.

Table 13.

Comparison of Mean Heart Rate and Heart Rate Variability Indices between KCH and IH During the Four Phases to Test KC Effects in the HRV Subgroup (n =14)

	Z	df	p
Heart Rate			
Baseline	2.20	1 (19)	**.028***
Heel Warming	0.11	1 (18)	.916
Heel Stick	2.43	1 (16)	**.015***
Recovery	1.44	1 (18)	.150
Low Frequency Power			
Baseline	2.59	1 (19)	**.009****
Heel Warming	0.60	1 (18)	.549
Heel Stick	3.43	1 (17)	**.000*****
Recovery	1.46	1 (18)	.144
High Frequency Power			
Baseline	2.00	1 (19)	**.045***
Heel Warming	1.12	1 (18)	.262
Heel Stick	1.24	1 (17)	.217
Recovery	1.71	1 (18)	.088
Low/High Frequency ratio			
Baseline	0.77	1 (20)	.443
Heel Warming	0.95	1 (18)	.340
Heel Stick	0.07	1 (17)	.941
Recovery	3.59	1 (18)	**.000*****

Note. KCH = Kangaroo Care Heel stick condition; IH = Incubator Heel stick condition; HRV = Heart Rate Variability. * p < .05, ** p < .01, *** p < .001.

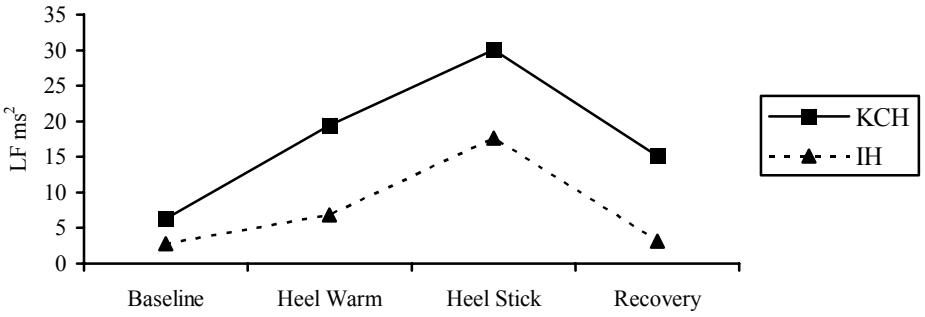
Table 14.

Comparison of Mean HR and HRV Indices Across the Four Phases in KCH and IH Conditions to Test Phase Effects in the HRV Subgroup (n =14)

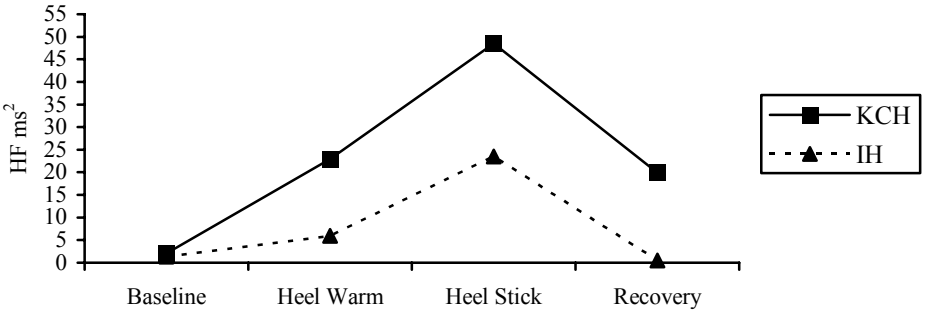
	F	df	P
Heart Rate			
KCH	4.426	3 (12)	**.010***
IH	12.164	3 (9)	**.000*****
Low Frequency Power			
KCH	4.063	3 (10)	**.016***
IH	4.748	3 (9)	**.009****
High Frequency Power			
KCH	6.175	3 (10)	**.002***
IH	4.302	3 (9)	**.000*****
Low/High Frequency ratio			
KCH	6.067	3 (10)	**.002****
IH	11.628	3 (9)	**.000*****

Note. KCH = Kangaroo Care Heel stick condition; IH = Incubator Heel stick condition; HRV = Heart Rate Variability. * p < .05, ** p < .01, *** p < .001.

A. Low Frequency Power



B. High Frequency Power



C. Low/High Frequency Power Ratio

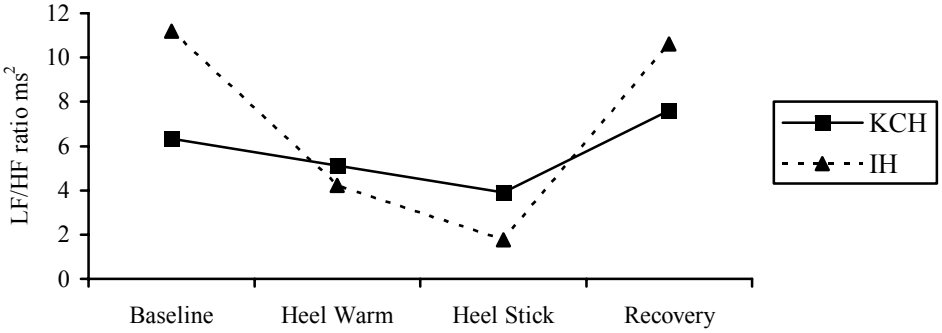


Figure 7. HRV indices across the four phases in the KCH and IH conditions. LF = low frequency power (predominately sympathetic activity); HF = high frequency power (parasympathetic activity); LF/HF = Low/High frequency power ratio (balance of sympathetic-parasympathetic activity); KCH = Kangaroo Care Heel stick condition; IH = Incubator Heel stick condition.

Additional Findings

Heart rate (HR) and oxygen saturation (SaO_2) data were also obtained using the Masimo SET Radical pulse oximeter system during the four study phases in KCH and IH conditions. The data were continually recorded every 2 seconds and downloaded to a laptop computer by the research nurse at the end of each study day. Descriptive and repeated measures ANOVA analyses were conducted using SPSS 13.0. To test for KC treatment effects, condition (KCH vs. IH) was entered as the repeated factor and order of conditions (Group A vs. Group B) as an additional factor. To test for phase (time) effects, study phase (Baseline vs. Heel Warming vs. Heel Stick vs. Recovery) was entered as the repeated factor and order of conditions (Group A vs. Group B) as an additional factor.

Heart Rate

Mean HR increased 15 – 23 beats/min during Heel Stick from the Baseline in both KCH and IH conditions in both the 80-min protocol and 30-min protocol subgroups, and in the Total Sample. When testing for KC treatment effects, no significant differences were found between KCH and IH during the four study phases in the 80-min protocol and 30-min protocol subgroups, and in the Total Sample (Table 15).

When testing for phase (time) effects, repeated measures ANOVA showed that HR was significantly higher during Heel Stick than other phases: 1) in KCH, $F(3, 16) = 33.46$, $p < .001$, and IH, $F(3, 16) = 27.19$, $p < .001$, in the 80-min protocol subgroup; 2) in KCH, $F(3, 9) = 18.44$, $p = .001$, and IH, $F(3, 8) = 16.73$, $p < .001$, in the 30-min protocol subgroup; 3) and in KCH, $F(3, 26) = 52.64$, $p < .001$, and IH, $F(3, 25) = 40.86$, $p < .001$, in the Total Sample.

Bradycardia and Tachycardia

Furthermore, the number of infants who had bradycardia (HR < 100 beats/min) and/or tachycardia (HR > 180 beats/min) was recorded and calculated (Table 16). A trend was seen in which during KCH, infants had less episodes of bradycardia and/or tachycardia than during IH, but these differences did not reach statistical significance. No significant differences of bradycardia or tachycardia occurred in number of infants were between KCH and IH during the four study phases in the 80-min protocol subgroup, 30-min protocol subgroup, and the Total Sample. However, the total duration (in seconds) of all bradycardiac episodes was significantly shorter in KCH than IH across the four phases in the 80-min subgroup, $F(1, 17) = 4.59$, $p = .040$ and in the Total Sample, $F(1, 51) = 5.07$, $p = .029$, but not in the 30-min subgroup (Table 17). The total duration of all tachycardia episodes was not significantly different between IH and KCH in the 80-min protocol and 30-min protocol subgroups and Total Sample.

Table 15.

Mean and Standard Deviation of Heart Rate (beats/min) During the Four Phases in the 80-min and 30-min Protocol Subgroups and the Total Sample

Phase	80-min Protocol Subgroup (n = 18)				30-min Protocol Subgroup (n = 10)				Total Sample (N = 28)			
	KCH		IH		KCH		IH		KCH		IH	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Baseline	148.52	9.31	150.44	10.70	150.33	12.51	154.28	10.51	149.17	10.37	151.86	10.59
Heel Warming	151.47	9.29	153.55	10.39	153.78	13.22	155.54	12.76	152.29	10.67	154.29	11.12
Heel Stick	171.88	16.33	171.22	15.73	170.28	14.58	168.84	12.62	172.03	14.12	172.06	13.09
Recovery	151.25	10.31	150.06	11.46	153.59	15.98	155.04	11.39	151.92	12.36	152.43	11.57

Note. KCH = Kangaroo Care Heel stick condition; IH = Incubator Heel stick condition. No significant differences in HR were found between KCH and IH conditions during the four phases in the 80-min protocol subgroup, the 30-min protocol subgroup, and the Total Sample.

133

Table 16.

Frequency and Percentage of Number of Infants Having Bradycardia and/or Tachycardia During the Four Study Phases in the 80-min and 30-min Protocol Subgroups and the Total Sample

	80-min Protocol Subgroup (n = 18)				30-min Protocol Subgroup (n = 10)				Total Sample (n = 28)			
	KCH		IH		KCH		IH		KCH		IH	
Phase & Variable	n	%	n	%	n	%	n	%	n	%	n	%
Baseline												
Bradycardia	1	5.9	2	11.8	1	10.0	2	20.0	2	7.4	4	14.8
Tachycardia	4	23.5	3	17.6	1	10.0	2	20.0	5	18.5	5	18.5
Heel Warming												
Bradycardia	1	5.9	0	0.0	1	10.0	2	20.0	2	7.4	2	7.4
Tachycardia	1	5.9	3	17.6	1	10.0	3	30.0	2	7.4	6	22.2
Heel Stick												
Bradycardia	1	5.9	2	11.8	0	0.0	3	30.0	1	3.7	5	18.5
Tachycardia	13	76.5	16	94.1	5	50.0	5	50.0	18	66.7	21	77.8
Recovery												
Bradycardia	2	11.8	3	17.6	0	0.0	2	20.0	2	7.4	5	18.5
Tachycardia	5	29.4	8	47.1	4	40.0	3	30.0	9	33.3	11	40.7

Note. KCH = Kangaroo Care Heel stick condition; IH = Incubator Heel stick condition. No significant differences of having bradycardia and/or tachycardia in number of infants were found between KCH and IH conditions during the four phases in the 80-min protocol subgroup, the 30-min protocol subgroup, or the Total Sample.

134

Table 17.

Mean and Standard Deviation of *Duration (Seconds) of Bradycardia and Tachycardia During the Four Study Phases in the 80-min Protocol and 30-min Protocol Subgroups and the Total Sample*

Condition	80-min Protocol Subgroup (n = 18)				30-min Protocol Subgroup (n = 10)				Total Sample (N = 28)			
	KCH		IH		KCH		IH		KCH		IH	
Phase & Variable	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Baseline												
Brady duration (s)	0.94	3.88	0.94	2.75	2.60	8.22	1.80	4.47	1.56	5.78	1.26	3.43
Tachy duration (s)	8.12	25.80	6.47	16.38	19.00	60.08	177.40	551.91	12.15	41.09	69.78	335.68
Heel Warming												
Brady duration (s)	0.12	0.49	0.00	0.00	2.00	6.32	12.60	26.60	0.81	3.85	4.67	16.83
Tachy duration (s)	3.18	13.10	2.47	6.54	10.00	31.62	10.60	23.27	5.70	21.52	5.48	15.16
Heel Stick												
Brady duration (s)	0.12	0.49	12.24	41.29	0.00	0.00	23.00	58.86	0.07	0.38	16.22	47.71
Tachy duration (s)	94.82	76.77	90.94	73.99	50.60	67.80	64.60	90.72	78.44	75.44	81.19	79.91
Recovery												
Brady duration (s)	1.29	4.41	5.18	12.31	0.00	0.00	4.20	8.97	0.81	3.52	4.81	11.01
Tachy duration (s)	17.65	57.66	43.41	142.86	36.60	81.60	25.40	57.43	24.67	66.62	36.74	111.39

Note. KCH = Kangaroo Care Heel stick condition; IH = Incubator Heel stick condition. Brady duration = bradycardia duration in seconds; Tachy duration = tachycardia duration in seconds. Using RM-ANOVA, duration of bradycardiac was longer in IH than KCH across the four phases in 80-min subgroup [(1, 31) = 4.59, p = .040] and in the Total Sample [F(1, 51) = 5.07, p = .029], but not in the 30-min subgroup. The duration of tachycardia was not significantly different between IH and KCH conditions in the 80-min protocol and the 30-min protocol subgroups and in the Total Sample.

135

Oxygen Saturation

In the KCH condition, mean Oxygen Saturation (SaO_2) dropped from Baseline to Heel Stick by 1.59% in the 80-min subgroup and by 0.82% in the Total Sample, but did not change at all in the 30-min protocol subgroup (Table 18). In the IH condition, mean SaO_2 decreased from Baseline to Heel Stick by 2.47% in the 80-min protocol subgroup, 2.67% in the 30-min protocol subgroup, and 2.25% in the Total Sample.

To test for KC treatment effects, infants in KCH had higher SaO_2 than IH infants during Heel Warming, $F(1, 8) = 5.49$, $p = .047$, and Heel Stick, $F(1, 8) = 8.69$, $p = .018$, in the 30-min protocol subgroup, but not in the 80-min protocol subgroup and the Total Sample. In the 30-min protocol subgroup, infants in KCH had no change in SaO_2 from Baseline to Heel Stick, whereas infants in IH dropped 3% from Baseline to Heel Stick, $F(1, 8) = 7.42$, $p = .026$.

To test for phase effects, SaO_2 was statistically lower during the Heel Stick phase than other phases in KCH, $F(3, 16) = 6.26$, $p = .009$, in the 80-min protocol subgroup and in IH, $F(3, 16) = 5.23$, $p = .016$, in the Total Sample. However, the differences were not clinical important, because the SaO_2 level was maintained at 95% - 97% across the four study phases. No differences from one phase to another were seen in the IH condition in the 80-min protocol subgroup, in both the KCH and IH conditions in the 30-min protocol subgroup, and in the KCH condition in the Total Sample.

Oxygen Desaturation

The number of infants who desaturated ($SaO_2 < 90\%$) was recorded and calculated (Table 19). Clinically significant oxygen desaturation events were defined as any decrease in SaO_2 below 90% for more than or equal to 1 second (Wong, 2004).

Desaturation events were categorized as "mild" (85 – 89%), "moderate" (81 – 84%), and "severe" (≤ 80%) (Thoyre & Carlson, 2003). No significant differences in the number of infants who desaturated were found between KCH and IH conditions during the four study phases in the 80-min and 30-min protocol subgroups, and in the Total Sample.

However, in relation to the duration in seconds of "mild" desaturation, KCH infants had no desaturation, whereas IH infants had 19.60 seconds "mild" desaturation during Heel Stick in the 30-min protocol subgroup, $F(1, 8) = 6.67$, $p = .032$. The total duration of desaturation (the sum of duration of "mild", "moderate" and "severe" desaturation) was less in KCH (3.53 seconds) than in IH (15.76 seconds) during Baseline in the 80-min protocol subgroup, $F(1, 15) = 5.18$, $p = .038$. In the 30-min protocol subgroup, infants in KCH had no desaturation at all during Heel Stick compared to 39.20 seconds total desaturation in IH, $F(1, 8) = 8.00$, $p = .040$.

Table 18.

Mean and Standard Deviation of Oxygen Saturation During the Four Study Phases in the 80-min and 30-min Protocol Subgroups and in the Total Sample

	80-min Protocol Subgroup (n = 18)				30-min Protocol Subgroup (n = 10)				Total Sample (N = 28)			
	KCH		IH		KCH		IH		KCH		IH	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Baseline	96.70	1.25	96.90	1.71	96.44	3.06	96.38	2.74	96.63	2.04	96.80	2.02
Heel Warming	96.50	1.46	96.18	2.13	**96.85***	2.07	**94.59***	3.17	96.60	1.66	95.76	2.61
Heel Stick	95.11	2.77	94.43	4.90	**96.83***	2.13	**93.71***	3.54	95.81	2.57	94.55	4.36
Recovery	97.06	2.04	96.39	1.71	96.44	2.58	95.93	2.51	96.81	2.32	96.28	2.00

Note. KCH = Kangaroo Care Heel stick condition; IH = Incubator Heel stick condition. Comparison of KCH and IH * $p < .05$

138

Table 19.

Frequency and Percentage of Number of Infants Having Oxygen Desaturation During the Four Study Phases in the 80-min and 30-min Protocol Subgroups and in the Total Sample

	80-min Protocol Subgroup (n = 18)				30-min Protocol Subgroup (n = 10)				Total Sample (N = 28)			
	KCH		IH		KCH		IH		KCH		IH	
Phase & Variable	n	%	n	%	n		n	%	n	%	n	%
Baseline												
"Mild" desat	6	35.29	6	35.29	4	40.00	4	40.00	10	37.03	10	37.03
"Moderate" desat	1	5.88	3	17.64	2	20.00	2	20.00	3	11.11	5	18.52
"Severe" desat	1	5.88	4	23.53	2	20.00	2	20.00	3	11.11	6	22.22
Heel Warming												
"Mild" desat	3	17.64	3	17.64	2	20.00	6	60.00	5	18.52	9	33.33
"Moderate" desat	1	5.88	2	11.76	1	10.00	2	20.00	2	7.41	4	18.41
"Severe" desat	1	5.88	2	11.76	1	10.00	1	10.00	2	7.41	3	11.11
Heel Stick												
"Mild" desat	9	52.94	4	23.53	1	10.00	5	50.00	10	37.03	9	33.33
"Moderate" desat	5	29.41	3	17.64	0	0.00	3	30.00	5	18.52	6	22.22
"Severe" desat	2	11.76	3	17.64	0	0.00	2	20.00	2	7.41	5	18.52
Recovery												
"Mild" desat	6	35.29	4	23.53	3	30.00	5	50.00	9	33.33	9	33.33
"Moderate" desat	3	17.64	4	23.53	2	20.00	2	20.00	5	18.52	6	22.22
"Severe" desat	0	0.00	1	5.88	1	10.00	1	10.00	1	3.70	2	7.41

Note. KCH = Kangaroo Care Heel stick condition; IH = Incubator Heel stick condition. desat = oxygen desaturation; "mild" desat = SaO_2 85 – 89%; "moderate" desat = SaO_2 81 – 84%; "severe" desat = $SaO_2 \leq 80\%$. No significant difference between KCH and IH.

139

Table 20.

Mean and Standard Deviation of Duration of Oxygen Desaturation in Second During Four Study Phases in the 80-min and 30-min Protocol Subgroups and in the Total Sample

Duration of Desat (Seconds)	80-min Protocol Subgroup (n = 18)				30-min Protocol Subgroup (n = 10)				TotaL Sample (N = 28)			
	KCH		IH		KCH		IH		KCH		IH	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Baseline												
"Mild" desat	2.71	4.58	13.76	31.85	34.60	89.21	58.60	172.11	14.52	54.90	30.37	106.60
"Moderate" desat	0.35	1.46	0.71	1.72	38.60	120.67	7.40	21.38	14.52	73.45	3.19	13.07
"Severe" desat	0.47	1.94	1.29	2.73	3.20	7.73	0.60	1.35	1.48	4.98	1.04	2.31
"Total" desat	**3.53***	6.98	**15.76***	32.98	76.40	216.57	66.60	194.62	30.52	132.48	34.59	120.03
Heel Warming												
"Mild" desat	0.94	2.93	2.35	8.22	0.80	1.93	38.40	91.24	0.89	2.56	15.70	56.90
"Moderate" desat	0.35	1.46	0.82	2.92	0.60	1.90	3.80	9.54	0.44	1.60	1.93	6.24
"Severe" desat	0.59	2.43	1.41	4.23	0.40	1.26	2.20	6.96	0.52	2.05	1.70	5.28
"Total" desat	1.88	6.76	4.59	15.01	1.80	4.05	44.40	91.46	1.85	5.82	19.33	58.46
Heel Stick												
"Mild" desat	14.24	25.44	9.76	19.27	**0.20***	0.63	**19.60***	27.82	9.04	21.12	13.41	22.80
"Moderate" desat	4.47	10.26	2.94	6.71	0.00	0.00	9.60	17.88	2.81	8.34	5.41	12.21
"Severe" desat	0.94	3.40	5.76	15.81	0.00	0.00	10.00	21.17	0.59	2.71	7.33	17.70
"Total" desat	19.65	35.78	18.47	39.63	**0.20***	0.63	**39.20***	64.90	12.44	29.66	26.15	50.29
Recovery												
"Mild" desat	5.41	10.97	3.88	8.87	8.60	16.36	16.40	33.18	6.59	13.01	8.52	21.62
"Moderate" desat	0.47	1.12	2.82	6.67	3.00	8.18	1.60	3.86	1.41	5.05	2.37	5.74
"Severe" desat	0.00	0.00	0.71	2.91	0.40	1.26	7.80	24.67	0.15	0.77	3.33	15.10
"Total" desat	5.88	12.07	7.41	17.55	12.00	20.68	25.80	44.50	8.15	15.71	14.22	30.93

Note. KCH = Kangaroo Care heel stick condition; IH = incubator heel stick condition desat = oxygen desaturation; "mild" desat = SaO_2 85 – 89%; "moderate" desat = SaO_2 81 – 84%; "severe" desat = $SaO_2 \leq 80\%$. Comparison of KCH and IH * < .05

140

Summary of the Results

In testing KC effects, premature infants had less pain as measured by PIPP in the KCH condition than in the IH condition during Heel Stick and Recovery phases in the 30-min protocol subgroup and in the Total Sample. The HRV indices were higher in the KCH condition than in the IH condition during Baseline (LF, sympathetic activity and HF, parasympathetic activity) and Heel Stick (LF, sympathetic activity) phases, and the LF/HF ratio (balance of sympathetic-parasympathetic activity) was lower in KCH than IH during Recovery. Additionally, infants in the KCH condition had shorter duration of bradycardia than in IH across the four study phases in the 80-min protocol subgroup and in the Total Sample. Infants in the KCH condition had higher mean SaO_2 during Heel Warming and Heel Stick phases and had less decrease from Baseline to Heel Stick than infants in IH condition in the 30-min protocol subgroup. The total duration of oxygen desaturation was less in the KCH condition than in the IH condition during Baseline in the 80-min protocol subgroup and during Heel Stick in the 30-min protocol subgroup.

In testing phase effects, PIPP scores indicated “moderate” to “severe” pain during Heel Stick in the 30-min and 80-min protocol subgroups and in the Total Sample. Heart rate, LF and HF were higher and LF/HF ratio was lower during Heel Stick than the other phases in both KCH and IH conditions in the 30-min and 80-min protocol subgroups and in the Total Sample. Oxygen saturation was lower during Heel Stick than other phases in the KCH condition in the 80-min protocol subgroup and in IH in the Total Sample.

CHAPTER FIVE

Discussion

The analgesic effects of Kangaroo Care (KC) on 28 premature infants undergoing heel stick pain were examined using Premature Infant Pain Profile (PIPP), heart rate variability (HRV) indices, heart rate (HR), and oxygen saturation (SaO_2) as indicators of pain response during Baseline, Heel Warming, Heel Stick, and Recovery phases in both KC heel stick (KCH) and incubator heel stick (IH) conditions. Main effects were defined as KC effects on pain responses between the two study conditions (KCH vs. IH) and phase effects were defined as heel stick effects on pain responses across the four study phases (Baseline vs. Heel Warming vs. Heel Stick vs. Recovery phase). Main effects and phase effects precede description of outcome variable results.

Main Effects

In relation to **KC effects**, premature infants in the KCH condition had lower pain scores (PIPP) during Heel Stick and Recovery phases than in the IH condition in the Total Sample and during Recovery in the 30-min protocol subgroup. The HRV indices LF (sympathetic activity) and HF (parasympathetic activity) were higher in the KCH condition during Baseline than IH condition, showing that both sympathetic and parasympathetic activities were increased in the KCH condition during Baseline. LF was higher during Heel Stick phase in KCH than IH, indicating that infants had more sympathetic response in KCH than in IH during Heel Stick. LF/HF ratio was lower during Recovery in KCH than IH, reflecting that infants had greater parasympathetic activity than sympathetic activity, and so a better balance of sympathetic-parasympathetic activity resulted.

When infants were in the KCH condition, HR showed a shorter duration of bradycardia across the four study phases than in the IH condition in the Total Sample and in the 80-min protocol subgroup. Infants had higher level of SaO_2 during Heel Warming and Heel Stick in KCH than in IH and KCH infants had no drop at all whereas IH infants dropped 3% in SaO_2 from Baseline to Heel Stick in the 30-min protocol subgroup. Infants in the KCH condition had no desaturation occurred at all, whereas infants in the IH condition had significant more desaturation in the 30-min protocol subgroup. The total duration of oxygen desaturation was less during Baseline phase in KCH than in IH in the 80-min protocol subgroup.

Phase Effects

In relation to **phase effects**, PIPP scores indicated "moderate" to "severe" pain during Heel Stick in both KCH and IH conditions indicating a clear pain response caused by the heel stick procedure. Heart rate, LF (sympathetic response) and HF (parasympathetic response) were higher and LF/HF ratio was lower during Heel Stick than other phases in both KCH and IH conditions which indicated autonomic responses to the heel stick pain. Oxygen saturation was lower during Heel Stick than other phases in the KCH condition in the 80-min protocol subgroup, and in the IH condition for the Total Sample, supporting other evidence that a heel stick is a stressful event even when in the presence of KC.

KC Effects on Premature Infant Pain Profile Scores

Testing hypothesis one. The hypothesis was that the Premature Infant Pain Profile (PIPP) scores would be lower in the KCH condition than in the IH condition. Hypothesis one was supported in the data from the 30-min protocol subgroup and from the Total

Sample (80-min + 30-min protocol subgroups data) based on PIPP scores for the Heel Stick and Recovery phases, the only phases for which PIPP scores were more than six, indicating "mild" or "moderate/severe" pain occurred.

In the **Heel Stick phase**, PIPP scores were significantly lower in the KCH condition than in the IH condition at the first time point (0.5 minutes into the heel stick) for the total sample. Through the remaining time points in the Heel Stick phase, the PIPP scores did not statistically differ between the KCH and IH conditions. Clinically significant differences in PIPP (any PIPP score difference ≥ 2 between KCH and IH) (Johnston et al., 2003; Stevens & Gibbins, 2002) occurred in the 30-min protocol subgroup: KCH condition PIPP scores were two or more points lower than IH condition scores at all 11 time points except two time points (at 2.0 and 4.5 minutes in the 30-min subgroup) during the 5.5 minutes of the Heel Stick phase, indicating 4.31% to 71.43% (average of 27% for 11 time points) less pain in the KCH condition than in the IH condition. The average effect size of the 11 time points was 0.84 (large effect) between KCH and IH (Cohen, 1988) showing that KC had a strong effect on reducing pain during Heel Stick phase.

Not only were PIPP scores different, so were **the number of time points** spent in "mild" versus "moderate-to-severe" pain levels. Ninety-one percent (10/11 time points) of the time pain was "mild" in the KCH condition versus 27% (3/11 time points) of the time in the IH condition. "Moderate-to-severe" pain occurred 9% (1/11 time points) of the time in KCH and 73% (8/11 time points) of the time in IH. In summary, in the Heel Stick phase, pain was less intense and less pervasive in the KCH condition than in the IH

condition for the 30-min protocol subgroup, showing a clear effect of KC on pain during its infliction. Clinical responses to pain were less extreme in the presence of KC.

In **the Recovery phase,** PIPP scores in KCH were lower than in IH at the 2.0, 3.5, 4.0, and 4.5 minute time points in the 30-min protocol subgroup and at the 4.0 and 4.5 minute time points in the Total Sample. At the 0.5 and 1.5 minute time points, KCH condition PIPP scores were two points (21% and 25%) less than IH condition scores, showing clinically significant lower levels of pain in KCH. The effect sizes also showed that KC had medium effect (Cohen, 1988), 0.59 at 0.5 minutes and 0.68 at 1.5 minutes, on reducing pain during Recovery in the 30-min protocol subgroup.

In relation to recovery from pain, with recovery defined as how soon post-Heel Stick the PIPP score dropped to 6.0 or less (the value that indicates "minimal or no pain"), recovery appeared shorter in KCH than IH (1.5 minutes vs. 4.5 minutes, respectively) in the 30-min protocol subgroup. In summary, pain was lower in KCH than in IH. Predominantly throughout the **Recovery phase** responses were less extreme. The KCH condition clearly facilitated recovery in the 30-min protocol subgroup.

The PIPP results discussed above are similar to and extend those found in Johnston's (2003) crossover study of older (32 - 36 weeks gestational age), healthier preterm infants who received 30 minutes of KC (or incubator care) before a heel stick and remained in KC during a heel stick (and had a later or earlier heel stick in an incubator). In Johnston's study, PIPP scores were lower at 30.0, 60.0, and 90.0 second time points during KC heel stick than they were during incubator heel stick and the PIPP score for the Heel Stick phase pain was 1.5 - 2.2 PIPP scores lower in KC than in the incubator. The results of the study reported here showed lower PIPP score during Heel

Stick at the 30 second time point, and extended Johnston's findings based on clinically significant decrements in pain during Heel Stick phase that can be ascribed to KC.

Thus, 30 minutes of Kangaroo Care before the Heel Warming phase reduced heel stick pain in prematures as measured by PIPP scores. The underlying mechanisms of the effect of KC on pain are not understood, but recent data from both humans and rodents provide insight into a potential molecular mechanism. Mooncey and associates (1997) and Modi and Clover (1998) found that serum and salivary cortisol and serum β-endorphin dropped significantly after 20 minutes of KC, indicating that pain responses may be diminished due to KC's ability to deactivate the HPA axis. The drop in β-endorphin after KC suggested an attenuation of the stress reaction induced by pain, at least in terms of its opioid components. The present data that showed lower pain scores in KCH during Recovery phase also suggested attenuation of the stress response. Weller and Feldman (2003) suggested that KC is a touch-based therapy that promotes infants' ability to moderate the effects of painful factors by increasing cholecystokinin (CCK) and opioid peptides secretion. Gitau and associates (2002) found that 20 minutes of KC attenuated stress responses. Another conceivable explanation is that KC may activate the pleasure center of the brain, thereby increasing release of dopamine, a neurotransmitter that dampens the pain responses (Gradin & Schollin, 2005). Olausson and associates (2002) found that caress-like, skin-to-skin contact between individuals may activate unmyelinated C tactile afferents and produce a faint sensation of pleasant touch. The findings identity C tactile as a system for limbic touch that may underlie infants' emotional, hormonal and affiliative responses to KC. Rodent studies demonstrated that maternal tactile stimulation affects stress responses in her offspring throughout the rest of

offspring's lives suggesting long-term down regulation of stress reactivity. The long term changes in stress reactivity reflect permanently altered gene expression, so-called "environmental programming" that changes HPA function (Meaney & Szyf, 2005).

However, results from the 80-min protocol subgroup (when phlebotomist #1 conducted the heel stick) did not show significant differences in PIPP scores between the KCH and IH conditions, and findings that were inconsistent in previous studies. One reason may be related to the phlebotomist's discomfort and tension with her inability to do blood collection in the KC position. The data were examined to determine length of the heel stick procedure when performed by the two phlebotomists. Phlebotomist #1 had significantly longer duration of heel stick in the KCH condition than in the IH condition in all infants in the 80-min protocol subgroup, $t(16) = 2.75$, $p < .05$. The mean length of heel stick by phlebotomist #1 was 4.24 ± 1.14 minutes in KCH and 3.47 ± 1.18 minutes in IH, and by phlebotomist #2 was 4.10 ± 1.60 minutes in KCH and 4.70 ± 2.83 minutes in IH. No differences in the duration of heel stick occurred between KCH and IH conditions when phlebotomist #2 performed the blood sampling. Phlebotomist #2 reported comfort in drawing blood in the KC position. Johnston (2003) acknowledged this moderator variable when she reported concern that there could have been some influence of position on the ability of the technicians who took the blood sample on her PIPP results (p 1087). The technicians in Johnston's study also reported that taking blood was more difficult in KC than when the infant was prone in the incubator. Therefore, if KC position negatively influenced the results due to technician's capability to optimally sample blood, the results would likely have been in the opposite direction,

which may have contributed to the explanation of no significant difference between conditions and KC infants crying more in the 80-min protocol subgroup.

Study design may have contributed to the unexpected findings in the 80-min protocol subgroup. Eighty minutes of KC prior to heel stick has not been tested in previous studies, but was used here to promote deep sleep and fully relaxed state when heel stick manipulations were begun. However, the 80 minutes of sleep appeared to contribute to more crying in the KC condition than in the incubator. One explanation for the increased cry may be the difficulty or agitation KC infants experienced when being aroused for heel warming after being deeply and quietly asleep in KC. Arousals that result in awakenings are generally accompanied by infant irritability and crying (Holditch-Davis & Edwards, 1998; Mirmiran, Maas, & Ariagno, 2003). Further studies are needed to test the effects of variations in duration of KC on infants' depth of sleep and pain responses.

Summary of PIPP results. Consistent with previous KC studies, the present results showed that KC is effective in reducing heel stick pain in preterm infants. KC had a large effect size during Heel Stick phase and reduced an average of 27% of the pain compared to incubator care at all 11 time points. KC continued to have a medium effect in the Recovery phase and the infant in KCH had 21% - 25% less pain than in IH at 0.5 and 1.5 minute time points, but not the other eight time points. Therefore, KC is a remarkably analgesic intervention to reduce heel stick pain. Overall, the data supported 30 minutes of KC rather than longer KC. Though one may think an explanation maybe that mothers became restless with 80 minutes of KC, observations of the mothers

confirmed that most fell asleep and remained asleep. So maternal stress being conveyed to the infant with the 80-min protocol was not supported by our data and observations.

KC Effects on Pain as Measured by HRV

Testing hypothesis two. The hypothesis that preterm infants will have decreased autonomic pain responses, specifically decreased sympathetic responses as measured by heart rate variability indices (LF, HF, L/H ratio), to KCH compared to IH was supported in the data from the HRV subgroup. The results showed a trend for mean HR (R-to-R intervals) to be lower in the KCH condition than in the IH condition during the four study phases. The lower mean HR was statistically significant during Baseline (KCH HR= 146, IH HR = 152, p = .028) and Heel Stick (KCH HR = 159, IH HR = 165, p = .015) phases. The magnitude of the HR differences between KCH and IH was only 6 beats/minute for both Baseline and Heel Stick phases. Although the differences were not considered clinically important, they supported an autonomic response (increased parasympathetic activity) in the expected direction. Heart rate was higher during Heel Stick than other phases in both KCH and IH conditions indicating a pain response to heel stick. Heart rate returned to the Baseline value during Recovery and did so quickly in both conditions.

Low frequency power (LF). The KC effects on LF (sympathetic activity) showed a pattern of mean LF being consistently higher in the KCH condition than in the IH condition during the four study phases. The differences was statistically significant during Baseline (KCH LF = 6.30 ms^2, IH LF = 2.83 ms^2, p = .009) and Heel Stick (KCH LF = 30.05 ms^2, IH LF = 17.62 ms^2, p = .000) phases. The LF region of HRV reflects predominantly sympathetic and partially parasympathetic activities. Higher LF in KCH

Baseline indicated that sympathetic influences were heightened during KC as compared to incubator. Higher LF during KCH Heel Stick than IH Heel Stick indicated activation of predominantly sympathetic influences in response to pain.

The increase in LF during KC Baseline and Heel Stick is consistent with previous literature (Schrod & Walter, 2002) in which LF increased when infants were tilted into the KC position, with a relatively greater increase in sympathetic than vagal activation due to upright positioning. LF tended to be higher during two hours of KC than during two hours in an incubator in preterm infants with bronchopulmonary dysplasia, but the increase failed to meet statistical significance (Smith, 2003).

Increases in sympathetic tone may be explained by several factors, i.e., maturation (gestational age and postnatal age), sleep state, maternal presence and body temperature, and change in body position (Chatow et al., 1995). A significant positive correlation was found between HRV indices (both LF and HF) and the gestational and postnatal ages of the preterm and full-term neonates (Longin, Schaible, Lenz, & Konig, 2005; Rosenstock, Cassuto, & Zmora, 1999). LF seems to increase with increasing gestational and early postnatal age and preterm infants have lower LF than term infants. Preterm infants still have lower LF than term infants even when they reach term age, suggesting that premature extrauterine life may delay maturation (Rosenstock et al., 1999). The cross-over design ensured equivalence regarding postnatal age and gestational age among subjects exposed to different conditions in the present study. Therefore, the higher LF in KCH cannot be explained by infant age.

Sleep state was found to affect both sympathetic and parasympathetic activities. LF is increased more during Active Sleep (Verklan & Padhye, 2004). However, our

behavioral state data did not show differences in Active Sleep between the KCH and IH conditions. Thus, the higher LF in the KCH Baseline cannot be explained by infants' sleep state.

The changes in LF have been found in response to thermoregulation influences and stimulation in preterm infants. An increased LF is associated with increasing temperature, and preterm infants require longer duration of stimuli than full-term and older infants in order to affect their vasomotor activity and react by changing LF (Davidson, Reina, Shefi, Hai-Tov, & Akselrod, 1997; Lindqvist, Oja, Hellman, & Valimaki, 1983; Shefi, Davidson, Maayan, & Akselrod, 1998). Infant sympathetic activity is increased in mother-infant bed-sharing environment compared to solitary-sleeping, which might be partly explained by thermal stimulation and thermoregulation when mothers are presence (Richard & Mosko, 2004). An increased environmental air temperature due to the mother's body temperature also causes LF to rise in KCH. The most likely explanation is that increased environmental temperature in KCH increased infant central temperature causing a concurrent increase in LF compared to IH.

The change in body position from horizontal to head-up results in pooling of blood in the lower body, decreasing venous return and slowing the ventricular filling rate, which in turn activate the baroreceptor reflex, causing an increase in sympathetic tone (Schrod & Walter, 2002). On the contrary, prone positioning reduced infants' LF compared to supine position and the reason is unclear (Jean-Louis et al., 2004; Sahni et al., 2000). The infants in the present study had equally controlled upright and prone position in both KCH and IH condition, however, LF was still higher in KCH than IH. Therefore, the higher LF in KCH than IH may not be explained by position, but was most

probably due to thermoregulation as previously noted. An increased LF is a positive finding because higher LF (higher sympathetic activation) enables infants to arouse from deep sleep in the presence of life-threatening events.

Apnea accompanied by desaturation/hypoxia is common in preterm infants (Martin & Abu-Shaweesh, 2005) and is common during sleep in preterm infants (sleep relate to breathing). Thus, prolonged apneas may easily constitute a life-threatening event for any preterm infant. However, those infants who experienced apnea during sleep had higher LF in the KC position than in the incubator and should be better able to arouse and spontaneously correct life-threatening events.

High frequency power (HF). High frequency results also showed a trend for HF to be higher in KCH than IH during all four of study phases. HF was significantly higher during KCH Baseline, M = 2.05, than IH Baseline, M = 1.38, p = .045. Higher HF in KC Baseline indicated that parasympathetic influences were heightened during KC as compared to incubator care. Parasympathetic influence reduces infant stress.

The effects of KC on HF during Baseline are similar to Schrod and Walter's (2002) findings, in which HF increased when infants were tilted into the KC position. Higher HF trended also to be higher during two hours of KC in comparison to two hours in an incubator in preterm infants with bronchopulmonary dysplasia, but the increase failed to meet statistical significant (Smith, 2003).

Several possible explanations for a higher HF during KC exist. Increased gestational age and postnatal age are accompanied by increased HRV parameters, both LF and HF, suggesting a maturation-related elevation of HRV (Longin et al., 2005; Rosenstock et al., 1999). However, infant postnatal age and gestational age were

equivalent in the KCH and IH conditions in the present cross-over design study. Therefore, the higher HF in KCH cannot be explained by infants' age.

Higher HF during KCH Baseline may also relate to infant behavioral state. During quiet sleep, healthy infants have increased power in HF, suggesting dominant parasympathetic activity during the deep, Quiet Sleep stage (Sahni et al., 2000; Smith, 2003; Villa et al., 2000). The results reported here showed 10% more time in Quiet Sleep during KCH Baseline than IH Baseline. Kangaroo Care increased infants' Quiet Sleep, and this increase was accompanied by a healthy increase in parasympathetic activity.

Another explanation for higher HF during KCH Baseline may be that KC activated pressure receptors that increased parasympathetic activity. A study of moderate-pressure massage therapy showed that parasympathetic activity peaked during massage and remained significantly higher throughout the 15-min post-massage period compared with infants who received sham, light-pressure massage (Diego, Field, & Hernandez-Reif, 2005; Ireland & Olson, 2000). Animal studies also indicate that tactile interactions between rat pups and their mother, a type of pressure receptor stimulation, activated pups' parasympathetic responses and prevented all the changes associated with maternal deprivation (Kuhn et al., 1991; Pauk, Kuhn, Field, & Schanberg, 1986; Schanberg, Ingledue, Lee, Hannun, & Bartolome, 2003). Moderate-pressure massage activates the infant's pressure receptors and KC may do this also, because the pressure receptors located in the infant's chest, abdomen, and extremities are activated by the full body touch between the infant and mother in KC position and the intensity of pressure in KC may be similar to moderate pressure. Increased HF in KCH may be due to KC stimulating infants' pressure receptors in the full body and is a positive outcome of KC.

Infants during KC experienced a higher level of HF than during the incubator. Higher level HF indicates increased parasympathetic activity on heart rate. Parasympathetic control is credited with stress reducing and relaxation effects and reflects a restorative, calming function to conserve energy (Verklan & Padhye, 2004). The NICU has long been recognized as a stressful environment (Aucott et al., 2002). Any thing can relax infants and reduce the stress associated with NICU hospitalization is a desirable intervention. Mooncey (1997) and Modi and Glover (1998) found that as little as 20 min of KC did reduce stress as measured by serum cortisol by 67% and 72% respectively. Activation of parasympathetic power (higher HF) in the Baseline phase may be a benefit to the whole heel stick process, because higher HF allows wide deviation from the mean HR and allows infants to have more flexibility to adjust to a stimulus such as pain (Verklan & Padhye, 2004). The higher HF presented in the data is a positive finding that suggests infants' vagal control is present in all phases and may be exerting its influence to promote recovery from the stressful heel stick.

Low frequency/high frequency ratio (LF/HF). The mean LF/HF ratio was significantly lower in KCH, M = 7.61, than IH, M = 10.60, p = .000, during the Recovery phase, indicating that when in KCH rather than IH, infants had a better balance between LF and HF. The lower LF/HF ratio during KCH Recovery reflected an increase in HF to counter balance the high LF during heel stick to re-establish a baseline heart rate. The effects of KC on LF/HF ratio are similar to Smith's study (2003), in which a trend of lower LF/HF ratio occurred during two hours of KC compared to incubator care in bronchopulmonary dysplasia infants.

Declines in the LF/HF power ratio are associated with maturation as postnatal age and gestational age increase, indicating an increase in parasympathetic contribution to control of HR with maturation (Chatow et al., 1995; Mazursky, Birkett, Bedell, Ben-Haim, & Segar, 1998). The cross-over design in the present study ensured equivalence (e.g., postnatal age and gestational age) among subjects exposed to different conditions. Therefore, the lower LF/HF ratio in KCH cannot be explained by the infant's age. Another explanation for lower LF/HF ratio in KCH is that the ratio is minimal at environmental temperatures that maintain infants' temperatures within the neutral thermal zone (Davidson et al., 1997). Most recently, infant temperature has been found to increase to the thermoneural range when infants were cool, but also decreased if infants were too warm, suggesting that mothers may have the ability to modulate their infant's temperature during KC (Chiu et al., 2005; Ludington-Hoe et al., in press; Ludington-Hoe et al., 2000). The environmental temperature in KC position maintains the infant's thermal neutral zone (K. Bauer et al., 1997; Chiu et al., 2005; Karlsson, 1996; Ludington-Hoe et al., 2004; Ludington-Hoe et al., 2000) suggesting the possibility that KC may contribute to a lower LF/HF ratio through the thermal mechanism.

Therefore, a more mature response (lower LF/HF ratio) to the painful heel stick painful procedure (during Recovery) was present in KCH than in IH. Autonomic maturation has been ascribed to KC that was provided for only 3 hours KC (Ludington-Hoe et al., In press) and to KC that has occurred repeatedly over the course of hospitalization (Feldman & Eidelman, 2003; Ohgi et al., 2002; Tessier et al., 2003).

Variance in LF and HF. Variances in LF and HF were much higher in the KCH condition than IH condition during the four phases: KCH LF SD = 10.38 – 54.37 vs. IH

LF SD = 2.46 – 24.55; KCH HF SD = 3.77 – 71.04 vs. IH HF SD = 0.50 – 23.52. Variance in LF and HF were also higher during KC in Smith's (2003) study. Wide variation in the LF and HF region suggests within-subject differences that may be due to a number of factors. The use of sedative medications may have an effect on the autonomic nervous system which may be reflected in the LF and HF power (Smith, Doig, & Dudley, 2004). Sedatives, vasopressors, and analgesics are associated with wide variance of autonomic responses in infants. However, no infants receiving these medications were recruited in the present study. Therefore, this explanation is not valid for this sample. Another explanation of wide variance of HRV in KCH may be the wide range of infants' responses to KC with mother. Reasons are unknown, but KC appears to influence autonomic measures more individually than the incubator environment does.

HRV varied across all four phases. Pain effects on HRV were evident in both KCH and IH conditions. Mean LF and mean HF increased from Baseline and Heel Warming phase values to Heel Stick values, confirming that heel stick is indeed a painful stimulus. Paradoxically, mean LF/HF ratio was lower during Heel Stick than other phases in both the KCH and IH conditions. Heart rate variability indices returned to Baseline values during Recovery on the incubator day but remained elevated on the KC day. An explanation for why LF and HF did not return to baseline during the 20 min KCH Recovery phase may be the magnitude of response to the heel stick. Low frequency power response to Heel Stick was 30.05 in the KCH condition and only 17.62 in the IH Heel Stick. Similarly, HF increased to 48.50 in the KCH Heel Stick and to 23.52 in the IH Heel Stick. The results demonstrated that heel stick blood sampling procedure moderately activated sympathetic and highly activated parasympathetic

nervous system responses under both KCH and IH conditions. Over a 20 minutes period, given the differences in peak response, decrement that occurred was in proportion to peak value, 50% KCH LF vs. 82% IH LF drop and 60% KCH HF vs. 98% IH HF drop from Heel Stick phase to Recovery phase. Still, the higher LF and HF values in KCH Recovery phase versus IH Recovery phase support heightened autonomic response to a to a painful stimulus in the KCH condition, and a heightened autonomic response to a stressor is better than a diminished autonomic response, at least in the short term.

Though the *effect of KC* on heart rate variability indices is similar to other studies, the *effect of pain* on heart rate variability indices reported here differs from those in the literature. LF and HF has been reported to drop during heel stick in preterm infants (Lindh et al., 1999; Lindh et al., 1997; Oberlander, Grunau, Fitzgerald, Ellwood et al., 2002; Oberlander, Grunau, Fitzgerald, & Whitfield, 2002; Oberlander et al., 2000). In each of the studies reporting decreased LF and HF with Heel Stick, the heel stick occurred while infants were horizontal and supine. All heel sticks in the study reported here were conducted while infants were at a 30 degree incline and prone. Effect of incline on LF and HF is an increase (Goto et al., 1999), whereas effect of prone on LF and HF is a decrease (Jean-Louis et al., 2004). The present study showed that in the prone and tilt position (both KCH and IH) a preterm infant had increased LF and HF from Baseline to Heel Stick, whereas, previous studies showed that LF and HF decreased from Baseline to Heel Stick in the supine and flat position. A clear stress response induced by heel stick is indicated by increased HR and decreased parasympathetic activity (HF), and increased HF may indicate a blunted pain response (Lindh et al., 1999). Therefore, infants in the prone and tilt position may have less pain response during a heel

stick than infants in the supine and flat position. Though this explanation is speculative, the deduction from evidence provided in this dissertation study is logical. Further studies are needed to examine effects of different positions on infant pain response.

The present study showed that LF/HF ratio dropped from Baseline to Heel Stick in both KCH and IH conditions in response to the pain stimulus, which is dissimilar to previous studies that no change in LF/HF ratio occurred from Baseline to Heel Stick (Lindh et al., 1997; Oberlander, Grunau, Fitzgerald, & Whitfield, 2002; Oberlander et al., 2000). The LF/HF dropping from Baseline to Heel Stick may be interpreted as an increase in parasympathetic contribution to HR control, which would be inconsistent with the expected increase in sympathetic activity and parasympathetic withdrawal accompanying a noxious stimulus (Oberlander & Saul, 2002). The underlying mechanism is unknown. The change in the normal respiratory pattern during painful procedure and its relative contribution to HRV may, in part, contribute to the decrease in the LF/HF ratio (Chatow et al., 1995; Oberlander et al., 2000).

Summary of HRV results. Consistent with previous KC studies, the present study showed that the warmth of the mothers' chest and the positioning inherent in KC increased LF (sympathetic activity) and HF (parasympathetic activity) and decreased LF/HF ratio indicating a better balance of sympathetic and parasympathetic activity. Increases in LF and HF and a decrease in LF/HF ratio are signs of maturation and good adaptability and indicate that the individual has well-functioning neural control mechanisms and more flexibility and stability, abilities that the individual needs to respond to external or internal demands in preterm LBW infants (Chatow et al., 1995; Jean-Louis et al., 2004; Verklan & Padhye, 2004). Increased maturity of the autonomic

nervous system permits the cardiac system to respond to stressors, such as painful procedures, with greater variance from baseline without becoming overwhelmed by the stimulus (Verklan & Padhye, 2004). Increased LF and HF also potentially decrease the vulnerability of the infant to Sudden Infant Death Syndrome (SIDS) (Goto et al., 1999). Decreased HRV is associated with SIDS in preterm infants during prone sleep (Ariagno et al., 2003; Goto et al., 1999). Although the mechanism has not been elucidated, one speculation is that lower HRV may increase the likelihood for cardio-respiratory failure, thereby increasing the risk of SIDS (Ariagno et al., 2003). The increased HRV in KC positioning may reduce the risk of SIDS and suggests that KC is a safe intervention, even though the infant is in a prone sleep position; however, the incline inherent in KC in this study may have exerted an influence.

KC Effects on Pain as Measured by Pulse Oximeter Data

Heart rate. No significant differences in infant HR (obtained from pulse oximeter) were found between KCH and IH conditions during any of the four study phases, a finding in contrast to Ludington-Hoe and associates' (2005) finding of less rise in HR from Baseline to Heel Stick in the KC condition (3 - 13 beats/min) than in the incubator condition (8 - 24 beats/min) after three hours of either the KC or incubator care. The lack of HR difference in the data reported here is dissimilar to Gray and colleagues' (2000, 2002) findings in which full-term KC infants had substantially less HR change from Baseline to Heel Stick than control infants. Another study of pain in full-term infants undergoing hepatitis B vaccine injection showed that KC infants had 7% lower mean HR than control infants (Kostandy, 2005). The equivocal findings may be due to

differences in the gestational age, postnatal age, and/or the duration of KC (Charpak, Ruiz et al., 2005).

Heart rate was significantly higher during Heel Stick than during other phases. Mean HR increased 15 - 23 bpm from Baseline to Heel Stick in both KCH and IH conditions in the 80-min protocol subgroup (mean HR change KCH = 23 bpm, IH = 21 bpm), the 30-min protocol subgroup (KCH = 20 bpm, IH = 15 bpm), and the Total Sample (KCH = 23 bpm, IH = 20 bpm). Heart Rate accelerates from baseline during a heel stick for blood sampling, indicating a clear pain response in preterm infants, which is consistent with other findings (Grunau et al., 2004; Holsti et al., 2005; Lindh et al., 1997). As soon as the infant's foot is touched, heart rate increases, and when lancing occurs, HR rises more. Following the lance, HR rises further with squeezing, producing a more pronounced change in HR in preterm infants than in full-term infants (Lindh et al., 1997; Walden, 2001). Squeezing causes the biggest increase in HR (Lindh et al., 1999).

Bradycardia. The total duration (in seconds) of bradycardia was shorter in the KCH condition than IH condition across the four phases in the 80-min protocol subgroup and in the Total Sample. Infants in the 30-min protocol subgroup also showed a similar trend: i.e. less bradycardia duration in KCH than IH. No previous reports could be found related to KC effects on bradycardia during heel stick pain in preterm infants. However, fewer bradycardia episodes occur in KC preterm infants than controls (Eichel, 2001; Ludington-Hoe et al., 2004), supporting the results of the study reported here. In contrast, two studies showed that infants had more bradycardia in KC than in control condition (Bohnhorst et al., 2004; Bohnhorst et al., 2001). The equivocal findings of KC effects on bradycardia may relate to duration of KC and infants' demographic and

medical characteristics. The result of less bradycardia in KCH than IH reported here supports findings that infants have better balance of sympathetic and parasympathetic activities in KCH than in IH, which was reported in the HRV section. A better balance of the autonomic nervous system may lead to a stable physiological response to stressors, such as painful procedures, whereas an imbalance of sympathetic-parasympathetic control may be manifested by apnea, bradycardia, color changes, and/or blood pressure abnormalities (Verklan & Padhye, 2004).

Oxygen saturation. Infants in the KCH condition had significantly higher SaO_2 than when in IH during Heel Warming (KCH SaO_2: 96.85%; IH SaO_2: 94.59%) and Heel Stick (KCH SaO_2: 96.83%; IH SaO_2: 93.71%) in the 30-min protocol subgroup. Mean dropped significantly less from Baseline to Heel Stick in the 30-min protocol during KCH than IH condition (KCH: no decrease at all; IH: 2.67% drop, $p < .05$) and a trend of smaller drops in the 80-min protocol (KCH: 1.59% drop; IH: 2.47% drop). The results indicated that KC reduced the decrease in SaO_2 in response to a heel stick and better maintained infants' SaO_2 at an optimal level ($\geq$ 95%) than incubator care. The dampened SaO_2 response during KCH is supported by findings of improved SaO_2 during KC in healthy preterm infants (Bier et al., 1996; Fohe et al., 2000; Ludington-Hoe et al., 1998).

That oxygen saturation decreased in response to pain in preterm infants is similar to other study findings (Butt & Kisilevsky, 2000; Huang et al., 2004; Lindh et al., 1997; Rush et al., 2005). With pain, SaO_2 commonly drops 3% to 4% below the infant's baseline and does so for the duration of the painful procedure (Ludington-Hoe et al., 2005). Oxygen saturation dropped less in the current study (0 - 2.67%) than in previous studies, probably due to prone positioning in both the KCH and IH conditions. Prone

positioning is significantly more beneficial to oxygen saturation than supine position (Chang, Anderson, & Lin, 2002; Wells, Gillies, & Fitzgerald, 2005).

Oxygen desaturation. No significant differences in the number of infants who desaturated were found between KCH and IH conditions during the four study phases in the 80-min and 30-min protocol subgroups and in the Total Sample. However, "mild" desaturation did not occur in the KCH condition, but did in the IH condition (19.60 seconds, $p < .05$), during Heel Stick in infants receiving the 30-min protocol. The total duration of desaturation (the sum of durations of "mild", "moderate" and "severe" desaturations) was less in KCH ($M = 3.53$ seconds) than in IH ($M = 15.76$ seconds, $p < .05$) during Baseline in the 80-min protocol subgroup. In the 30-min protocol subgroup, KCH infants had no desaturation occurred at all, but IH infants had a total desaturation in 39.20 seconds, $p < .05$ during Heel Stick. The results indicate that oxygen saturation was more stable in the KCH condition than IH condition in response to the heel stick. No study has been conducted to test the effect of KC on oxygen desaturation during a heel stick. The results of KC effects on oxygen desaturation are equivocal (Charpak, Ruiz et al., 2005). Two studies showed that preterm infants in KC had fewer desaturation episodes than in incubator (Bier et al., 1996; Eichel, 2001), and two other studies showed that infants had more desaturation episodes during KC than in the incubator (Bohnhorst et al., 2004; Bohnhorst et al., 2001). Thus, more studies are needed to test effects of KC on pain reduction as measured by SaO_2 levels and desaturation episodes.

Behavioral State Findings

During Baseline. Infants showed a trend toward more percentage of time in Quiet Sleep in KCH (59% - 65%) than in IH (49% - 60%), and less Active Sleep (5% - 24%) in

KCH than in IH (18% - 26%) in both the 80-min and the 30-min protocol subgroups. The difference in Active Sleep time (KCH baseline: 5% vs. IH Baseline: 18%) was significant in the 30-min protocol subgroup. The results are similar to findings in investigations of the effect of KC on behavioral state (Chwo et al., 2002; Feldman & Eidelman, 2003; Lai et al., 2005; Ludington, 1990; Ludington-Hoe & Swinth, 1996), in which the uniform results have been that preterm infants quickly settle down and fall asleep, demonstrating Quiet Sleep predominately and diminished Active Sleep (Charpak, Ruiz et al., 2005).

During Heel Stick. In the 80-min protocol subgroup, infants cried more in KCH (79% of the time) than in IH (51%) and the difference was significant. In the 30-min protocol subgroup, infants cried less in KCH (55%) than in IH (66%) over both days but the difference was not statistically significant. Further infants cried less during the Recovery phase in the KCH condition on the first day of study than during all other phases; and on the second day infants cried less during Heel Stick phase in the KCH than IH condition. The conflicting results from the two subgroups may be explained by the heel stick procedure as performed by the two phlebotomists (discussed in the PIPP result section, p147) and different study protocol. The result that infants in the 80-min protocol subgroup cried more in KCH than in IH may be explained the interruption and Quiet Sleep the 80-min protocol subgroup infants might have experienced. Arousal latencies have been found longer in Quiet Sleep compared to Active Sleep (Parslow, Harding, Adamson, & Horne, 2004), supporting that infants in Quiet Sleep during KC Baseline may be hard to be awaken and very irritated when Heel Warming and Heel Stick occur.

The results of the 30-min protocol subgroup are consistent with findings in another preterm infant study (Ludington-Hoe et al., 2005) and in two full-term studies (Gray et al., 2002; Gray et al., 2000), in which KC infants cried less than control infants during Heel Stick phase. Infant crying is a stress behavior (Ludington-Hoe et al., 2002) and crying clearly occurs in response to pain in preterm infants. Kangaroo care infants cried less than incubator care infant indicating that a heel stick in KC may be less stressful than a heel stick in the incubator care and supporting that the multi-sensory effects of KC calm the infant and reduce pain reactivities.

During Recovery. In the 80-min protocol subgroup, infants cried more in KCH (4.69%) than in IH (0.29%) possibly due to the longer duration of the heel stick in KCH than in IH. In the 30-min protocol subgroup, infants had more Quiet Sleep (74%) and less Active Sleep (6%) in the KCH condition than in the IH condition (Quiet sleep: 63%; Active Sleep: 21%) suggesting that KC calmed infants responding to a heel stick. This finding is similar to those of Gray et al, (2000, 2002), Kostandy (2005), and Ludington-Hoe et al, (2005).

Limitations

The data should be considered with caution because of the following limitations. First, mothers who agreed to participate in this study may have been mothers who believed they would be comfortable in the KC situation during a heel stick on their infants in the NICU, thus selection bias may have existed.

Second, another selection bias may be that 71% of participated infants had cesarean section births. Pharmacological effects of analgesia can be detected as long as three days after birth (Kuhnert, Linn, Kennard, & Kuhnert, 1985) and are known to

depress neurobehavioral organization. Thus HRV data may have been influenced as well as PIPP data. The infant postnatal age in the present study was 2 to 9 days with a mean of 6 days. The effects of delivery analgesia on infant pain response needs to be considered in future studies.

Third, the data reported here were collected when infants were 2 to 9 days post-birth, creating the possibility that of prenatal stressors and fetal condition may have played a role in the results. The "developmental origins of adult disease" paradigm proposes that prematurity and impaired fetal growth reflect a fetal strategy to cope with an impaired fetal environment, e.g. maternal stress and undernutrition (Gluckman, Cutfield, Hofman, & Hanson, 2005). Exposure to an adverse fetal environment leads to an altered risk of disease in adult life, a condition termed "programming" (D. J. Barker, 2000). Programming is a result of the channeling of developmental plasticity by adaptive responses so a match between developmental and responses to a later environment is enhanced. The plastic period extends from the fetal period into the neonatal period for the premature infant. Thus, the prenatal environment may have exerted an influence on neonatal reactivity to pain and this possibility should be measured in future study.

Fourth, observers, PIPP coders, and ABSS scorers could not be blinded to the treatment and control conditions. The researchers tried videotaping and "whiting-out" the background to make scores "blind", but maternal chest movement during KC still moved the infant on the screen making "blinding" impossible. In regard to PIPP data, only the infant's face was focused on the screen, but the mother's respiratory movement, the glare of room light on the incubator walls and the sound of opening incubator doors still identified the KCH or IH condition. Double coding by two coders was then

implemented to minimize bias effects. The two PIPP scores for the same subject were compared and corrected when they were not identical.

The fifth limitation was the small sample size for the HRV subgroup and the 30-min protocol subgroup. The HRV subgroup was small due to unavailability of the HRV equipment; the 30-min protocol subgroup was small due to financial constraints. Thus, comparisons between the subgroups have been interpreted conservatively.

Implications for Theory

Levine's Conservation Model. The findings showed that KC was a nursing intervention that reduced preterm infants' pain measured by PIPP, HRV, SaO_2 level, bradycardia events, and oxygen desaturation episodes. Kangaroo Care appears to have stabilized preterm infants' physiologic status and conserved their energy during a heel stick. Heart rate variability data also showed that infants had a better balance (parasympathetic maturation) of autonomic nervous responses to pain during KC than during incubator care. Improved parasympathetic tone is believed to promote growth and restoration and conserve energy (Porges, 1995). The results extend Levine's Conservation Model, especially the Principle of Conservation of Energy component, to include the benefits of KC in reducing infant pain.

In relation to the *Gate Control Theory of Pain and the Neuromatrix Theory of Pain,* KC was shown to be effective in deceasing heel stick pain in preterm infants, suggesting that KC may activate non-nociceptive tactile nerve impulses (large nerve fibers), close the gate in the dorsal horn, and inhibit nociceptive transmission, thus attenuating infant pain experience and responses as described by the Gate Control Theory. Kangaroo Care is a multi-sensory stimulus which serves as competitive stimuli

during a heel stick, minimizing pain response as described by the Neuromatrix Theory of Pain. KC appears to have activated the central nervous system (behavioral component of the PIPP and crying time changed) supporting KC modulates and inhibits pain perception and contributes to the output of the neuromatrix in such a way that pain responses (PIPP, HRV indices, bradycardia, SaO_2, and oxygen desaturation) are reduced.

Implications for Research

Based on the findings and the limitations of the present study, future studies should be conducted in relation to pain assessment scales, skin assessment with repeated painful procedures, HRV as a pain response measure, and KC treatment time. The recommendations for future studies are discussed in the following sections.

Pain assessment scales. Despite the progress made in neonatal pain research since the early 1980s, no gold standard for pain assessment in the neonate exists (Anand et al., 2005). Determining the presence of pain in the neonatal population remains problematic for health professionals because of the subjective nature of pain, the lack of accurate indicators of pain, and the infants' inability to verbally communicate their pain. A variety of physiologic, behavioral, biochemical, contextual, and other indicators have been used, either singly or in combination, to develop tools for the study of neonatal pain.

The PIPP, which combines bio-behavioral responses, is a well-developed and widely used composite measure of procedure pain in preterm and term infants. However, one limitation of the composite measures is that the relationships between biologic and behavioral responses are not clear, particularly in regard to interpretation of pain responses in extremely premature infants (Anand et al., 2005). Reports that behavioral and physiological responses are either uncorrelated or weakly correlated across situations

and studies are common (Barr, 1998; Grunau et al., 2005; Johnston, Stevens, Yang, & Horton, 1995; Stevens et al., 1994). This dissociation impedes decision-making about the effectiveness of interventions, leaving nurses or physicians uncertain about relying on behavioral, physiologic or composite pain outcomes. Physiologic measures alone may not be specific to pain, and they may or may not increase along with behavioral response. Behavioral responses generally are more consistent and specific to pain than physiologic measures alone.

Assessment is the cornerstone of adequate pain management and it is the responsibility of health researchers to develop, test and use the best measures to assess infant pain. Further studies are needed using PIPP and other scales to test KC effects on pain. PIPP scores can be compared to pain responses measured by behavioral assessment tools such as Neonatal Facial Coding System (NFCS) (Craig, Hadjistavropoulos, Grunau, & Whitfield, 1994; Pereira et al., 1999), and to other composite scales including Neonatal Infant Pain Scale (NIPS) (Lawrence et al., 1993), Pain Assessment in Neonates (PAIN) (Hudson-Barr et al., 2002), CRIES (Krechel & Bildner, 1995; McNair, Ballantyne, Dionne, Stephens, & Stevens, 2004), and the Pain Assessment Tool (PAT) (Hodgkinson, Bear, Thorn, & Van Blaricum, 1994; Spence, Gillies, Harrison, Johnston, & Nagy, 2005). A study comparing responses according to different measurement tools would help determine the most appropriate instrument for assessing pain in neonates.

Skin assessment with repeated pain procedures. The present study was the first to incorporate skin assessment in infant heel stick research. In the present study heel skin assessment was conducted for each infant before the heel stick using the Neonatal Skin Condition (Lund & Osborne, 2004). Infants who had any signs of tissue breakdown or

inflammation/necrosis of either heel were excluded from the study, because tissue damage increases pain responsiveness and has long term consequences on nociceptive neuronal circuit development (Lindh et al., 1999; Ruda et al., 2000). Thus, uninjured neonatal skin condition was used as an inclusion criterion in the study reported here, but in future studies, the relationship between infant skin condition and pain responses, and interventions that can reduce pain in infants who have breakdown skin due to repeated pain procedures should be examined.

Heel stick vs. venipuncture. The heel stick method for blood sampling may be an inappropriate method for routine use in neonatal practice due to marked pain responses during heel lance and squeezing (Lindh, 1999). Venipuncture seems to be a less painful alternative (Larsson, 1998). A recent study demonstrated that venipuncture is less painful and more effective than heel stick for blood sampling in full-term newborns and oral sucrose is not necessarily required when venipuncture is chosen as the blood sampling method (Ogawa, 2005). Further studies are needed to compare pain responses to the heel stick and venipuncture in preterm infants and to determine KC effects on venipuncture pain. Future studies should also compare pain responses to KC alone and in combination with other nonpharmacologic interventions to determine which treatment is the best.

Heart rate variability as a measure of pain response. Heart rate variability is a unique non-invasive measure of the sympathetic and parasympathetic activity of the nervous system in responses to pain. However, infants' maturation (gestational and postnatal age), behavioral state, medication use, and health conditional contribute to potentially altering autonomic responses to painful events (Oberlander & Saul, 2002). Thus, the methodology used and its application must be flexible. Furthermore, both time

domain and frequency domain analyses of HRV are based on the assumption of stationarity of the data (Oberlander & Saul, 2002). Stationarity requires that the mean of HR and variance remain stable over time. However, in most clinical research settings, particularly in studies of infant pain reactivity, stationarity is rarely possible where environmental and infant physical conditions and behavioral state may not be stable. Therefore, having environmental conditions as stable as possible during the recording period and careful selection of HR epochs for stationarity is critical, as was practiced in the study reported here. Analytical methods using linear or polynomial trend removal have also been used to remove nonstationary trends (Cowan et al., 1993; Longin et al., 2005; Porges, 1995). A new HR measure developed by Cao and associates (2004) has been using in detecting nonstationary HR series in clinical diagnosis of sepsis, and is a promising method for future studies in examining infant pain responses as measured by HR characteristics.

Kangaroo Care treatment time. The duration of KC treatment for infant pain needs further investigation. Kangaroo Care has now been shown to be effective in reducing heel stick pain when given for 30 minutes (in the present study and Johnston's study [2003]) and 180 minutes (in Ludington-Hoe's study [2005]) before the stick in preterm infants. In the full-term infants, 5 minutes of KC combined with breastfeeding (Gray et al., 2002) and 15 of minutes KC before the heel stick (Gray et al., 2000) effectively reduced infants' pain responses. Thirty minutes of KC before an injection also reduced pain from hepatitis B vaccination in full-term infants (Kostandy, 2005). The most effective duration of KC for maximum pain reduction in neonates, especially in preterm infants, is not clear.

Maternal stress during Kangaroo Care. Maternal stress and maternal behavioral state during Kangaroo Care should be measured and controlled in future studies. Maternal response to the stress has been found to be "communicated" or conveyed to the infant and the mother-infant dyad has a synchronized parallel response to the stress (Coplan et al., 2005). Therefore, a maternal stress response may affect infant pain response. In the present study 57% of the infant-mother dyads had not had KC experience before the study. Both the mother and infant during KC could have had stress during their first KC session as Morelius et al. (2005) found in their study comparing maternal stress with the first and forth KC sessions. Infant autonomic responses may be blunted if the infant was stressed, which is possible in the first KC session (Morelius et al., 2005). Future study is needed to segregate data provided by dyads experiencing KC for the first time KC from data provided by dyads with previous KC experience.

Infant candidate for Kangaroo Care. Not all neonates are equally sensitive to variations in maternal care (Liu, Diorio, Day, Francis, & Meaney, 2000). Thus, KC might not be equally effective in all infants due to influence of individual genetic expression and prenatal stress exposure on newborn reactivity. NICU infants could be stratified in groups by umbilical cord cortisol level to determine which infants are the best candidates for KC, or prenatal condition and postnatal status can be used as stratifiers because response may be different in these groups. Estimating infant genotype and how infants respond according to genotype should also be considered.

Implications for Practice

Pain intervention. The behavioral and physiological responses to repeated painful procedures in neonates can be life threatening and are related to long-term sequelae. For

instance, pain causes oxygen desaturation while increasing intracerebral pressure and blood pressure, both of which can lead to brain hemorrhage (Anand & Hickey, 1987; Bellieni, 2005). Every effort needs to be made to reduce the number of painful events and blunt the painful responses during neonatal intensive care (Anand, 1998). The past research coupled with results from the present study show that KC is effective in reducing infant pain. The high rate of cooperation from the mothers in this study suggests that KC can be implemented readily in standard hospital settings as a strategy to minimize pain.

Some concerns may be expressed by health providers or parents about the potential risk of negative conditioning if KC was employed repeatedly for painful procedures. Animal studies suggest that simultaneous close contact and suckling prevent activation of afferent pathways to the brain, preventing cortical activation and, thus, associative memory (Ren, Blass, Zhou, & Dubner, 1997; Ren & Dubner, 2002). Both animal and human studies suggest that maternal touch and contact have the potential to reverse the negative impact of separation from the mother and painful procedures on the infant's emotion regulation capacities which may be evident on the structural, neurochemical, and behavioral levels (Weller & Feldman, 2003). The vast majority of parents, regardless of ethnic backgrounds – white, black, or Hispanic, would prefer to remain present even for highly invasive procedures and coach and soothe their child during the procedure (Jones, Qazi, & Young, 2005). Studies in older children being held by their mothers for surgical procedures showed that both the mother and child had less anxiety (Kain, Caldwell-Andrews, & Wang, 2002) and parents of children undergoing surgery in the United States were allowed to be present more often during induction of

anesthesia as compared with 1995 (Kain et al., 2004). Long periods of maternal-child contact prior to painful procedures and intensification of maternal-infant contact by holding the infant more firmly and adding soothing vocalizations and stroking after the procedure may be useful for preventing the negative associations in preterm infants too (Ludington-Hoe et al., 2005).

Conclusion

In summary, KC decreased pain (PIPP) scores during both Heel Stick and Recovery phases. Infants in the KCH condition had improved autonomic nervous system activity in the Baseline phase and demonstrated better balance in autonomic control in the Recovery phase too. Infants in KC had higher oxygen saturation level and less bradycardia, and shorter duration oxygen desaturation than in the incubator. The present study examined KC effects on reducing preterm infant pain using PIPP and using HRV indices as pain indicators during heel stick. Kangaroo Care (skin-to-skin contact) was shown as an effective and safe intervention for reducing pain in preterm infants. Though preterm infants have limited ability to respond and adapt to environmental challenges, KC helped infants in their response to the pain associated with heel stick. KC's use as a pain management strategy in preterm infants undergoing heel stick should be encouraged.

Appendix A

Neonatal Skin Condition Score

Study ID#_____ Data Collector _____

Date_____ Time_____

Observations	1	2	3	Total Score
Dryness	Normal, no sign of dry skin	Dry skin, visible scaling	Very dry skin, cracking/fissures	
Erythema	No evidence of erythema	Visible erythema < 50% of heel surface	Visible erythema > 50% of heel surface	
Breakdown/ Excoriation/ Scab/ Bruises	None evidence	Small localized areas	Extensive	
Perfect score = 3; worst score = 9.				

Appendix B

Demographic and Medical Data Chart for Preterm Infants.

Date _____ Data Collector_____ Study ID _____

1. Date of birth (MM/DD/YY and time).....Date:_____/_____/_____
Time:_____:_____
2. Infant's postconceptional age (Weeks and Days)….....week:_____Day:_____
3. Postnatal age (Days).....
4. Gender… .....
(1) Male
(2) Female
5. Race.....
(1) White
(2) Black or African American
(3) Asian
(4) American Indian or Alaskan Native
(5) Native Hawaiian or Pacific Islander
(6) 2 or more selected
(7) Do not know
(8) Refused
6. Is baby's ethnic background Hispanic?.....
(1) Yes
(2) No
7. Type of birth… .....
(1) Vaginal
(2) Caesarean Section
8. Birth weight (in Grams).....
9. Weight at the time of testing (in Grams).....
10. APGAR scores.....1-minute_____; 5-minutes _____
11. Feeding .....
(1) Yes (2) No
12. If #11 is Yes, then type of feeding.....
(1) Bottle (2) Gavage
(3) Breast (4) Mixed _____
13. Type of milk.....
(1) Breast milk
(2) Formula
14. Severity of illness (SNAP II Score)… .....
* The Score for Neonatal Acute Physiology: Version II (SNAP-II) is used to measure the severity of clinical condition. The score can be obtained from medical chart.
15. Documented Intraventricular Hemorrhage (IVH).....
(1) No (2) Grade I (3) Grade II
16. Pre-study KC experience.....
(1) Yes (2) No

Demographic Information for Mothers.

Date _____ Data Collector_____ Subject ID _____

1. What is your age in years at the birth of this child ....._____
2. Race....._____
   (1) White (2) Black or African American
   (3) Asian (4) American Indian or Alaskan Native
   (5) Native Hawaiian or Pacific Islander (6) 2 or more selected

3. Is your ethnic background Hispanic ....._____
   (1) Yes (2) No

4. Employment situation before delivery....._____
   (1) Employed - Full time (2) Employed - Part time
   (3) Full time homemaker (4) Full time student
   (5) Disabled (6) Unemployed
   (7) Refused

5. Occupation status....._____
   Job Title_____
   Kind of work_____

6. Yearly income…....._____
   (1) Under $5000 (2) $5000 - $9,999
   (3) $10,000 - $19,999 (4) $20,000 - $29,999
   (5) $30,000 - $39,999 (6) $40,000 - $49,999
   (7) $50,000 - $59,999 (8) $60,000 - $69,999
   (9) $70,000 - $79,999 (10) $80,000 - $89,999
   (11) $90,000 - $99,999 (12) $100,000 or more
   (13) Don't know (14) Refused

7. Educational level....._____
   (1) Completed Graduate school
   (2) Completed (4 year) College
   (3) Some College(at least 1 year but less than 4)
   (4) Completed High School
   (5) Some High School (completed 10th or 11th grade)
   (6) Junior High School (completed 7th though 9th grade)
   (7) Less than 7 years of school (did not complete 7th grade)
   (8) Refused

8. Marital status....._____
   (1) Married (2) Never married (3) Divorced
   (4) Separated (5) Widowed (6) Refused

Appendix C
Premature Infant Pain Profile (PIPP) – Baseline, Heel Warm, and Heel Lance Procedure

Subject ID#:_____ Scorer initial: _____ Study Date: _____ Study: Day 1 / Day 2
GA birth _____wks _____days Day #1_____ Day # 2_____

Process	Indicator	0	1	2	3	Heel warm 4min & 30s ___-___	Heel warm last 30s ___-___	30s post HS ___-___
Chart	Gestational age	36 wks and more	32-35 wks, 6 days	28-31 wks, 6 days	less than 28 wks			
Observe infants 15 s	Behavioral state	active/awake eyes open facial movements, Cry +/-	quiet/awake eyes open no facial movements	active/sleep eyes closed facial movements	quiet/sleep eyes closed no facial movements			
Observe baseline Avg 20 min HR_____ Sao2_____								
Observe infants 30 s	Heart Rate Max_____	0-4 bts/min increase	5-14 bts/min increase	15-24 bts/min increase	25 bts/min or more increase			
	Oxygen saturation Min_____	0-2.4% decrease	2.5-4.9% decrease	5.0-7.4% 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	0 none 0-9% of time	minimum 10-39% of time	moderate 40-69% of time	maximum 70% of time or more			
					Total score			

177

Premature Infant Pain Profile (PIPP) – Squeezing Procedure (Score q 30 secs until the blood draw is complete)

Subject ID#:_____ Scorer initial: _____ Study Date: _____ Study Day 1 / Day 2

Process	Indicator	0	1	2	3	30s _____- _____	60s _____- _____	90s _____- _____	120s _____- _____	150s _____- _____	180s _____- _____	210s _____- _____
hart	GA	36 wks and more	32-35 wks, 6 days	28-31 wks, 6 days	less than 28 wks							
Observe infants 15 s	Beh state	active/ awake eyes open facial movement cry +/-	quiet/ awake eyes open no facial movement	active/ sleep eyes closed facial movement	quiet/ sleep eyes closed no facial movement							
Observe baseline HR_____ SaO2_____												
Observe infants 30 s	HR Max___	0-4 bts/min increase	5-14 bts/min increase	15-24 bts/min increase	25 bts/min or more increase							
	SaO2 Min___	0-2.4% decrease	2.5-4.9% decrease	5.0-7.4% 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	0 none 0-9% of time	minimum 10-39% of time	moderate 40-69% of time	maximum 70% of time or more							
Total score												

178

Premature Infant Pain Profile (PIPP) – Recovery Procedure (Score every 30 secs until the infant has returned to base line x2 scores. This may be different for each infant!)

Subject ID#:_____ Scorer initial: _____ Study Date: _____ Study Day 1 / Day 2

Process	Indicator	0	1	2	3	30s _____- _____	60s _____- _____	90s _____- _____	120s _____- _____	150s _____- _____	180s _____- _____	210s _____- _____
Chart	GA	36 wks and more	32-35 wks, 6 days	28-31 wks, 6 days	less than 28 wks							
Observe infants 15 s	Behav state	active/ awake eyes open facial movement cry +/-	quiet/ awake eyes open no facial movement	active/ sleep eyes closed facial movement	quiet/ sleep eyes closed no facial movement							
Observe baseline HR_____ SaO2_____												
Observe infants 30 s	HR Max___	0-4 bts/min increase	5-14 bts/min increase	15-24 bts/min increase	25 bts/min or more increase							
	SaO2 Min___	0-2.4% decrease	2.5-4.9% decrease	5.0-7.4% 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	0 none 0-9% of time	minimum 10-39% of time	moderate 40-69% of time	maximum 70% of time or more							
Total score												

179

Appendix D

Anderson Behavioral State Scoring System (ABSS)*

November 14, 1996

EYES OPEN OR CLOSED

HC	(12)	**Hard Crying**. Very prolonged exhalation; audible or silent cry; entire body very tense; (very red face; clenched fists)
C	(11)	**Crying**, Prolonged exhalation; audible or silent cry; general body tension; (red face).
F	(10)	**Fussing**. Normal color; single or frequent slightly prolonged exhalation; whimper; (precry grimace; snorts).

EYES OPEN

VAW	(9)	**Very Active Awake**. Total body movement; (twisting or lifting head or trunk; turning head side to side).
AW	(8)	**Active Awake**. Whole body movement; (twisting or lifting head or trunk slowly or slightly).
QW**	(7)	**Quiet Awake**. Eyes do not fix and follow; no movement or slight slow movement of head, face, forearm, hand, fingers, lower leg, foot, or toes.
AI**	(6)	**Alert Inactivity.** Eyes are wide open, quiet, luminous, fixated and/or following; no or slight slow movement of head, face, forearm, hand, fingers, lower leg, foot, or toes.

EYES OPENING AND CLOSING SLOWLY

D	(5)	**Drowsy**. Quiet or some movement; eyes dull or glazed; heavy lidded.

EYES CLOSED

VAS	(4)	**Very Active Sleep**. Total body movement; (twisting or lifting head or trunk; turning head side to side).
AS	(3)	**Active Sleep**. Whole limb movement; (twisting or lifting head or trunk slowly or slightly).
IS	(2)	**Irregular Sleep**. Irregular respirations; no movement or slight; slow movement of head, face, forearm, hand, fingers, lower leg, foot, or toes; (brief apnea).
RS	(1)	**Regular Quiet Sleep**. Regular, deep, and even respirations; faint or no movement; no rapid eye movement (slight mouthing or movement of fingers/ toes).

SUCKING ON: F = finger; G = tongue; H = hand; O = object; P = pacifier; T = thumb

OTHER: C = hiccough; J = jitter; M = mouthing; S = startle; W = twitch; Y = yawn; Z = sneeze

Notes:
Items in parentheses need not be present.
Whole limb movement involves shoulder or hip; forearm or lower leg involves elbow or knee.
Wait two minutes after any intervention before first state assessment.
Score the highest state attained during the 30 seconds.
If state 6 occurs, score as a 6 even if a higher number occurs.
If eye patches are on, assume eyes are closed unless seen to be open.
States 2 to 12 have irregular respirations.

* Adapted from Parmelee, Bruck, & Bruck (1962); Parmelee & Stem (1972).
** Activity is the same; only eyes differ.

Appendix E

SNAP-II
(Scores for Neonatal Acute Physiology Version II)

Study ID#_____ Data Collector_____

Date_____ Time_____

Variables	**Values**	**Points**
Mean blood pressure (mmHg)	>= 30 20 – 29 < 20	0 9 19
Lowest temperature (°F)	> 96 95 – 96 < 95	0 8 15
PO_2 (mmHg)/FiO_2 (%)	> 2.49 1.0 – 2.49 0.3 – 0.99 < 0.3	0 5 16 28
Lowest serum pH	> = 7.20 7.10 – 7.19 < 7.10	0 7 16
Multiple seizure	No Yes	0 19
Urine output (mL/kg.h)	> = 1 0.1 – 0.9 < 0.1	0 5 18
Total score		

Note: Data are collected within the first 12 hours after admission to the NICU (Richardson et al., 2001)

Appendix F

Data Collection Chart

Subject # _____
Date: _____
Condition:_____ (1 = KC, 2 = Incubator)
Page# _____

Minute-by-Minute Observation Record

1. Time (Take from HRV screen)	2. ABSS	3. Skin TEMP	4. COMMENTS

1. Time: Data collection to begin immediately 20 minutes before the heel warming and to end approx 25 minutes later.
2. ABSS = Anderson Behavioral State Scale Score. Begin scoring at top of min. (i.e.10:50:**00**).
3. Skin TEMP: record temp from incubator screen.

Appendix G

Study Protocol Manual
STEP-BY-STEP STUDY DAY PROCEDURE

Day 1 Day 2

0800 – Research Associate to arrive in NICU:

☐ ☐ Bring cart with equipment on it to the bedside
☐ ☐ Place Masimo probe on Lt. foot on KC day, Rt. foot on incubator day, or on a hand if IV is in a foot.
☐ ☐ At the same time, do the skin assessment of the heel.
☐ ☐ Clean skin with water and dry well.
☐ ☐ Attach HRV electrode patches. Place one electrode on baby's right side on lower rib cage, *directly in line with the armpit.* Place second electrode on baby's left side *directly opposite first electrode & directly in line with the armpit.* Hints: Use your hand to determine where the best respiratory excursion occurs.
☐ ☐ Plug power strip into an outlet that has nothing in the other plug.
☐ ☐ Attach lead wires to electrodes.
☐ ☐ Turn on the C-R monitor and be sure there is a good heart & respiratory signal. When it is determined that the signal is appropriate, turn the monitor off.
☐ ☐ ***If this is a KC day be sure mother is in NICU***

No instruments need to run until 0930.

0830 – position infant in correct position:

☐ KC day – in KC position on mother, with mother in recliner at a 30° angle.
☐ Incubator day – infant nested prone in incubator or on warming table inclined at a 30°angle.

As infant is positioned be sure there is space to position video camera to capture face, and that blankets do not obstruct viewing face.

No pacifiers during study

0830-0930 – Leave infant undisturbed:
During this hour:

☐ ☐ Bring Video equipment to the bedside & set up – Be sure we have good, close-up view of baby's face. HINT: Turning the spot lights on that are above the infant's bed usually will give a better video image of the face. It eliminates some of the shadows on the face.

☐ ☐ Check Masimo settings, & be sure previous previous data deleted.
Questions??? See sheet in set-up manual

☐ ☐ Attach Masimo probe to patient cable. Be sure patient cable is plugged into the Masimo, and the Masimo is plugged into electricity. DO NOT run the Masimo on battery if at all possible

0930 – Begin data collection:

☐ ☐ Turn on Masimo.
☐ ☐ Turn on HRV equipment (C-R monitor & computer).
☐ ☐ Place microphone for Video Camera near baby's face. Turn on the microphone.
☐ ☐ Be sure the Video Camera is on record.
☐ ☐ Focus camera on the time of day on the Masimo for approx. 10 sec.
☐ ☐ Focus camera on the time of day on the computer screen for approx. 10 sec., then refocus in on the baby's face.
☐ ☐ Begin hand collecting the Anderson Behavior State Scale and infant temp. Use the time on the HRV computer screen, & begin at the "top" of the minute (10:04:**00**).

☐ ☐ **0950 – Warm heel for heelstick:**

☐ ☐ Say out loud "Heel warmer applied"
☐ ☐ Note beginning of heel warming in the comments column of data collection sheet.

☐ ☐ **0955 – Heel lance and blood draw:**

☐ ☐ Say out loud "Heelstick" as heel is lanced.
☐ ☐ Collect serum cortisol, & AM Labs if applicable
☐ ☐ Note beginning of heelstick in comments column of data collection sheet

0958 (approx.) – End of blood draw:

☐ ☐ State out loud "End of blood draw" when draw completed & Coban secured.
☐ ☐ Note end of draw in comments column of data collection sheet.
☐ ☐ Continue collecting all data (HRV, video, sats)

☐ ☐ **1020 (approx.)**

☐ ☐ Discontinue all data collection.
☐ ☐ Turn off the video camera microphone
☐ ☐ BE SURE TO SAVE THE DATA ON THE COMPUTER
☐ ☐ While still at the bedside, and before turning off the computer, print all spectra screens, numerical data, & summary analysis.

☐ ☐ Remove all our equipment (Masimo sat probe can be saved, & electrodes can stay on, for the 2nd day of study,)

Upon completion of study:

☐ ☐ Download data from Masimo to Computer.
☐ ☐ Check computer program to be sure Masimo data has transferred.
☐ ☐ Delete data from Masimo after you are sure data has transferred to computer.
☐ ☐ Print all good spectra screens, numerical data, & summary analysis from HRV computer
☐ ☐ Clip the printed data from the HRV computer together & place in the subject's chart. (Each day separate).
☐ ☐ Clean & put away our equipment.
☐ ☐ Remove cassette from video camera and label with subject # + study day.
☐ ☐ Place green sheet in subject's chart at end of second day.

Please do not leave Masimo or the video equipment unattended at any time.

Thank you, bless you, may the rest of your day be delightful!!

Appendix H

Informed Consent

You and your baby are being asked to participate in a research study about using skin-to-skin contact (Kangaroo Care) as an intervention to reduce preterm infant's pain. You were selected as a possible participant because your infant is preterm. We have obtained permission from your physician to talk to you about this study. Please read this form and ask any questions that you may have before agreeing to be in the research.

PURPOSE OF THE STUDY

This study is being done to determine if being held skin-to-skin, also called Kangaroo Care (KC), can reduce a premature infant's pain level induced by the daily heelstick for routine blood draws. Skin-to-skin contact is described as a mother holding her baby bare skin to bare skin with the baby wearing only a diaper. Your baby and you are being included in this study because you are healthy, and your baby is stable and staying in an incubator in the step down unit of the Neonatal Intensive Care Unit (NICU).

PROCEDURES

The study will be conducted over two days (kangaroo care day and control day) with all heel sticks as routine daily blood draws by the unit phlebotomist. Your baby will receive a heel sticks for the study purpose. You are being asked to participate in the Kangaroo Care day by holding your infants in skin-to-skin contact for one and half hours. You will wear a skirt or pants and have the baby on your chest beneath a receiving blanket folded in fourths that covers your baby's back. A hospital gown will be closed over the blanket throughout Kangaroo Care. You will sit in a recliner at an angle 40 degree at the side of the incubator. Your baby will be observed by researchers with heart rate electrodes attached to his/her chest and attached to a machine that will print out the heart rate pattern while your baby lies in the incubator or on your chest. Videotape will be used to record and evaluate your baby's behavioral pain responses before, during, and after the heel stick procedures in the kangaroo care day and control day, which will take approximately 15 minutes each day. Video camera #1 will be positioned so only the infant's face fills the view finder, and video #2 will capture your baby's pulse oximeter numerics.

RISKS

The risks or discomforts of the study are the following and the precautions will be exercised.

1. Your baby's skin may experience a temporary drop in temperature when he/she is moved from the incubator onto your chest. The researcher will use a blanket to cover your baby and quickly transfer your baby to lessen the risk.
2. Sitting without movement for one and half hours may increase the risk of clot formation in your leg. You may elevate your legs and slightly shift your position at any time sitting in the recliner when you are holding your infant.

BENEFITS

There will be no direct medical benefit to you or your baby from participating in this study. You may learn about your baby's behavior during the study. Bonding and attachment between you and your baby may be enhanced by Kangaroo Care.

COST / COMPENSATION

The study will provide you a total of $30 for your presence at the hospital in the Kangaroo study day. There are no costs to you or your baby for being in this study.

ALTERNATIVES TO PARTICIPATION

You do not have to participate in this study. Pain medication is not given to infants for routine heel sticks. With the approval of the medical staff caring for your infant, Kangaroo Care can be used without being part of this study.

CONFIDENTIALITY

The records of this research will be kept private. They will be kept in a locked file and any report we publish will not include any information that will make it possible to identify a participant. Access to research records will normally be limited to the researchers. However, the University's Institutional Review Board (IRB) and other regulatory agencies, and funding agencies may review the research records to ensure that the rights of human subjects are being adequately protected. Only the participant ID number will be used on the videotape as identification method, and both the infant and mother's name will be not identified on the video. The video tapes will be kept for 5 years for the research purposes and then they will be destroyed.

SUMMARY OF YOUR RIGHTS AS A PARTICIPANT IN A RESEARCH STUDY

Your participation in this research study is voluntary. Refusing to participate will not alter your usual health care or involve any penalty or loss of benefits to which you are otherwise entitled. If you decide to join the study, you may withdraw at any time and for any reason without penalty or loss of benefits. If information generated from this study is published or presented, your identity will not be revealed. In the event new information becomes available that may affect the risks or benefits associated with this study or your willingness to participate in it, you will be notified so that you can decide whether or not to continue participating. If you experience physical injury or illness as a result of participating in this research study, medical care is available at UHC or elsewhere; however, University Hospitals of Cleveland will not provide free care or compensation for lost wages.

CONTACT INFORMATION

_____ has described to you what is going to be done, the risks, hazards, and benefits involved, and can be contacted at _____. Further information with respect to illness or injury resulting from a research procedure as well as a research subjects' rights is available from the Office of the Chief Medical Officer at (216) 844-3695.

SIGNATURE

Signing below indicates that you have been informed about the research study in which you voluntarily agree to participate; that you have asked any questions about the study that you may have; and that the information given to you has permitted you to make a fully informed and free decision about your participation in the study. By signing this consent form, you do not waive any legal rights, and the investigator(s) or sponsor(s) are not relieved of any liability they may have. A copy of this consent form will be provided to you.

_____ _____

Printed Name of Participant Child's Signature if this form is used to obtain assent

_____Date_____ _____

Parent or Legal Guardian signature Relationship to Child

_____Date_____ _____

Signature of Person Obtaining Consent Printed Name of Person Obtaining Consent

(Must be study investigator or individual who has been designated in the Checklist to obtain consent.)

_____ Date_____

Signature of Principal Investigator (Affirming subject eligibility for the study and that informed consent has been obtained.)

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