EFFECT OF A SENSORY MINIMIZATION INTERVENTION ON THE PHYSIOLOGICAL STABILITY AND PAIN RESPONSE OF PRETERM INFANTS

By
Marilyn Aita
School of Nursing
McGill University, Montreal

October 2008

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Ph.D. in Nursing

Abstract

Light and noise in the neonatal intensive care unit (NICU) may be stressful to infants who are born preterm. The goals of this research were twofold: a) to evaluate the *physiological stability* (heart rate, heart rate variability, and oxygen saturation) of 28 to 32 gestational age preterm infants while wearing eye goggles and earmuffs for a 4-hour period, and b) to evaluate their *pain response* (heart rate and heart rate variability) during a painful procedure (heel lance) following the 4-hour period that they had worn the eye goggles and earmuffs. Preterm infants were recruited from four university-affiliated teaching hospitals in the Montreal region that have a level III NICU. A cross-over trial allowed the evaluation of physiological stability in a sample of 54 infants, and a randomized controlled trial with 44 infants was used to evaluate pain response. For the cross-over trial, preterm infants were randomized in one of the following sequences: intervention - control *or* control - intervention. In the RCT, the first randomized study period (A *or* B) of the cross-over trial determined whether preterm infants were or were not wearing eye goggles and earmuffs prior to a heel lance procedure. Data were collected using the Somté^{TM} device allowing the continuous recording of outcome measures and infants were videotaped during the study periods for evaluation of potentially confounding variables. Results of the RM-ANOVA revealed that infants were more physiologically unstable while wearing the eye goggles and earmuffs. This was shown by signs of stress, such as a significantly higher maximum heart rate and a significantly lower high frequency power (heart rate variability), during the intervention period compared with the control period.

Also, the results of the ANCOVA indicated that infants who wore eye goggles and earmuffs before the heel lance did not show a significant reduction in their pain response in comparison with those who did not wear the material prior to the procedure. This intervention, conducted with the aim of reducing preterm infants' exposure to light and noise in the NICU, is therefore not recommended for the clinical practice. Control of these stimuli by environmental modifications of the NICU is preferable.

ABRÉGÉ

La lumière et le bruit dans l'unité néonatale peuvent être particulièrement stressants pour les prématurés. Les buts de cette étude étaient d'évaluer chez des prématurés de 28 à 32 semaines d'âge gestationnel : a) leur *stabilité physiologique* (rythme cardiaque, variabilité du rythme cardiaque et saturation d'oxygène) lorsqu'ils portent des lunettes et des couvre-oreilles pendant une période de 4 heures, et b) leur *réponse à la douleur* lors d'une prise de sang au talon *(*rythme cardiaque et variabilité du rythme cardiaque) après la période de 4 heures où ils ont porté les lunettes et les couvre-oreilles. Les prématurés ont été recrutés dans quatre centres hospitaliers de la région de Montréal qui possèdent une unité néonatale de soins intensifs. La stabilité physiologique a été évaluée à l'aide d'un plan croisé avec un échantillon de 54 prématurés alors que la réponse à la douleur a été évaluée à l'aide d'un essai clinique randomisé avec un nombre de 44. Pour le plan croisé, les prématurés ont été randomisés dans l'une des séquences suivantes : intervention - contrôle *ou* contrôle - intervention et pour l'essai clinique randomisé, la première période de la randomisation réalisée pour le plan croisé (A *ou* B) a déterminé si les prématurés portaient ou non les lunettes et les couvre-oreilles avant la prise de sang au talon. Les données ont été collectées à l'aide d'un appareillage (Somté^{TM}) qui permettait un monitorage continu des variables dépendantes et des enregistrements vidéo des prématurés ont été réalisés pour l'évaluation de variables potentiellement confondantes. Les résultats des analyses de variance à mesures répétées ont révélé que les prématurés qui avaient porté les lunettes et les couvre-oreilles pendant la période de quatre heures montraient moins de stabilité physiologique comparé à

lorsqu'ils ne portaient pas ces éléments, ce qui se traduit par des signes de stress tels qu'un rythme cardiaque maximum plus élevé et une diminution de la haute fréquence de la variabilité du rythme cardiaque. Aussi, des analyses de covariance indiquent que les prématurés qui ont porté les lunettes et les couvre-oreilles avant la prise de sang au talon n'ont pas démontré une diminution de leur réponse à la douleur au moment de la procédure comparés à ceux qui n'ont pas porté les éléments avant le prélèvement. Cette intervention réalisée dans le but de réduire l'exposition des prématurés à la lumière et au bruit dans les unités néonatales n'est donc pas recommandée pour la pratique clinique. Le contrôle de ces stimuli dans l'environnement néonatal est préférablement souhaité.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

This doctoral thesis marks the end of an eventful journey and there are many people I would like to acknowledge for their support along the way. First and foremost, I would like to express my gratitude to my thesis supervisor Dr. Celeste Johnston who gave me all the confidence needed to accomplish this endeavour. With her continuous support, encouragement, enthusiasm, and personal and professional guidance, she has truly been an inspiration throughout my doctoral studies.

I would also like to thank my thesis committee members, Dr. Céline Goulet, Dr. Laurie Snider, and Dr. Tim Oberlander for the support, thoughtful comments, and advice provided along the way. I am also grateful to several people who helped me through the preparation and implementation phases of my study. I wish to thank Françoise Filion, Annie Bessette, Rasha Sjouri, Terry Suss, Linda Bell, Sara Audet-Pelletier, Monique Lorrain, and Françoise Picard for their help and support in data collection and coding, as well as Marie-Claude Guertin, Miguel Chagnon, Louise Fortier, and Marie-Eve Cossette for their statistical analysis assistance.

I would like to express my gratitude to the parents who gave me their trust and agreed to have their infants participate in this study. I also have valued the collaboration and contribution of the neonatal nurses, administrators, and physicians in the clinical settings where this research was conducted.

I wish to acknowledge a special thank you to Marie-Claire Richer for her friendship and continuous support during my doctoral studies. I also want to thank Maryse Pelletier-Hibert, Marjolaine Héon, Caroline Longpré, Sylvie Dubois,

and Nathalie Gélinas for being there for me during this journey. I would also like to thank my husband, Benoit, for his tremendous support and understanding throughout, and, most importantly, my son Eliott who gave me the strength to conduct this journey to its end.

Finally, I am grateful to the Montreal Children's Hospital Research Institute (MCH-RI), The Order of Nurses of Québec (OIIQ), the Canadian Nurses Foundation (CNF) / the Canadian Institute of Research in Health (CIHR) / the Hospital for Sick Kids Foundation, the Fonds de la recherche en santé du Québec (FRSQ), as well as the Ministère de l'éducation, du loisir et du sport for their financial support during my doctoral studies. In addition, I would like to thank the Groupe de la recherche interuniversitaire en sciences infirmières de Montréal (GRISIM) / Canadian Nurses Foundation who funded this doctoral research project.

DEDICATION

To the two loves of my life:
my husband *BENOIT*, for believing in me &
my son *ELIOTT* born July 11th 2007
and who was at risk of being born preterm.

REFLECTION

"The important thing is not to stop questioning"

Albert Einstein
(1879 - 1955)

PROBLEM STATEMENT

Introduction

Over the last two decades, there has been increased interest in studying the development and growth of preterm infants[1] in the Neonatal Intensive Care Unit (NICU). To meet preterm infants' developmental needs, neonatal nurses and other professionals are encouraged to modify or control the NICU environment, in an effort to create a milieu comparable to the maternal womb. However, light and noise in the NICU are identified as environmental factors creating stress (Als, 1982; 1986; Blackburn, 1998; Bowden, Greenberg, & Donaldson, 2000; Holditch-Davis, Blackburn, & Vandenberg, 2003; Glass, 1999; Goldson, 1999; Lotas, 1992; Perlman, 2001; Warren, 2002) and potentially causing neurobehavioral impairments in preterm infants (Perlman). Light (Blackburn & Patteson, 1991; Shiroiwa et al., 1986) and noise (Zahr & Balian, 1995; Zahr & de Traversay, 1995) are also reported as contributing to physiological instability in preterm infants and possibly engendering significant long-term detriment to their visual (Fielder & Moseley, 2000; Glass, 1993; Gonzalez & Dweck, 1994; Graven, 2004) and auditory development (American Academy of Pediatrics [AAP], 1997; Blackburn; Holditch-Davis et al.). There is also growing empirical evidence (Anand & Scalzo, 2000; Porter, Wolf, & Miller, 1998) supporting the importance of reducing light and noise in the NICU since non-painful sensory stimulations may affect the pain response of preterm infants. These findings justify the need to

---

[1] Infants who are born before or on the last day of the 37th week of gestation (AAP & ACOG, 2007).

develop and evaluate interventions reducing the preterm infants' exposure to light and noise in the NICU to promote their development and growth.

Background

The NICU environment is continuously bright and noisy, in stark contrast to the dark intrauterine environment, where perceptible ambient sounds consist of maternal heart and voice filtered through amniotic fluid. The amniotic fluid and uterine walls are the only tactile stimulations experienced by the fetus. Whereas in the NICU preterm infants periodically undergo necessary painful and non-painful procedures performed for therapeutic purposes (Barker & Rutter, 1995; Simons et al., 2003; Johnston, Collinge, Henderson, & Anand, 1997). The light and noise of the NICU environment are particularly stressful to infants born before term and there is a strong probability of visual and auditory overstimulation, since vision and hearing are the last two senses to develop (White-Traut, Nelson, Burns, & Cunningham, 1994).

Ceiling lights, treatment and phototherapy lamps as well as daylight and sunlight provide continuous lighting in the NICU. To prevent exposing preterm infants to excess light, the AAP and the American College of Obstetricians and Gynecologists [ACOG] (2007) as well as the Committee on Recommended Standards for Newborn ICU Design (2006) recommend that the ambient light level at each infant bedside be adjustable from ten to 600 lux[2]. Light intensity in NICUs has been reported to range between 236 and 905 lux during the day (Robinson, Moseley, & Fielder, 1990) and, more recently, was measured at 82.11

[2] Lux divided by 10 approximately equals foot-candle (Blackburn, 1996), i.e. 10 lux equals around 1 foot-candle.

foot-candles (fc) (i.e., ~821.1 lux) during intense lighting periods (Lee, Malakooti, & Lotas, 2005), thereby exceeding the recommended level.

NICU noise emanates around the clock from both inanimate sources such as mechanical equipment, as well as animate sources such as professionals and patients (Nzama, Nolte, & Dörfling, 1995). Noise levels should not exceed 45 decibels (dBA) (AAP, 1997; Committee on Recommended Standards for Newborn ICU Design, 2006) but there are reports of noise varying from 38 to 75 dBA[3] (Philbin, 2000) and noise levels inside incubators in particular were recently calculated as exceeding 50 dBA (Thomas & Uran, 2007). Given these reports it is clear that ambient noise levels frequently exceed the recommended level.

In addition, it is reported that neonatal nurses do not always cover incubators and cribs nor restrain their conversation near the incubators/cribs to prevent visual and auditory overstimulation in preterm infants (Aita & Goulet, 2003). These data further suggest that light and noise levels may not be properly modified or controlled in the NICU environment.

For infants born preterm, these issues are important to consider since light and noise have been reported to affect their physiological stability. For example, infants who are exposed to continuous lighting in the NICU show increased mean heart rate and motor activity levels compared to a cycled light group (day/night lighting) (Blackburn & Patteson, 1991). Noise in the NICU increases infants' heart and respiratory rates and significantly decreases their oxygen saturation (Zahr & Balian, 1995). Blackburn (1998) explains that sensory inputs in the NICU, such as light and noise, may alter physiologic processes and central nervous system

[3] Measured on A-weighted decibel scale.

(CNS) organization in preterm infants. This alteration of CNS organization may provoke hypersensitivity in infants to stressful NICU stimuli, resulting in poor modulation or in the inability to respond to stimulation (Blackburn, 1998). This perspective suggests that excessive exposure to NICU stimuli may increase exhaustion and decrease responsiveness to the environment among preterm infants.

Moreover, light and noise exposure may contribute to significant negative long-term consequences for neonates' visual and auditory development. Visual impairment may result from the exposure of the immature visual system to bright lights (Fielder & Moseley, 2000; Glass, 1993; Gonzalez & Dweck, 1994; Graven, 2004), while exposure to intense sounds may directly damage the fine, delicate hairs of the preterm infant's cochlea and cause hearing loss (AAP, 1997; Blackburn, 1998; Holditch-Davis et al., 2003). Such severe consequences justify the importance of controlling light and noise in the NICU environment with the objective of limiting preterm infants' energy loss and favoring their development and growth. However, published reports reveal that NICU light (Lee et al., 2005; Robinson et al., 1990) and noise (Philbin, 2000; Thomas & Uran, 2007) are exceeding the recommended environmental levels, suggesting inappropriately high levels of stimuli for preterm infants.

According to the Synactive Theory of Development (Als, 1982; 1986), the NICU environment influences the preterm infant's brain development and organization through the senses. The assumption underlying this theory is that preterm infants are competent organisms who are continuously interacting with their environment by reacting to it with behaviors through the autonomic, motor,

state organizational, and attention-interactive systems, with the objective of self-regulation. The autonomic system, which modulates the basic functioning of infants born preterm, exhibits a physiologic stress response when the infants are exposed to environmental stress. Previous studies, which have associated preterm infants' physiological instability to the exposure of light and noise in the NICU, suggest that these stimuli are stressful environmental stimulation. Inspired by Als' theory, NICU environmental light and noise should therefore be modified and controlled to reduce stress behaviors in hospitalized preterm infants to promote brain development and organization.

There are reports of benefits associated with reducing environmental light and noise on the physiological stability of preterm infants. For example, preterm infants exposed to reduced lighting in the evening and night, demonstrated greater physiological stability than infants exposed to continuous lighting (Blackburn & Patteson, 1991; Shiroiwa et al., 1986). Infants wearing earmuffs for a 4-hour period had a significantly higher oxygen saturation levels and spent more time in a state of quiet sleep than when they were not wearing earmuffs (Zahr & de Traversay, 1995). Empirical articles (Anand & Scalzo, 2000; Porter et al., 1998) imply as well that reducing the preterm infant's exposure to sensory stimulations in the NICU environment might be beneficial in reducing their pain response while undergoing painful stimulation.

Anand and Scalzo (2000) contend that repetitive pain and abnormal sensory stimulation in the NICU care could cause a hyperexcitability in preterm infants. Even though abnormal sensory stimuli other than pain are not made explicit in Anand and Scalzo's empirical work, environmental NICU light and

noise are also believed to be causing hyperexcitability in the CNS (Anand, personal communication, February 5, 2008). This process is particularly important for preterm infants with neurological immaturity. Indeed, Anand (1998) explains that excitability in preterm infants may increase their vulnerability to other stressful stimulation, which may cause physiologic stressful responses. This viewpoint is empirically supported by Porter et al. (1998) who report that tactile stimulations, such as handling the infant before performing a heel lance increases their pain response during the procedure. Taken together, these perspectives support the hypothesis that non-painful sensory stimulations in the NICU environment may influence pain response in preterm infants.

Painful procedures such as heel lancing also cause physiological disorganization in preterm infants (Craig, Whitfield, Grunau, Linton, & Hadjistavropoulos, 1993, Harrison, Evans, Johnston, & Loughnan, 2002; Lindh, Wiklund, Sandman, & Håkansson, 1997; Johnston & Stevens, 1996; McIntosh, Van Veen, & Brameyer, 1993; Stevens & Johnston, 1994; Stevens, Johnston, & Horton, 1993). It has also been postulated that repetitive pain in preterm infants may in the long-term decrease pain sensitivity and create hyperactivity which may be associated with subsequent abnormal adult behaviors such as cognitive impairment, specific drug preferences and poor socialization skills (Anand & Scalzo, 2000). Because of the adverse effects of repeated pain, preventing pain should be the goal of NICU healthcare professionals (AAP and Canadian Paediatric Society [CPS], 2006) and pain management should be aimed at maximizing the infant's abilities to handle and recover from painful experiences,

and to provide effective interventions with reduced risks for them (Stevens & Franck, 1995).

In order to prevent and manage pain in preterm infants, one strategy would be to reduce exposure to environmental stress such as light and noise in the NICU (Franck & Lawhon, 1998; Anand and the International Evidenced-Based Group for Neonatal Pain, 2001). However, limited research has been conducted to evaluate the effects of environmental interventions on the pain response of infants in the context of a brief, acute and single-event such as heel lancing (Stevens, Gibbins, & Franck, 2000).

In summary, there is some empirical evidence that light and noise cause physiological instability in preterm infants and that the effect of this continuous stimulation may be detrimental to their visual and auditory development. Even though previous research has indicated physiological benefits for infants born preterm associated with the reduction of light (Blackburn & Patteson, 1991; Shiroiwa et al, 1986) and noise (Zahr & de Traversay, 1995) in the NICU, published reports suggest that this environment nonetheless provides inappropriately high levels of light (Lee et al., 2005; Robinson et al. 1990) and noise (Philbin, 2000; Thomas & Uran, 2007). However, these studies have some methodological limitations such as small sample size and absence of randomization thereby limiting the scientific evidence related to interventions reducing light and noise in the NICU. Growing evidence also points to the fact that reducing environmental sensory stimulation in preterm infants may decrease their pain response (Anand & Scalzo, 2000; Porter et al., 1998) and prevent long-term sequelae associated with pain However, to date, no study has evaluated the

effect of reducing *both* light and noise on the physiological stability of preterm infants as well as their response during a painful procedure. Therefore, this study proposes to evaluate an intervention minimizing sensory exposure to light and noise in the NICU environment by having preterm infants wear eye goggles and earmuffs.

Purpose of the Study

The purpose of this study is twofold: a) to evaluate the *physiological stability* of 28 to 32 week gestational age preterm infants wearing eye goggles and earmuffs for a 4-hour period in the NICU; b) to evaluate the *pain response* of 28 to 32 week gestational age preterm infants during a painful procedure (heel lance) following the 4-hour period wearing the eye goggles and earmuffs in the NICU.

LITERATURE REVIEW

This chapter presents a comprehensive review of the theoretical and empirical literature pertaining to the proposed research. The first section presents an overview of the nervous system development of preterm infants, followed by a description of the NICU environment, revealing the common types of sensory stimulation found there. The third section summarizes interventions decreasing NICU environmental light and noise and managing pain in preterm infants. The final section describes the framework of Als (1982; 1986) and the empirical work of Anand and Scalzo (2000) which serve as a theoretical basis for the proposed research.

The development of the nervous system is presented first in this literature review to highlight the neurological immaturity of preterm infants and how they may react when exposed to environmental stimuli in the NICU. This section starts with a description of the development of the central nervous system (CNS), then the autonomic nervous system (ANS), and ends with the hierarchical development of the sensory nervous system (SNS). Figure 1 illustrated at the end of this section summarizes the critical developmental steps of the CNS, ANS, and SNS, which are subsequently presented.

Nervous System Development

The intrauterine environment is optimal for the development and growth of the fetus and a premature delivery may have an important influence on the development of the infant's fundamental systems, particularly brain development. Anand and Scalzo (2000) acknowledge that unfavorable experiences around birth may affect the course of brain development and even predispose the infant

to later abnormal behavior. Critical periods of human brain development occur around birth when the establishment of neuronal circuitry is more subject to disturbing events at that time than at any other period in life (Anand & Scalzo, 2000). The nervous system may also be vulnerable at numerous points to environmental disturbances as the events in neural development are spread over a long period (Pomeroy & Ullrich, 2004). Major events in human brain development and their peak times of occurrence (Volpe, 2000) are presented in Table 1. This shows that ongoing brain organization and myelination are major developmental events of preterm infants hospitalized in the NICU.

Major Developmental Events	Peak Time of Occurrence
▪ Primary neurulation	▪ 3 - 4 weeks of gestation
▪ Prosencephalic development	▪ 2 - 3 months of gestation
▪ Neuronal proliferation	▪ 3 - 4 months of gestation
▪ Neuronal migration	▪ 3 - 5 months of gestation
▪ Organization	▪ 5 months of gestation - years postnatal
▪ Myelination	▪ birth - years postnatal

Table 1.

Major Events in Human Brain Development and Peak Times of Occurrence. Reproduced from Volpe (2000, p. 2) with permission from the author (see Appendix A).

Central Nervous System (CNS)

White-Traut et al. (1994) propose that the environment of the NICU may specifically jeopardize the organization and maturity of the preterm infants' CNS. Gressens, Rodigo, Paindaveine, and Sola (2002) identify that the critical periods

of human cortical brain development occur from 24 to 32 weeks postconceptional age (PCA)[4]. Blackburn (1998) also agrees that the organizational stage of the CNS development, which goes through a critical period of growth from five months of gestation to one year of age, is especially vulnerable in infants born preterm. This period of organization consists of establishing the circuitry of the brain and preparing the axonal myelination. For an infant born preterm, these critical periods occur during their hospitalization in the NICU (Gressens et al., 2002) while exposed to light and noise, and undergoing necessary repetitive painful procedures for therapeutic purposes. In preterm infants, an alteration of the CNS organization may result in hypersensitivity, poor modulation, or all-or-nothing responses when they react to the environment (Blackburn). Hypersensitive infants may, for example, be startled by minimal noise or conversely may not respond at all to loud noises. The organization of the CNS also plays an important role in the integration and processing of environmental sensory stimulation (Blackburn). The ANS, which is responsible for the infants' basic physiological functioning, is one of the main systems affected by environmental stress exposure (Als, 1982). The following sections describe the function and role of the ANS and the SNS as well as the responses of infants born preterm when they are exposed to environmental stimulation.

Autonomic Nervous System (ANS)

The ANS is composed of the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS) and regulates the body systems' function

[4] Conceptional age is two weeks shorter than gestational age (Committee on Fetus and Newborn, 2004).

of homeostasis (Porges, 1992). According to White-Traut et al. (1994), while the SNS and PNS branches of the ANS are believed to be functional around the last two months of gestation (32th to 40th weeks), the SNS would still be immature at that time. Clairambault, Curzi-Dascalova, Kauffmann, Médigue, and Leffler (1992) report that the sympathetic tone increases periodically from 31 to 41 weeks PCA while the parasympathetic tone increases suddenly at 37th to 38th weeks PCA. It is also believed that preterm infants begin to show physiological homeostasis by controlling sympathetic function between 28 and 32 weeks of gestational age (Holditch-Davis et al., 2003). The SNS deals with environmental challenges by increasing the system's metabolic outputs while the PNS promotes growth and restoration in systems (Porges). More specifically, the PNS controls homeostatic processes and would therefore be more responsive to stress (Porges). Johnson, Kamilaris, Chrousos and Gold (1992, p. 115) define stress as a "state of threatened "homeostasis"" (Greek for "steady state") or threatened harmony, balance, or equilibrium.

Physiological stress responses exhibited by the ANS consist of changes, among others, in heart rate (Anand, 1993; Cheng & Chapman, 1997; Bowden et al, 2000; Modrcin-McCarthy, McSue, & Walker, 1997), respiratory rate, color, and visceral signs such as hiccuping, sneezing, etc. (Als, 1982; Bowden et al.; Cheng & Chapman; Modrcin-McCarthy et al.). These physiological stress responses, though not specific, can all be noticed in preterm infants exposed to sensory stimulation in the NICU environment. More specifically, under challenging conditions, a withdrawal of PNS tone on the heart (i.e. vagal tone), may create stress responses which can be quantified physiologically (Porges, 1992).

Sensory Nervous System (SNS)

The preterm neonatal brain seems to be operational for transmitting and integrating visual, auditory, and tactile sensory stimuli (Anand & Scalzo, 2000). The transmission of sensorial stimuli to the preterm infant's CNS is ensured by two specific components: a) the peripheral receptors; and, b) the pathways that these stimuli follow to reach the cerebral neurons (Campbell, 1985). According to Graven (2000), it is assumed that the SNS development in humans follows the same sequential pattern as in animals. It begins with sensations to skin (touch), followed by kinaesthetic (movement), chemosensory (taste and olfactory), auditory, and visual. As a comprehensive description of the tactile, auditory, and visual sensory development is essential in this study, it will subsequently be presented to support the notion that infants born preterm are, at the time of their initial hospitalization in the NICU, competent at sensing environmental stimuli.

Tactile System

The tactile system is the first to develop following conception (Graven, 2000; Vanhatalo & van Nieuwenhuizen, 2000; White-Traut et al., 1994) and includes sensory receptors which react to painful stimuli, pressure, and temperature (Glass, 1999). Cutaneous sensory perceptions appear in the mouth area around the 7.5^{th} week and are reported to spread to almost all body surfaces by the 17^{th} week of gestational age (Humphrey, 1964). According to Glass, the cortical pathway is integrated by 20 to 24 weeks of gestation with the presence of some myelin. Based on existing anatomical evidence, it is also believed that pain pathways required for pain perception are present by 26 weeks of gestational age (Glover & Fisk, 2007). Studies have recently confirmed that pain activates

somatosensory cortex responses in preterm infants at early as 25 weeks gestational age (Bartocci, Bergqvist, Lagercrantz, & Anand, 2006; Slater et al., 2006).

Auditory System

The human cochlea and the structures for peripheral sensory integration are in place at 24 weeks of gestation (AAP, 1997; Hack, 1983). Following a review of the literature on sound and the developing infant, Graven (2000) concludes that the auditory system would be sufficiently mature between the 23rd and 25th weeks of gestation to react to environmental sound, this being apparent through physiological signs.

Visual System

The layers of the retina are in place by 22 weeks of gestation and the immature rods and cones are distinguished by the 23rd week (Hack, 1983). Myelinization of the optic nerve begins at 24 weeks, and neurons of the visual cortex are present at the 25th and 26th week of gestation (Hack). The neurons of the visual cortex undergo important growth between the 28th and 34th week of gestation (Hack). Eyelids are fused until 24 to 25 weeks PCA (Birch & O'Connor, 2001). By 26 weeks of gestation, Hack substantiates that visual stimuli are transmitted from the cornea to the visual cortex. White-Traut et al. (1994) speculate that the visual system is the last system to develop anatomically and is believed to be functional at around the beginning of the third trimester (i.e. 28 weeks of gestational age).

Tactile, auditory, and visual systems of infants born preterm are functional but still immature at birth. They seem to be able to integrate and process sensory

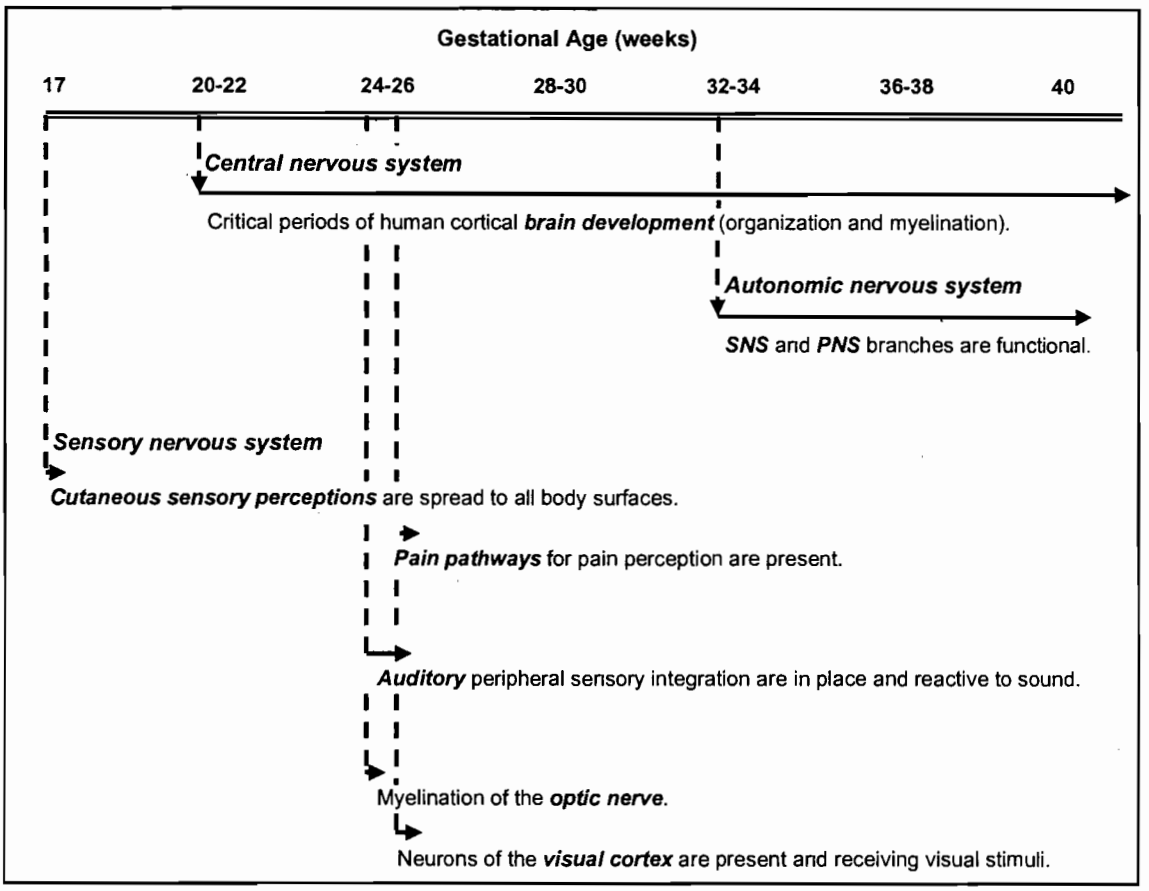

Figure 1. Summary of critical periods of CNS, ANS and SNS development.

stimulation from the environment but their immature CNS leads them to demonstrate physiological signs of stress emitted by the ANS. While preterm infants are both dependent on and vulnerable to the NICU environment, the goal is to maintain a developmentally supportive environment without introducing stressful stimulation. The following section describes the NICU environment and the common types of sensory stimulation to which preterm infants are exposed during hospitalization.

NICU Environment and Sensory Stimulation

After preterm birth, development continues in the NICU environment during a lengthy hospitalization rather than in utero. Survival may be

accompanied by an increased incidence of physical, neurological, sensory, and developmental impairments (D'Agostino & Clifford, 1998). According to Gressens et al. (2002), moderate cognitive impairments, attention deficits, and behavioral disorders in preterm infants not associated with periventricular leukomalacia (PVL) or cerebral palsy, may be partially attributable to the exposure of preterm infants' immature brains to noxious stimuli in the NICU. Significant numbers of preterm infants display impairments of visual, auditory, and tactile processing which would justify the importance of appropriately timed sensory stimulation during prematurity (Warren, 2002). Therefore, strategies for reducing neurosensory impairments in extremely low birth weight infants (< 1250 g) hospitalized in the NICU are considered a priority (Gressens et al.). As a result, identification of stressful stimuli in the NICU environment and interventions to promote adaptation is a critical component of neonatal nursing care (Johnson, 2001).

The comparison of sensory development of the fetus in utero versus the sensorial experiences of the NICU environment has been made by White-Traut et al. (1994). Figure 2 shows that the visual and auditory systems are the last to develop and therefore the least mature of the sensory systems with infants born preterm. Accordingly, light and noise seem to be environmental NICU stimuli that may cause overstimulation in preterm infants. Nonetheless, they are continuously exposed to visual and auditory stimulation in the NICU environment. Anand (1998) specifies that the nature of neonatal intensive care exposes preterm infants to invasive procedures and handling that may result in acute and chronic pain, and prolong stress during periods of brain developmental epochs.

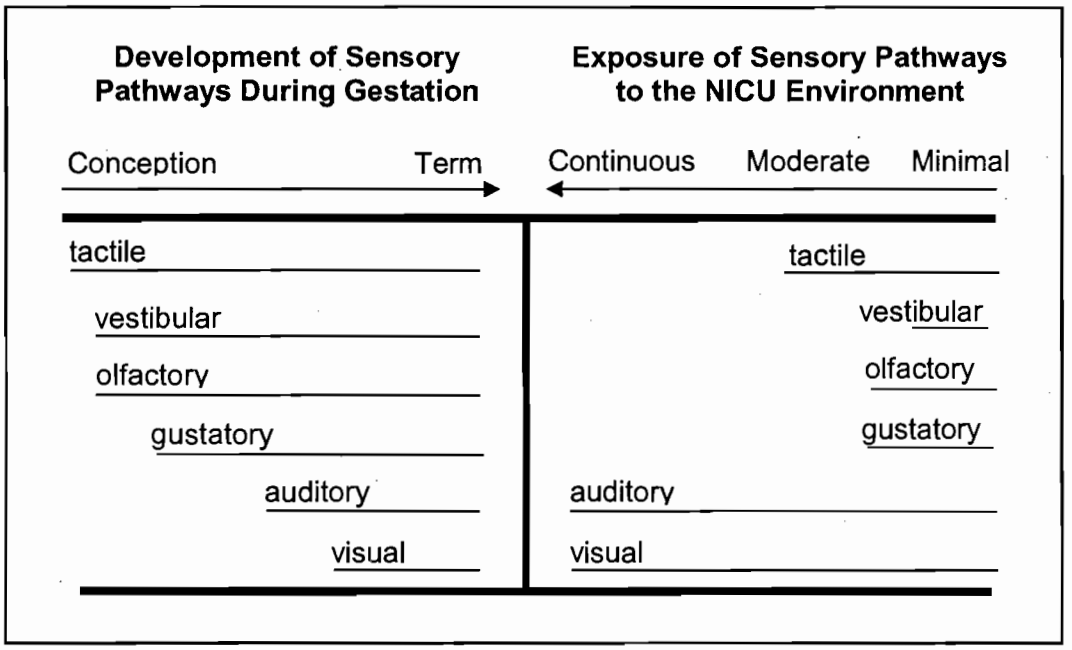

Figure 2. Hypothetical comparison of sensory pathway development to sensory exposure in the NICU. Reproduced from White-Traut et al. (1994, p. 396) with permission from the author and Blackwell Publishing (see Appendix A).

The extent to which sensory stimulation in the NICU environment influences the neurological development of preterm infants during phases of rapid brain growth and differentiation is of concern (Warren, 2002). Experiences of pain and other sensations during the development of pain pathways appear to shape the development of the overall pain system and may determine the final structure of the adult pain system (Anand & Carr, 1989). Philbin, Roberston, and Hall III (1999) state that animal studies confirm that the sensory nervous system will develop normally if environmental stimulation is moderate during periods of rapid growth and organization occurring during the third gestational trimester. Lickliter (2000) concludes after reviewing psychobiological work that early sensory development is dependent on experiences encountered by infants in their

surrounding environment. For preterm infants, these environmental experiences consist of the light, noise, and pain stimulation which are commonly identified as sources of stress and abnormal stimulation in the NICU environment (Blackburn, 1998; Glass, 1999) that may occur simultaneously or independently. Lickliter (2000) additionally highlights that the sensory experiences of one sensory system will also influence the other developing sensory systems. Indeed, based on animal studies, Turkewitz and Kenny (1982) had hypothesized that reducing the stimulation of an earlier developing sensory system, for example the tactile, may lead to the premature utilization of a system which should be developing later, such as the visual. In other words, reducing only the stimulation of the auditory nervous system in preterm infants could lead to the untimely utilization of their immature visual system. This hypothesis strengthens therefore the purpose of this study which proposes to evaluate an intervention reducing both the auditory and visual stimulation of preterm infants. The following sections characterize light, noise, and painful stimulation in the NICU environment, as well as their short and long-term effects on the development and growth of preterm infants.

NICU Environmental Light

After birth, preterm infants are admitted to a bright NICU environment as compared to the dark intrauterine environment. A review of how preterm infants may perceive lighting in this environment is followed by a description of light characteristics to provide a better understanding of ambient NICU light level.

Light and Preterm Infants

Biological or physiological factors, such as pupil diameter and frequency of eyelid opening in preterm infants, play an important role in how much light

reaches the infant's retina (Fielder & Moseley, 2000; Robinson & Fielder, 1992). Preterm infants are more susceptible to light exposure as they have a larger pupil diameter because they have not developed any reflex to light (Robinson & Fielder, 1990). This reflex to light, translated as the ability to constrict the pupils in reaction to illumination, would only start developing at 30 weeks gestational age (Blackburn, 1996; Gonzalez & Dweck, 1994; Robinson & Fielder, 1990). Robinson and Fielder reported that 86% of 34-week preterm infants constricted their pupil in reaction to an increase in illumination and this reflex was present in all 35-week infants studied. Blackburn also states that light is more likely to reach the preterm infant's retina as they have an increased lens translucency. The eyelid opening of preterm infants seems to vary accordingly to lighting in the NICU. Indeed, Robinson, Moseley, Thompson, and Fielder (1989) report that preterm infants exposed to continuous lighting in the NICU opened their eyes less significantly than infants exposed to reduced lighting such as in a day and night nursery.

NICU Lighting

The level of lighting to which the neonate is exposed in the NICU environment is influenced by factors such as the intensity and spectral characteristics of the lights, as well as the duration of exposure (Fielder & Moseley, 2000; Robinson & Fielder, 1992). NICU lighting varies from one unit to another. Based on a survey of seven neonatal units in Europe, the mean lighting was 470 lux with a range of 236 to 905 during the day and a mean of 348 lux varying from 192 to 690 at night (Robinson et al., 1990). NICU lighting is composed of ceiling lights, heat lamps, phototherapy lamps, and daylight or

sunlight. Heat lamps produce 200 to 300 fc (i.e. ~ 2,000 to 3,000 lux) while phototherapy lamps produce 300 to 400 fc (i.e. ~ 3,000 to 4,000 lux) (Glass et al., 1985). The phototherapy can be considered a safe treatment as preterm infants under these lamps have their eyes covered. The lighting of these lamps, however, radiates and contributes to ambient NICU light and other preterm infants located close by may be exposed to their intense brightness. Daylight and sunlight are reported to increase NICU lighting between1,000 fc (i.e. 10,000 lux) (Hamer, Dobson, & Mayer, 1984) and approximately 25,000 lux (i.e. ~ 2,500 fc) (Blackburn, 1996). Blackburn (1998) enumerates three factors influencing the preterm infant's exposure to NICU luminosity: a) the infant's individuality, location, and position in relation to light sources and windows; b) the unit design; and, c) the diurnal and seasonal variability in environmental light.

Professional neonatal groups (AAP & ACOG, 2007; Committee on Recommended Standards for Newborn ICU Design, 2006) advise that the level of light in the NICU should be adjustable from 1 to 60 fc as measured at each preterm infant's bedside. This recommended light level should be respected by NICU professionals with the objective of preventing the effects of exposing preterm infants to too much luminosity.

Physiological and Behavioral Effects of Light

The effects of light reported in this section are mainly interpreted from studies evaluating the effect of an intervention reducing light exposure of preterm infants in NICUs. Shiroiwai et al. (1986) reported that when preterm infants (n = 10) of an average mean gestational age of 33.1 weeks were exposed to continuous lighting in the NICU, they had significantly higher respiratory rates and

variability, as well as longer-lasting body movements compared to when they were blindfolded. Blackburn and Patteson (1991) have also reported that preterm infants between 29 and 33 weeks of gestation who were exposed to continuous lighting in the NICU have a significantly higher mean heart rate and increased activity level (facial and body movements) as compared to infants exposed to cycled lighting (day/night) over a 24-hour period.

Based on significant clinical observations, Shogan and Schumann (1993) conclude that a rapid increase in lighting may cause stress in preterm infants. They reported that 22% of infants suddenly exposed to 100 fc lighting after being exposed to a 30-minute period of reduced lighting (5 fc) showed a significant clinical drop in oxygen saturation (< 4% to 7%). Following their study, Miller, White, Whitman, O'Callaghan, and Maxwell (1995) reported that preterm infants with a mean gestational age of 28 weeks assigned to continuous lighting until discharge had lower daily weight gain, fed less rapidly orally, spent more time under phototherapy lamps, required more days of ventilation support, and demonstrated less motor co-ordination compared to a similar group of infants exposed to cycled lighting over a 24-hour period.

Long-term Effects of Light

According to Glass (1993), a premature exposure of preterm infants to NICU light could have a disruptive effect on their developing visual system. Based on animal studies, Gonzalez and Dweck (1994) and Fielder and Moseley (2000) also conclude that preterm infants in the NICU are exposed to a sufficient amount of lighting to damage their visual system, although Fielder and Moseley caution that there is still a need to confirm that the human visual system can be

damaged by NICU light in the premature period. Blackburn (1996) explains that the extent of photochemical injury to the retina depends on the light intensity and duration of exposure; explicit damage can occur with short exposure to bright lights or with long exposure to less intense light. Birch and O'Connor (2001) explain that a premature birth plays a potential role in visual development of preterm infants in two ways. First, postnatal visual experience and nutrition can modify the function of the visual system exposed to external visual stimulation, and second, the overall immaturity of the preterm infants creates a significant risk for visual impairment. Following a review of the literature on environmental light and the preterm infant, Fielder and Moseley (2000) conclude that NICU ambient light could be involved in subtle visual pathway sequelea that cannot be attributable to premature birth alone. Graven (2004) recently considers that the exposure of preterm infants' eyes to bright lights, among other things, may affect the preterm infant's early visual development.

Retinopathy of prematurity (ROP) has been one long-term outcome studied in relation to preterm infants' exposure to NICU light, while empirical studies are now confirming that light does not contribute to ROP. In a Cochrane review, Phelps and Watts (2001) concluded that decreasing preterm infants' exposure to ambient light will not be likely to reduce the incidence of ROP. However, as highlighted by Fielder and Moseley (2000), Silverman (1999, p. 129-130) rightly questions: "Does *early* light exposure have an adverse effect on the development of visual function – quite *apart* from any influence on the course of ROP?... The full story, I suspect, has not yet been told".

NICU Environmental Noise

Preterm infants may be more vulnerable to noise in the NICU because of their immaturity (Morris, Philbin, & Bose, 2000) but also because noise exposure in the NICU is very different from the experience in the mother's womb (Sparshott, 1995). Following a review of published articles and data by experts on NICU noises, it is concluded that the fetus is exposed to a background noise level greater than 50 dB at low frequency in the intrauterine milieu (Graven, 2000). In contrast, preterm infants in the NICU environment are exposed to both high levels of low and high-frequency sound (Graven) which are, at times, exceeding this level. A description of sound properties provides an understanding of research providing a measurement of NICU ambient sound levels, and how preterm infants may perceive environmental noise levels.

Noise and Preterm Infants

Noise in the NICU environment can be labeled as unwanted sound (Thomas & Martin, 2000). Philbin (1996) reports some basic principles that should be considered when evaluating hearing and sound in the NICU environment.

Sound enters the human ear as waves characterized by frequency and pressure. Adult human hearing is mostly acute between the 1000 and 3000 Hz frequency range, so a sound in this frequency range requires less pressure to be audible to humans. For example, NICU monitor alarms are in the 1000 Hz frequency and can be annoying for the human ear at low sound pressure (Philbin, 1996). The pressure corresponds to the sound loudness and is calculated in decibels. Decibels are measured on a logarithm scale where sound loudness

doubles with every six dB (Philbin, 1996; 2000), that is, the difference in pressure between 70 and 76 dB is lower than the difference between 90 and 96 dB (Philbin, 1996).

Hearing consists of the energy of a sound wave that is transferred from the tympanic membrane to the brain (Morris et al., 2000). Noises would not sound the same for preterm infants as for adults as their auditory system is still maturing after birth. Based on Als' work, Philbin (1996) advises that neonatal nurses and other NICU professionals should not evaluate NICU noise levels based on their own perceptions, which could be different from what the preterm infant perceives, but should observe signs emitted by the infant's motor, autonomic, and state-related systems in reaction to noise.

NICU Noises

NICU noises are produced by equipment such as incubator alarms, cardiorespiratory monitors, infusion pumps, and telephones, in addition to neonatal nurses and other NICU professionals carrying out procedures (i.e., closing incubator doors, laughing or talking loudly), and infants crying (Allen, 1995; Holditch-Davis et al., 2003; D'Agostino & Clifford, 1998; Elander & Hellström, 1995; Lotas, 1992; Zahr & de Traversay, 1995). In their study, Chang, Lin and Lin (2001) describe the most common source of noise recorded in the NICU environment was conversation between NICU professionals. Conversations accounted for 34.1% of noise during the observation time (48 hours) and were creating, on average, 70 dB. Monitor alarms were causing a mean of 68.5 dB and accounted for 9.2% of observations; while care at the infant's bedside caused a mean of 77.2 dB and was observed 4.7% of the time.

DePaul and Chambers (1995) also report that the highest ambient noise level in the NICU environment was created by routine care activities and that these were repetitive throughout day and night.

Philbin (2000) presents a summary of published sound levels and concludes that NICUs are dissimilar and vary from 38 to 75 dBA. Gray, Dostal, Ternullo-Retta, and Armstrong (1998) reported a baseline sound level of 64 to 70 dB with peaks up to 100 dB from equipment alarms. In their studies, Zwick (1993) reports a baseline mean ambient noise level of 63.12 dB in the NICU environment, while Saunders (1995) reports a mean of 57.98 dBA. Robertson, Cooper-Peel, and Vos (1999) measured a mean ambient sound level of 55.8 dB and when noise created by staff conversation, and heating, ventilation, and air conditioning airflow were eliminated, the noise level was reduced to 51.3 dB. Philbin et al. (1999) report that background sound intensity in an average NICU environment produces between 50 to 70 dBA. A significantly higher mean noise level was measured in the neonatal intensive care unit (M = 54.89 dB) versus the intermediate care unit (M = 49.07 dB) (Levy, Woolston, & Browne, 2003). Finally, Strauch, Brandt, and Edwards-Beckett (1993) indicate that the ambient noise level varies from day to day and shift to shift, where noise levels are higher during mid-week (M = 64 dB) versus week-ends (M = 52 dB) and higher on the night shift as compared to the evening shift, which was reported to be the quietest.

The interaction of the environments of the NICU and the incubator contribute to the sound environment of preterm infants (Johnson, 2001). According to Johnson, NICU noises filter through incubators' walls thereby intensifying the infant's sound environment. Infants in incubators are exposed to

particular noises such as closing portholes without care, or placing objects on the top surface of the incubator which may peak at over 100 dB (Glass, 1999). Saunders (1995) has found that noise levels inside an uncovered incubator were higher (M = 65.90 dB) than in the NICU environment (M = 57.98 dB). Apparatus such as ventilator and suction equipment were the main contributors to noises inside incubators. As well, Kent, Tan, Clarke, and Bardell (2002) report a mean hourly level of 61 dB inside the incubator versus 55 dB outside. Conversely, Elander and Hellström (1995) report that noise levels before the implementation of their intervention in the NICU were higher in an infant's crib (M = 57 dB) than in an incubator (M = 51 dB). The inconsistent measurements of incubator noise levels highlights that preterm infants in incubators are exposed to different levels of ambient noise than infants in cribs.

Infants receiving continuous positive airway pressure (CPAP) would also be exposed to an important increase in the level of noise. Using a probe microphone inserted inside infants' ears, Surenthiran et al. (2003) conclude that preterm infants on CPAP are exposed to significantly higher noise levels than those receiving no respiratory support or on ventilators.

The AAP (1997) and the Committee on Recommended Standards for Newborn ICU (2006) recommend that the ambient noise level in NICUs should not exceed 45 dBA hourly. Consequently, care activities should be reduced in order to decrease ambient noise below the recommended level toward the objective of preventing consequences related to the preterm infant's exposure to NICU noises.

Physiological and Behavioral Effects of Noise

The physiological effects of sound on the preterm infant have been mainly studied in the cardiovascular and respiratory systems (Morris et al., 2000). Factors influencing the responses produced by these systems are: a) intensity of sound; b) behavioral state; c) infants' maturity and postnatal age, and d) perinatal history (Morris et al.).

In a study of 55 preterm infants aged between 23 and 37 weeks gestation, Zahr and Balian (1995) report that NICU noise had the effect of significantly decreasing oxygen saturation and clinically provoking acute rises in heart and respiratory rates, in 16% and 13% of preterm infants, respectively. Long, Lucey, and Philip (1980) state that sudden loud noises caused periods of agitation and crying, followed by a decrease in oxygen saturation and then an increase in intracranial pressure in two preterm infants. More importantly, the researchers noted that the increase in intracranial pressure was observed even if a change in oxygen saturation did not happen.

Strauch et al. (1993) reveal that during the implementation of a quiet hour where noise levels were significantly reduced, preterm infants had improved sleep states, 84.5% of preterm infants were in light and deep sleep compared to 33.9% during the control period. Wharrad and Davis (1997) conducted a study to evaluate the physiological and behavioral responses of preterm and full-term infants to different sound intensities (80, 90, and 100 dB). Responses to sound were found to be different in preterm infants compared to full-term infants and for preterm infants, the mean change in heart rate was significantly related to the stimulus intensity. These researchers conclude that an acceleration in heart rate

(≥ 5 beats) was the most reliable variable to detect the effect of sound on preterm infants in their study.

Long-term Effects of Noise

Exposure to intense sounds may directly damage the fine, delicate hairs of the infant's cochlea leading to hearing loss (AAP, 1997; Blackburn, 1998; Holditch-Davis et al., 2003). Blackburn states that since anatomic structures are functional by 26 weeks of gestation, auditory stimulation that preterm infants experience in the NICU may interfere with the establishment of the auditory functional capability. Incidence rates of hearing loss were found to be higher in infants hospitalized in NICUs (2.4%), in very low birth weight infants (< 1000g) (1.1%), and in low birth weight infants (0.4%) (Thiringer, Kankkunen, Lidén, & Niklasson, 1984). Lotas (1992) also stipulates that preterm infants with the highest number of risks of sensorineural hearing loss are those exposed to NICU ambient noises for the longest time. Davis and Wood (1992) report that NICU infants are at least ten times more likely to experience hearing impairment compared to non-NICU infants. These statements were later supported by Borg (1997) who reports via a critical literature review that total length of stay in the NICU and length of artificial ventilation were the best predictors of hearing loss in the perinatal period. Surenthiran et al. (2003) confirm that following the insertion of a probe microphone in the post-nasal space which is close to the inner ear, sufficient noise is transmitted by the administration of higher oxygen flow rates to cause cochlear damage and increase the risk of hearing loss.

According to Purdy (2000), infants at risk of hearing loss are also vulnerable to developmental language disorders. After a review of long-term follow-up

studies on the effect of noise on preterm infants, Graven (2000) concludes that problems related to speech delay, learning, and language might be related to auditory functioning. The author adds that even though the association of long-term problems to NICU care is not confirmed, it still substantiates the concern for the effect of high noise levels in the NICU.

Pain in the NICU Environment

Mechanisms of Pain and Preterm Infants

Pain pathways and cortical structures essential for pain perception are present in late gestation and, at that time, the systems linked to pain transmission are believed to be functional (Anand & Hickey, 1987). Vanhatalo and van Nieuwenhuizen (2000) explain the neuronal pathways involved in neonatal pain and confirm the presence of nocioceptive activity in fetuses. Figure 3 shows the neuronal pathways participating in pain.

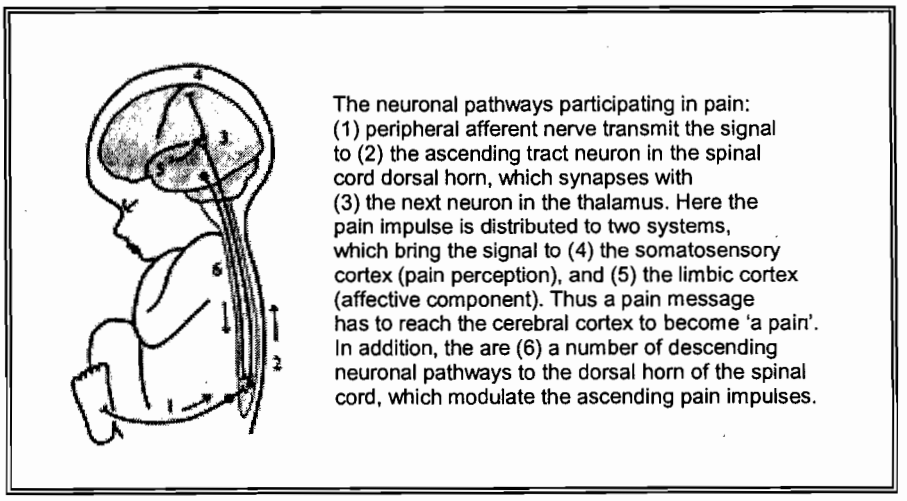

Figure 3. Neuronal pathways participating in pain. Reprinted from Brain & Development, 22(3), Vanhatalo & van Niuewenhuizen, Fetal Pain?, 145-150, Copyright (2000, p.146), with permission from Elsevier (see Appendix A).

Painful NICU Procedures

Pain and discomfort in preterm infants is caused by various elements of the NICU environment while procedural pain is defined by Halimaa (2003) as pain caused by nursing procedures or examinations. In a study conducted by Baker and Rutter (1995), over 3000 procedures were recorded on 54 infants admitted to the NICU, and 74% of these procedures were performed on infants aged less than 31 weeks of gestation. The most common painful tissue-damaging procedure that preterm infants undergo in the NICU is heel lancing (Barker & Rutter; Johnston et al., 1997). Other tissue-damaging procedures are the insertion of intravenous and arterial lines, lumbar punctures, and bladder taps; whereas insertion of feeding tubes, intubations, suctioning, and tape removal are non tissue-damaging painful procedures (Stevens et al., 2000).

Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (International Association for the Study of Pain, 1994). Anand and Hickey (1987) explain that "nocioceptive activity" should be used when discussing pain in preterm infants as "pain" implies emotional associations. Nociception is described as the activity in the nervous system provoked by noxious stimuli (Simons & Tibboel, 2006) and evidence demonstrates that activation of nociceptive systems in the neonatal period is associated with long-term alterations in the perception of pain (Grunau & Tu, 2007). A description of the physiological, behavioral, and hormonal signs of pain in preterm infants reveals how infants respond to painful stimulus in the NICU.

Physiological and Behavioral Signs of Pain

Physiological, behavioral, and hormonal (or biochemical) responses have been used to evaluate pain responses (Franck & Miaskowski, 1997). Stress caused by pain would be apparent with physiological signs (Anand, 1993). The heel lancing procedure has been identified with increased heart rate (Craig et al., 1993; Harrison et al., 2002; Lindh et al., 1997; Stevens & Johnston, 1994; Stevens et al., 1993), decreased respiratory rate (Craig et al.), increased intracranial pressure (Stevens et al.; Stevens & Johnston), as well as increased heart and respiratory variability (McIntosh et al., 1993). In addition, this procedure has been reported to decrease oxygen saturation (Craig et al.; Stevens et al.; Stevens & Johnston) in preterm infants. Lindh et al. reported a reduction in total power of heart rate variability and lower frequency power in preterm infants experiencing a heel lance reflecting a reduction of vagal tone and an activation of the SNS during a painful procedure. Some studies have also reported a sustained increase in heart rate following heel lancing (Craig et al.; Harrison et al.). The physiological changes caused by painful procedures and stress are also recognized as important factors that may contribute to early intraventricular hemorrhage (IVH) or periventricular leukomalacia (PVL) in preterm infants (Anand, 1998).

Behavioral signs responsive to pain in infants are facial expressions, body movements, and crying characteristics (Harrison et al., 2002; Stevens, Johnston, & Grunau, 1995). More precisely, the facial expressions observed in 32 to 34 week gestational age preterm infants were brow bulge, eye squeeze, nasolabial furrow, open lips, vertical and horizontal mouth stretch, taut tongue, and tongue

protrusion (Johnston, Stevens, Craig, & Grunau, 1993). Preterm infants demonstrate higher crying characteristics (Stevens, Johnston, & Horton, 1994) and greater facial expressions in response to a heel lancing procedure (Lindh et al., 1997; Stevens et al.). Stevens et al. reported that facial expressions were mainly influenced by infants' behavioral state while illness severity influenced crying characteristics. Franck (1998) cautions that a lack of behavioral responses of the preterm infant to painful procedures does not indicate that the infant is not feeling pain but rather that there may be an exhaustion of resources to communicate a response.

Long-term Effects of Pain

Effects of painful stimulation experienced by preterm infants while hospitalized in the NICU seem to persist during infancy. Preterm infants weighing less than 1000 grams at birth and spending the longest time in the NICU had lower pain sensitivity scores at 18 months, as rated by their parents, than infants weighing 1500 grams or more at birth (Grunau, Whitfield, & Petrie, 1994). Grunau, Whitfield, Petrie, and Fryer (1994) also report that very premature infants (< 1000 grams) who experienced the longest hospitalization and repeated interventions in early life had a significantly higher score of somatization at the age of 4½ years compared to full-term infants.

Preterm infants would also be hypersensitive to pain. Indeed, Fitzgerald, Millard, and McIntosh (1989) confirm that while preterm infants exhibit neurological immaturity, they appear to have an increased sensitivity to pain. This pain hypersensitivity is seen in instances where normally non-painful stimuli result in pain (allodynia) and normally painful stimuli evoke proportionately

greater pain and pain response (hyperalgesia) (Woolf & Mannion, 1999; AAP & CPS, 2006).

Repeated painful procedures are hypothesized as creating hyperexcitability and windup in preterm infants (Anand & Scalzo, 2000). Mendell and Wall (1965) were the first researchers to publish about the windup phenomenon. They noticed that repetitive low frequency stimulation in cats resulted in a progressive build-up in the amplitude of the response in the dorsal horn neurons. They called this build-up process a windup. Woolf and Salter (2000) explain that the stimulation of nocioceptive pathways can lead to an activity-dependent plasticity, which can be observed by a progressive increase of the organism's response to repetitive stimuli. The activity-dependent plasticity can be created in the terminals of nociceptors by reducing their thresholds (autosensitization) as well as in the dorsal horn neurons (windup).

Preterm infants have lowered reflex threshold (Fitzgerald, Shaw, & McIntosh, 1988), and so they are more vulnerable to the windup phenomenon. Windup or sensitization in infants resulting from repeated stimulation would only fade and develop in habituation by 32 weeks PCA (Fitzgerald et al.). CNS hyperexcitability and windup during the neonatal period would increase chemical activation processes that may subsequently damage developing neurons. In return, Anand and Scalzo hypothetically claim that this damage may lead to long-term negative effects as cognitive impairments, specific drug preferences, and poor socialization skills later in adulthood.

Factors Influencing Pain Response in Preterm Infants

Sizun, Ansquer, Browne, Tordjman, & Morin (2002) enumerated intrinsic and extrinsic factors that may influence the pain response of preterm infants. Gestational age is an example of an intrinsic factor, while handling before a painful procedure, the number of exposures to invasive procedures in the NICU, and behavioral states are identified as extrinsic factors.

Preterm infants that were handled and immobilized before a heel lancing showed a significant increase in mean heart rate and behavioral state and demonstrated more facial activity at the lancing phase of the procedure compared to infants that were not handled (Porter et al., 1998). These researchers hypothesized that the infants might be at a different stage of vulnerability as a function of previous exposure to stressful stimuli, which may affect their pain response to subsequent exposure to noxious stimulus. More recently, Holsti, Grunau, Whitfield, Oberlander, and Lindh (2006) reported that infants born between 30 and 32 weeks gestational age who had experienced clustering care (a series of interventions) prior to a painful procedure showed induced sensitized facial responses during the heel lance phase compared to when the procedure was preceded by a rest period. These empirical studies provide evidence for the windup phenomenon where infants show an increased response to painful stimulation following exposure to and experience of non-painful stimulation.

Porter, Wolf, and Miller (1999) reported later that physiological and behavioral pain responses of preterm infants of different gestational age increased with the invasiveness of the procedure. For infants less than 28 weeks

of age responses increased with postnatal age. These results confirm that preterm infants of different gestational ages respond to painful stimulus and are able to differentiate the stimulus intensity.

The NICU experience of preterm infants also seems to influence their pain response. Johnston and Stevens (1996) compared the pain response of two groups of preterm infants 32 weeks of PCA. One group of infants was born around 28 weeks and had spent four weeks in the NICU while the other group had just been admitted to the NICU. Infants who had been hospitalized in the NICU for four weeks had increased heart rates and lower saturation levels, as well as decreased behavioral response to heel lancing compared to the group of infants recently admitted. The number of previous invasive procedures experienced by the infant in the NICU explained most of the variance of these outcome measures. The researchers further point out that light and noise were not assessed during the study and recognize that these factors might also have influenced the infant's pain response.

Grunau, Oberlander, Whitfield, Fitzgerald, and Lee (2001) further support that the number of previous painful experiences in addition to gestational age were significantly related to a dampened pain response (behavioral signs) in preterm infants of 32 weeks PCA. Finally, Anand and Scalzo (2000) have hypothesized that repetitive painful experience in the NICU as well as other non-painful sensory stimulation in the NICU, such as light and noise, could indirectly influence pain responses of preterm infants. While previous, repetitive, painful experiences are empirically recognized as influencing pain response in preterm infants (Grunau et al. 2001; Johnston & Stevens, 1996), no study has evaluated

how exposure of preterm infants to other sensory stimulation such as light and noise in the NICU could influence their pain response.

Impact of Light, Noise, and Painful Procedures

In summary, there are important physiological and medical outcomes associated with the exposure of preterm infants to light, noise, and painful stimulation in the NICU environment. "Pain is always stressful, but stress is not necessarily painful; both require assessment, evaluation, and treatment" (AAP & CPS, 2000, p. 454). From their model of conservation of energy, Sammons and Lewis (1985) explained that both variation of cardiovascular and respiratory systems cause energy expenditure in preterm infants. Consequently, this energy is not maintained for growth, formation of tissues, muscles and different systems' organization, creating a delay in growth and development (Sammons & Lewis).

Preterm infants can be hospitalized for a period up to three months in the NICU and this environment may hinder cognitive, emotional, physical, sensory, and neurological development (White-Traut et al., 1994). Neonatal nurses and other NICU professionals should strive to minimize sensory stimulation in the NICU as a goal to promote preterm infants' growth and development. The following section presents the environmental interventions that have been empirically evaluated to decrease NICU light and noise, and managing pain in preterm infants.

Environmental NICU Interventions

Interventions Reducing Light Level

There have been two studies on the effect of covering the incubator with a blanket. Shogan and Schumann (1993) report that this intervention, in addition to

turning down ceiling lights, did not improve the oxygen saturation of preterm infants. They explain this finding by the possible habituation of infants to a 100 fc baseline level. It should be noted however that oxygen saturation measurements in this study were taken one and five minutes after lowering the lighting, which may not have been enough time to detect any significant difference in oxygen saturation levels in the infants. In addition, Hellström-Westas, Inghammar, Isaksson, Rosén, and Stjernqvist (2001) report that there was no significant difference in the duration and percentage of quiet sleep of infants over a 24-hour period with or without an incubator cover. The results of this study should be carefully interpreted as only nine preterm infants participated. As well, measurements of sleep state were only taken over two 24-hour periods which may not have been enough time to detect significant changes (Hellström-Westas et al.).

The most common environmental intervention evaluated empirically to control light in the NICU is cycled lighting which is believed to foster the development of circadian rhythms in preterm infants. Cycled lighting consists of reducing the preterm infant's exposure to light for a sequential period of time over 24 hours, usually in the evening and night. In the study conducted by Blackburn and Patteson (1991), the cycled condition consisted of turning off overhead lights as well as the individual infant's light during the evening hours (ranging from 16:00 p.m. to 00:26 a.m.) and then turning them back on in the morning (around 06:00 a.m. to 09:00 a.m.). The cycled lighting condition in Miller et al.'s (1995) study involved turning off a segment of fluorescent ceiling light at night from 23:00 p.m. until 07:00 a.m. This intervention has shown significant positive

effects on physiological and behavioral outcomes (Blackburn & Patteson, 1991) and medical and behavioral outcomes (Miller et al., 1995) of preterm infants. More recently, Brandon, Holditch-Davis, and Belyea (2002) report no significant difference in weight gain between 27 week-old preterm infants exposed to cycled lighting from birth or starting at 32 weeks PCA compared to infants exposed to the cycled lighting only at 36 weeks PCA. Rivkees, Mayes, Jacobs, and Gross (2004) found that preterm infants born < 32 weeks PCA who were exposed to cycled lighting in the NICU showed patterns of rest-activity ten days after discharge in comparison to 21 to 30 days for infants that had been exposed to dim lighting.

In contrast, Boo, Chee, and Rohana (2002) report that there was no significant difference between weight gains of preterm infants exposed to 12-hour cycled lighting compared to those exposed to a continuous dimmed environment. Mirmiran, Baldwin, and Ariagno (2003) reported that this intervention had no effect on the establishment of circadian rhythms (measured by rectal temperature) and sleep organization of preterm infants compared to those exposed to continuous dim lighting. These researchers still conclude that there seems to be no apparent adverse effects to cycled lighting and agree that this intervention could be performed in NICUs. Yet, other experts in neonatology state that generalization of the findings of these studies is limited because of some methodological limitations, including the difference in lighting conditions across studies, small sample sizes, and timing of data collection associated with PCA (Harrison, Lotas, & Jorgensen, 2004). Nevertheless, some studies have reported beneficial physiological effects associated with reducing light exposure of preterm

infants in the environment over a 24-hour period. Warren (2002) cautions that efforts made to reduce the ambient light level in the environment may be creating more lighting variability exposure for the infant as lights are continuously switched on and off.

There are few studies that have evaluated the effects of minimizing sensory exposure to light by covering the preterm infants' eyes. Shiroiwa et al. (1986) have evaluated the physiological and behavioral stability of blindfolded preterm infants in the NICU environment. Even though the sample size of this study was small (n = 10), the intervention has shown significant positive outcomes on physiological and behavioral stability of preterm infants. Conversely, Kennedy et al. (2001) report that preterm infants of a mean gestational age of 27 weeks randomly assigned to wear eye goggles until 31 weeks did not demonstrate a significant difference in weight gain, days of supplemental oxygen therapy, mechanical ventilation, hospitalization, or incidence of intracranial hemorrhage than infants who not did wear eye goggles. The researchers cite that they did not find any detrimental or beneficial effect of this method of light reduction. Roy et al. (1999) evaluated if reduction in light stimulation would affect the central visual development of preterm infants. Infants born before or at 29 weeks have wore eye goggles in the NICU for a period of three weeks (until 32 weeks). At term age, two months later the term age, and at three years, the preterm infants had their eyes examined by pattern visual-evoked potentials responses, which revealed that the maturation of their visual system was not different from the visual system of infants who did not wear the eye goggles. These findings are consistent with a previous randomized controlled trial

conducted by Kennedy et al. (1997). The researchers evaluated the effect of shielding the eyes of preterm infants of less than or equal to 31 weeks of gestation on their retinal development and visual acuity. The results also confirm that covering the infants' eyes did not have a deleterious effect on their visual development. This was evaluated by electroretinograms done at 36 weeks gestational age and visual acuity tests performed at 4 to 6 months corrected age. The results of these studies are particularly important in the context of this research proposal, which also proposes to cover the eyes of 28 to 32 week preterm infants with goggles.

Interventions Reducing Noise Level

Among the interventions empirically evaluated, Saunders (1995) reports that covering incubators with a blanket reduced the noise level in the incubator. Johnson (2001) also reports that when an acoustical foam was placed in the incubator, the noise level inside was significantly lower, and preterm infants showed significantly higher oxygen saturation level and improved sleep states. The researcher points out that the oxygen saturation level results must be interpreted carefully as the majority of the sample had supplemental oxygen treatment. Yet, the sleeping state of the infant persisted after the acoustical foam was removed from the incubator; suggesting that there might be lasting physiological effect associated with reducing the preterm infants' exposure to environmental noise.

Educational programs for neonatal nurses aimed at reducing the overall noise level in the NICU were also implemented. Elander and Hellström (1995) report after the implementation of a 1-hour educational program for intensive care

nurses that noise levels in the NICU environment were reduced. The minimum dB value had decreased from 45 to 35 and the maximum dB from 84 to 79. Researchers report that before the intervention program, the conversations between staff constituted 62% of observed time and following the intervention the amount of conversation decreased to 14%. Zwick (1993) describes that two weeks following the implementation of their intervention nurses had increased knowledge about noise and noise level in the NICU had significantly decreased from 63.12 to 59.46 dB with the same number of preterm infants. Warren (2002) cautions that noise levels can be altered by modifying the care behaviors of neonatal nurses and other NICU professionals, but benefits may be brief and limited.

To the knowledge of the investigator, only Zahr and de Traversay (1995) have evaluated the effect of having the preterm infants wearing earmuffs. These researchers have conducted two studies, a cross-over and a randomized controlled trial (RCT) where preterm infants wore earmuffs for a 4-hour period (two hours in the morning and two hours in the evening). In the cross-over study, they found that infants had significantly higher oxygen saturation with less variation, and spent more time in quiet sleep than when they were not wearing earmuffs. Even though there is no indication of the duration of the wash-out period between the intervention and the control study sequences, and there is a small sample size (n = 13), there still are beneficial physiological effects observed in reducing sound exposure of preterm infants. In the RCT, the only significant finding was that the experimental group showed more awake quiet periods than

the control group. The researchers highlight the importance of using preterm infants as their own control when conducting intervention research.

According to the Study Group on NICU Sound and the Expert Panel of the Center for the Physical and Developmental Environment of the High-Risk Infant, an appropriate environmental sound level in the NICU would promote the preterm infant's physiological stability, growth rate, consistent and age-appropriate neurological and sensory maturation, and lead to fewer long-term problems of speech and language (Graven, 2000).

Intervention Reducing Both Light and Noise Levels

In a longitudinal randomized controlled trial, Mann, Haddow, Stokes, Goodley, & Rutter (1986) compare preterm infants with a mean gestational age of 32 weeks assigned to a day and night nursery versus infants assigned to a control group (standard nursery). The day and night nursery had windows covered by dark curtains, lights and radio turned off, and professionals and visitors were advised to make as little noise as possible from 19:00 until 07:00. Infants assigned to the day and night nursery spent less time awake, took less time for feeding per day, and had greater weight gain than infants assigned to the standard nursery. These results became significant only after the infants were discharged from the hospital indicating that there might be long-lasting effects from both the modification and control of the lighting and environmental noise levels.

Non-pharmacological Interventions Managing Pain

The most effective way to manage pain in preterm infants is to reduce the frequency of the most common painful procedures that preterm infants undergo

while hospitalized in the NICU (Stevens et al., 2000). In addition, to prevent excessive pain in unstable infants, Stevens et al. advise that painful procedures should be performed by an experienced professional. The following section summarizes the non-pharmacological interventions managing pain in preterm infants in the context of a heel lancing procedure.

Non-pharmacological interventions to reduce pain response in preterm infants fall directly within the realm of nursing care and represent important strategies contributing to neonatal nurses' care practices. The most common non-pharmacological procedure studied to relieve pain in preterm infants is the administration of sucrose. Following a systematic review, Stevens, Yamada and Ohlsson (2004) conclude that the administration of sucrose is an effective method of reducing physiological and behavioral pain responses of preterm infants experiencing a heel lancing. Yet, if used repetitively, it may cause neurodevelopmental problems in preterm infants less than 31 weeks PCA (Johnston et al., 2002). Non-nutritive sucking (NNS) (Bo & Callaghan, 2000; Pinelli, Symington, & Ciliska, 2002; Stevens et al., 1999), music therapy (Bo & Callaghan), kangaroo care (Johnston et al., 2003; Ludington-Hoe, Hosseini, & Torowicz, 2005), and facilitated tucking (Corff, Seideman, Venkataraman, Lutes, & Yates, 1995) have been evaluated as effective methods for managing pain in preterm infants. Conversely, prone position has not been found effective in decreasing the pain response of infants to a heel lancing procedure (Grunau, Linhares, Holsti, Oberlander, & Whitfield, 2004; Stevens et al., 1999).

Franck and Lawhon (1998) as well as Anand and the International Evidenced-Based Group for Neonatal Pain (2001) advocate that decreasing the

stress to which the infant is exposed in the NICU could be an environmentally effective strategy in managing pain in preterm infants. Limited research, however, has been conducted to evaluate how this environmental strategy could reduce pain response of infants in the context of an acute painful event (Franck & Lawhon, 1998). More specifically, the effect of minimizing sensory exposure of preterm infants to light and noise in the NICU has not been evaluated on their pain response to a heel lancing procedure.

In summary, this section has highlighted how preterm infants may physiologically benefit from interventions reducing environmental light and noise. Some of these interventions have an environmental perspective while empirical studies reveal that NICU environmental light (Lee et al., 2005; Robinson et al., 1990) and noise levels (Philbin, 2000; Thomas & Uran, 2007) exceed the recommended levels. Furthermore, few interventions minimizing the preterm infant's sensory exposure to environmental light (Shiroiwa et al., 1986) and noise (Zahr & de Traversay, 1995) have been evaluated on their physiological stability. It appears that only one study has tested the effect of reducing both light and noise in the NICU on the outcome of preterm infants (Mann et al., 1986).

So far, no study seems to have evaluated the effect of having preterm infants wearing both eye goggles and earmuffs on their physiological stability and pain response. Stevens et al. (2000) point out that the effect of reducing other noxious stimuli in the NICU environment on pain response of preterm infants is still a research avenue to explore. Furthermore, there is also growing evidence that minimizing the preterm infant's exposure to visual and auditory stimulation in

the NICU may indirectly influence their response to painful stimulation (Anand & Scalzo, 2000; Anand, personal communication, February 5th, 2008).

The last section of this literature review begins by describing the Synactive Theory of Development (Als, 1982; 1986) that serves as a framework for this study. The empirical work of Anand and Scalzo is discussed as it supports the perspective that sensory stimulation (other than painful ones) in the NICU environment may influence the pain response of preterm infants. The combination of this theoretical framework and empirical work leads to the establishment of an intervention model for the proposed research.

Theoretical Framework

Synactive Theory of Development

The "Model of the Synactive Organization of Behavioral Development" was described in 1982 by developmental psychologist Dr. Heidelise Als who puts forward that there is a mismatch between the brain development of the neonate and the NICU environment that can lead to developmental impairments. Infants born preterm may particularly suffer from the impact of the environment through their various senses, such as visual, auditory, cutaneous, tactile, somatesthetic, etc. Als (1982) appropriately questions the range of environmental modification required to re-institute a more balanced, integrated state in preterm infants. Based on these premises, Als' theory offers a unique insight into the objective of assessing neurobehavioral development through behaviors shown by preterm infants in the NICU.

Assumptions

The assumptions underlying this theory are that there is a hierarchy of continuously interrelated dynamic body and attentional systems that allow the preterm infant to become accustomed to the NICU environment and work toward the ultimate goal of self-regulation. The five systems included in the model are the autonomic, the motor, the state organizational, the attention-interactive, and the regulation.

The autonomic system is observed through the physiological stability of the infant (e.g., respiratory changes). The infant's posture, tone and movements show the state of the motor system. The state organizational system is reflected through the sleeping and alert states of the infant. Observation of the infant's ability to interact socially, emotionally, and cognitively with the environment denotes the attention-interactive state. Finally, the regulatory state involves the behavioral efforts made by the infant to maintain self-regulation within the environment (Als, 1982). The model is termed "synactive" as each subsystem is interactive and evolved side by side. For example, physiological stability must be achieved for motor and attention-interactive control (Holdtich-Davis et al., 2003). The differentiation of preterm infants' signs through the four systems reflects brain maturity and organization.

The ANS, which modulates the basic functioning of the preterm infant, exhibits a stress response when the infant is exposed to environmental stress. Physiological signs of stress emitted by the ANS are changes in heart rate (Anand, 1993; Cheng & Chapman, 1997; Bowden et al., 2000; Modrcin-McCarthy et al., 1997), respiratory rate, color, and visceral signs such as hiccups (Als,

1982; Bowden et al., 2000; Cheng & Chapman, 1997; Modrcin-McCarthy et al., 1997). Based on Als' theory, when signs of stress are shown by preterm infants in the NICU, environmental modifications should be made to reduce stress, increase self-regulation, and thereby promote brain development and organization. Since previous research have linked physiological signs of stress to preterm infants' exposure to light and noise in the NICU, a strategic environmental intervention would be to reduce their exposure to light and noise in the NICU to promote physiological stability. This research proposes such an intervention to minimize preterm infants' sensory exposure to light and noise by having them wear eye goggles and earmuffs.

CNS Hyperexcitability

Minimizing the exposure of preterm infants to sensory stimulation in the NICU environment may also influence their pain response. Anand and Scalzo (2000) hypothesized that repetitive pain and NICU care could create a hyperexcitability in preterm infants (see right side of Figure 4). This process would cause an excessive activation of N-methyl-D-aspartate [NMDA] leading to serious long-term consequences in adolescence and adulthood. Conversely, maternal separation creating isolation and neglect may consequently lead to similar harmful consequences in infants (see left side of Figure 4). The latter part of Figure 4 will not be further explained in this chapter as the present study concentrates mainly on abnormal sensory stimulation to which preterm infants are exposed in the NICU.

In addition to repetitive neonatal exposure to pain and NICU care (see Figure 4), excessive and abnormal sensory stimulation through the visual and

auditory systems is also believed to cause hyperexcitability in the central nervous system of preterm infants (Anand, personal communication, February 5, 2008).

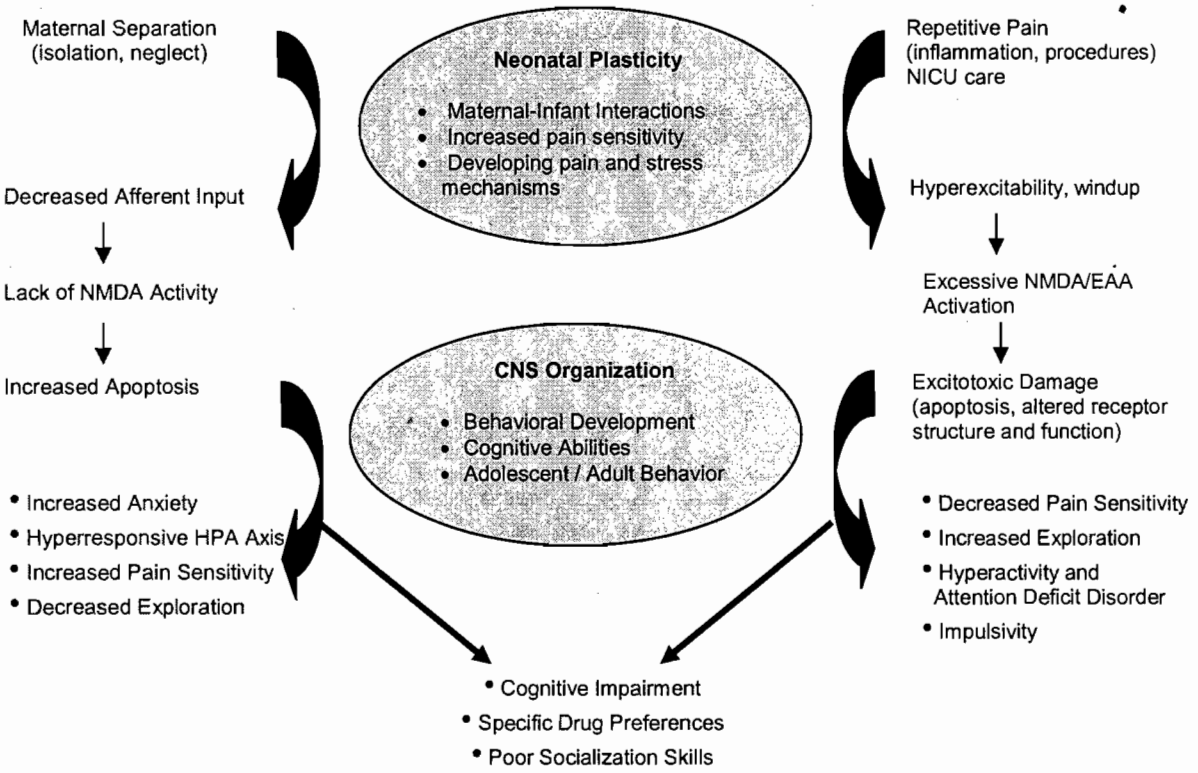

Figure 4. Anand and Scalzo's mechanistic hypotheses of neonatal pain. Figure reproduced from Anand and Scalzo (2000, p. 72) with permission of author and S. Karger AG, Basel (see Appendix A).

The process of hyperexcitability is particularly important for preterm infants with neurological immaturity. Indeed, Anand (1998) explains that because of the increased and prolonged excitability in preterm infants, they may exhibit physiological responses to stress when the nociceptive pathways are stimulated by non-painful events (i.e., handling, nursing care, etc.), long after a painful stimulus (i.e., a heel lance procedure). That is, when the nociceptors are stimulated, they send signs to the cortex through the thalamus eliciting pain

perception, as well as withdrawal reflex, increasing arousal and provoking emotional, autonomic or/and neurohumeral responses (Woolf & Salter, 2000). Arousal and autonomic stress responses are particularly important in preterm infants as they may respond by poorly-modulated or disorganized signs such as changes in heart rate and oxygen saturation levels.

Preterm infants wearing eye goggles and earmuffs should therefore be less exposed to abnormal sensory stimulation in the NICU which in turn should prevent CNS hyperexcitability. Consequently they should demonstrate reduced pain response when exposed to painful stimulus.

In summary, inspired by Als' Synactive Model of Development (1982; 1986), minimizing sensory stimulation to light and noise in the NICU environment may reduce stress behaviors in preterm infants and promote physiological stability. From the empirical work of Anand and Scalzo (2000), it can also be deduced that reducing the preterm infants' exposure to light and noise in the NICU may prevent hyperexcitability consequently reducing their pain response to painful procedures. Figure 5 proposes a sensory minimization intervention study framework for the proposed research based on Als and the empirical work of Anand and Scalzo.

Study Hypotheses

Thus, the following study hypotheses have been formulated:

a) Preterm infants born between 28 and 32 weeks gestational age show *greater physiological stability.* This is reflected in lower mean and maximum heart rate and higher minimum heart rate, improved oxygen saturation (mean, minimum and maximum), as well as improved heart

rate variability (higher mean, minimum and SDNN of R-R intervals, lower maximum of R-R intervals, higher LF and HF, and lower LF/HF ratio) while wearing eye goggles and earmuffs for a 4-hour period, than when unprotected from light and noise in the NICU;

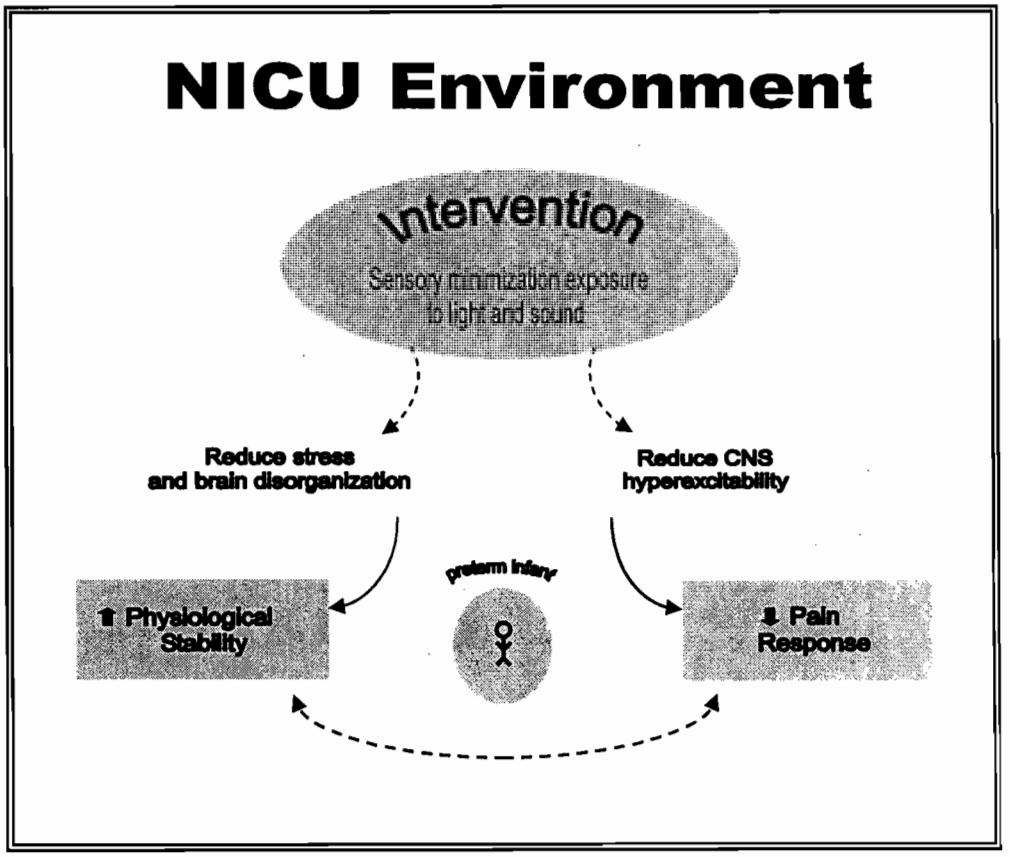

Figure 5. A sensory minimization intervention study framework

b) Preterm infants born between 28 and 32 weeks gestational age show a *reduced pain response* during heel lancing. This is reflected by lower mean and maximum heart rate, lower maximum R-R intervals and higher minimum R-R intervals as well as shorter time for heart rate to return to

baseline at the end of a 4-hour period wearing eye goggles and earmuffs, compared to when they are not protected from light and noise in the NICU;

c) Preterm infants born between 28 and 32 weeks gestational age showing *greater physiological stability* while wearing eye goggles and earmuffs for a 4-hour period, show also a more *reduced pain response* during heel lancing at the end of the 4-hour period.

METHOD

This chapter presents the research designs, the participants and settings, the independent variable (intervention), the data collection, the dependent variables (outcome measures), the data cleaning and editing procedure, the potential confounding variables as well as the statistical methods employed in this study. The ethical considerations related to this research are also discussed.

Research Designs

In this research, there are two studies involving the same participants and the same sensory minimization intervention, i.e. having preterm infants wear eye goggles and earmuffs for a 4-hour period. The first study consists of a cross-over trial measuring physiological stability and the second study is a randomized control trial (RCT) measuring pain response in these preterm infants. These two studies are a modification of an experimental research design (cross-over) that was initially planned to evaluate both physiological stability and pain response. After the initial implementation of the procedure in clinical settings, the design was modified to improve its feasibility. The most important modifications were to reduce the intervention time from 24 hours to four hours and to only measure pain response at the end of the first study period. Appendix B presents the modifications made to the original research design as well as the justifications for these changes. The modified research design allowed for both a comparison of the physiological stability of preterm infants between the 4-hour intervention and control periods with a cross-over trial and a comparison of pain response between infants within a randomized clinical trial. Figure 6 is a schematic representation of these study designs. This chapter is therefore separated in two

parts, the first part describes the methodology related to the evaluation of the physiological stability by the cross-over trial and the second part presents the methodology related to the assessment of pain response by the RCT.

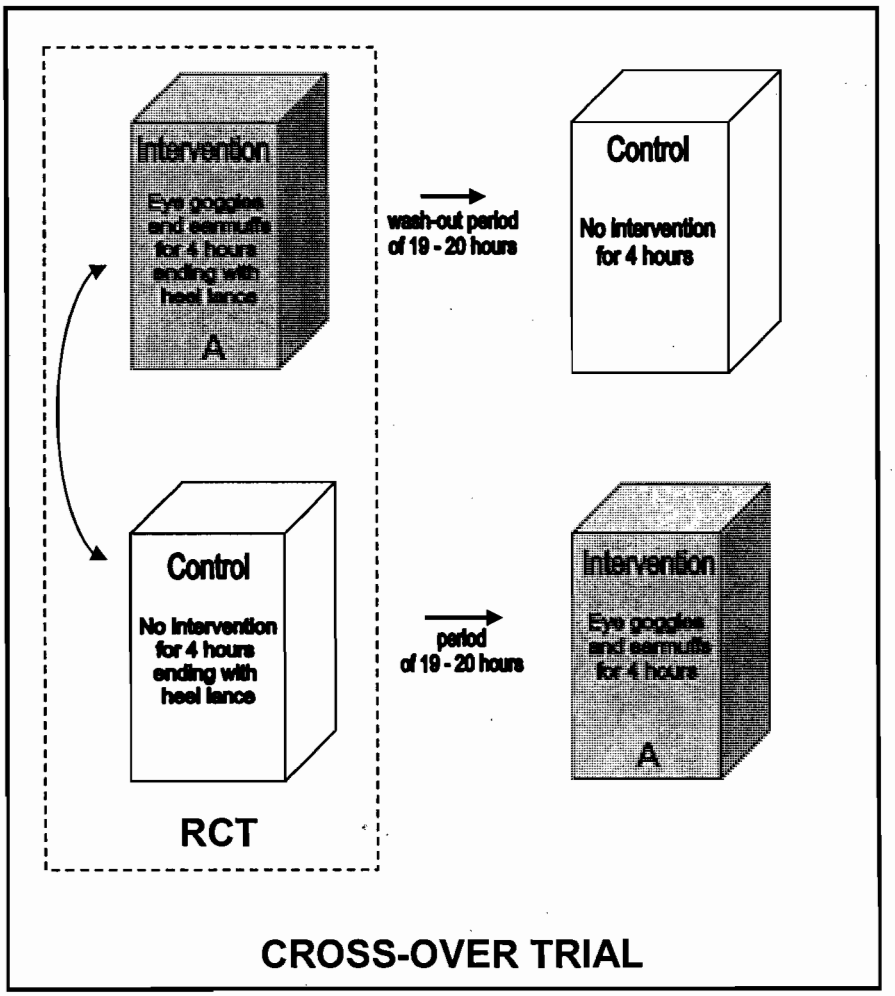

Figure 6. Schematic representation of the cross-over trial for physiological stability and the RCT for pain response.

Physiological Stability

Study Design – Cross-Over Trial

In a cross-over trial, participants serve as their own control. Participants are given a sequence of treatments, for example, intervention then control or control then intervention, with the purpose of studying the difference between the

treatments (Senn, 2002). The cross-over experimental trial evaluated physiological stability of the neonates, ensuring the highest possible equivalence among the participants being exposed to different conditions, i.e. same gestational age and birth weight (Polit & Hungler, 1999). This design increased control over the effect associated with the intervention (wearing eye goggles and earmuffs) and allowed for better control of the potential response variability that may exist between preterm infants of different gestational ages. Neonates were randomly assigned using sealed envelopes to one of the following group sequence: starting with intervention (A) then control (B) or starting with control (B) then intervention (A). The sealed envelopes were generated by an IT Security Officer who used a randomization website (www.randomization.com), and were opened by the parents after giving their approval for their infant's participation in the study.

A cross-over trial is also characterized by having a pre-determined wash-out period. This period of time is believed to eliminate the carry-over effect of the intervention in the subsequent trial period (Portney & Watkins, 2000; Senn; Jones & Kenward, 1989). Based on a previous study using a cross-over trial to measure physiological stability of preterm infants wearing earmuffs (Zahr & de Traversay, 1995), it was hypothesized that collecting data on two consecutive days within an interval of approximately 20 hours would be sufficient to wash-out the effect of the intervention and prevent any carry-over effect in the control period. The wash-out period used by Zahr and de Traversay was less than 20 hours. A period of time around 20 hours was also allowed for between the B-A sequence (control

then intervention) to prevent a maturation bias in infants and reduce threats to the internal validity of the study.

Participants and Settings

A convenience sample of 72 preterm infants was recruited into the study (see sample size in RCT section, p. 93). Data were collected in four university-affiliated teaching hospitals in the Montreal region that have a level III NICU. Combined, these hospitals had a capacity of approximately 130 NICU beds, where, at any given time, infants born between 28 and 32 weeks of age were admitted.

Sample size. The sample size required for the cross-over trial was calculated to be 48 participants. The sample calculation of physiological stability was based on the results of the cross-over study conducted by Zahr and de Traversay (1995) where the effect of earmuffs was evaluated by a measurement of heart rate and oxygen saturation in preterm infants. A significant difference was reported for the mean in oxygen saturation, which was 95.6% in infants wearing earmuffs compared to 92.3 % in infants not wearing the earmuffs. The standard deviations (*SD*) were 2.4% and 3.3% respectively, and the greater *SD* was used in the calculation. Using Colton's (1974, p. 145) formula with a two-sided alpha of 0.05 and a beta of 80% (.80), the sample required for the study was:

n per group = $\frac{2\,(Z\alpha - Z\beta)^2\,\sigma^2}{\Delta^2}$ where: $\alpha = 0.05$ and $\beta = 0.2$ (80%) then $Z\alpha = 1.96$ and $Z\beta = -0.84$; σ is the greatest *SD* of the two groups, in this case the group of infants without earmuffs: 3.3, and Δ in oxygen saturation levels was 3.3% (95.6%

- 92.3%) so: $\frac{2\,(1.96 + 0.84)^2 \times 3.3^2}{3.3^2} = 15.68$ so 16 infants.

Given that this calculated sample size is assuming a large effect size (i.e. 1); a calculation was also performed with a more conservative estimate of effect size of 0.5 increasing the sample requirement to 64. A mean of both sample sizes (i.e. 16 and 64) was then performed resulting in a sample of 40 infants. Based on two intervention studies conducted with preterm infants (Johnston et al., 2002; Johnston et al., 2003), an attrition rate of 20% was anticipated for this study. According to these calculations, the total number of preterm infants required for the physiological stability measurement was 48. Since both dependent variables, i.e. physiological stability and pain response were examines as outcomes in two different research designs, two sample sizes calculations were performed and the highest sample size, calculated for the pain response, was kept in order to adequately answer all research hypotheses (see sample size in the RCT section, p. 93).

Inclusion criteria. Preterm infants were recruited if they were born between 28 to 32 weeks of gestation as determined by ultrasound, last menstrual period, estimated time of delivery or clinical estimation. Data collection was performed from five to 24 days postnatal age allowing a 3-week window. The collection of data was not begun before five postnatal days to avoid any situation where the infants would receive a diagnosis in the first days of life that would exclude or withdraw them from the study. In order to maintain a homogeneous sample and prevent a threat to the internal validity of the study, data were collected only when respiratory support was terminated, and at least 24 hours after the end of the

phototherapy treatment, where the preterm infants' eyes were already protected by goggles.

Exclusion criteria. The infants were excluded if they: a) were born from mothers with a history of illicit substance or antidepressant use during pregnancy, b) required surgery, c) had congenital malformations or genetic disorders, d) had an intraventricular hemorrhage greater than grade II before data collection, e) received analgesics or paralyzing agents at time of data collection, f) had an APGAR of less than 6 at 5 minutes, and g) were not cared for in an incubator. The objective of all criteria except the last was to exclude any situations where the preterm infants' physiological stability might have been influenced by factors other than the intervention, while the last criterion was to control for the level of ambient noise exposure in the unit, which is reported to be different than directly in incubators (Elander & Hellström, 1995; Saunders, 1995; Kent et al., 2002).

Intervention

Materials. Eye goggles and earmuffs were applied to preterm infants in their incubators for a period of four hours by experienced neonatal nurses. The eye goggles were put on first, then the earmuffs. The entire installation procedure lasted approximately three to five minutes. The eye goggles used in this study were the Olympic Medical Bili-Mask provided by the Quick Medical Company. These eye protectors were chosen for the following two reasons: a) they were used in a previous study evaluating if covering preterm infants' eyes influenced the central visual system development (Roy et al., 1999), and they were the eye protectors used for preterm infants under phototherapy treatment in one of the main hospital centers where data collection was performed. The eye goggles

reduced 100% of the light intensity perceived by the infant. The earmuffs were the MiniMuffs® neonatal noise attenuators from the Natus Medical Company, which reduced sound levels by at least 7 dB, representing a reduction of sound pressure of approximately 50%. The MiniMuffs® are oval-shaped, designed with foam that utilizes a hydro gel adhesive to allow attachment and easy removal from the infant's skin.

Procedure. The intervention was set at four hours duration in the morning and was performed between 06:00 a.m. to 12:00 p.m. This window time was chosen for the following reasons: a) this time period covers a period of time when the lighting is increased due to daylight and when the noise intensity is particularly high and peak noises occur more frequently (Chang et al., 2001; Krueger, Wall, Parker, & Nealis, 2005), and b) no study with similar purposes (Blackburn & Patteson, 1991; Shiroiwa et al., 1986; Zahr & de Traversay, 1995) has continuously measured the physiological stability of infants for a period of four hours with both eye goggles and earmuffs.

Data Collection

Physiological stability was evaluated by measuring the following parameters: heart rate, heart rate variability (HRV) and oxygen saturation over the 4-hours intervention and control periods. The parameters were collected using the Somté™ device commercialized by the Compumedics Company. The Somté™ recorded electrocardiogram (ECG) tracings allowing both collection of heart rate and HRV on preterm infants. The oxygen saturation was collected by a pulse oxymeter applied to one of the infant's extremities, which was also

recorded into the Somté™ device. The accuracy and precision of the equipment measuring these physiological parameters were established by doing a sample analysis, i.e. comparing the heart rates provided by the Somté™ and the NICU monitors of infants recruited in the study for a few seconds at the beginning of data collection. At the end of the recording, the data were converted from the device into a computer using the Somté™ software and further exported into Excel files.

All preterm infants were videotaped with a digital camera in their incubators over the 4-hour periods of intervention and control for the evaluation of potential confounding variables. All videos recordings (n = 108) were incorporated into computer video files for further observation and analysis. Inter-rater and intra-rater reliability for the coders of the videos recordings were established by having the investigator, a researcher in the neonatal field, and a student nurse code one hour of the same videotape. The percent of agreement calculated initially between the three coders was 64.5%. A second one-hour video sequence was coded with an adequate 90.0% inter-rater reliability. The intra-rater reliability was then calculated by having the nursing student code the same video sequence one week apart, and the concordance between both samples was 92.5%, which was also considered adequate. Intra-rater reliability was performed over one-week presuming that the coder would not have memory (recall bias) of the beginning and ending time of the infant's handling (for the description of the handling's coding, see section on extraneous variables, p. 88).

Another coder was trained to code the research videos. The two different coders coded an equal portion of the videos, (i.e. 54 videos each). To establish

the inter-rater reliability between the two, a one-hour sequence of a video previously coded by the first coder was used. The initial percentage agreement obtained was 67.0%, and after clarifying the process, the percent agreement was 94%. The intra-rater reliability obtained was 96.5% and followed the same steps as with the first coder. In cases (n = 17) where some parts of the video recordings were missing due to equipment malfunction, electricity problems or camera obstruction, a pro-rating rule was applied to obtain equivalent time of video recordings in the comparison between both study sequences. In one case, the total handling time used for the analysis was the one written down by the nurse since the computer did not record during data collection. All handling that exceeded four hours of recording was cut off from the analysis and the same procedure was used for the infant's position.

Outcome Measures

Heart rate. Heart rate was assessed by calculating the mean, minimum and maximum recorded during the study periods. Even though the heart rate was collected by both the ECG tracings and the pulse oximeter, only the ECG tracings were used to summarize the results of heart rate since they provide greater precision than the pulse oximeter.

HRV. HRV quantification has been identified as a noninvasive measure of central nervous system integrity since the prevailing determinant of heart rate's response is the brainstem (Oberlander & Saul, 2002). The sympathetic nervous system accelerates the heart rate while the parasympathetic system slows it (Hainsworth, 1995).

There are two types of analysis that can be performed in order to calculate the HRV: the time-domain analysis and the frequency-domain analysis. The time-domain analysis is a broad measure of the autonomic nervous system balance, while the frequency-domain analysis separates the effects of the sympathetic and parasympathetic on the autonomic nervous system control (Cowan, 1995). The time-domain analysis allows the calculation of the distance in millimiseconds (ms) between the Rs of normal consecutive QRS complexes. Using time-domain analysis, HRV was assessed by the mean of normal R-R intervals, the standard deviation of consecutive normal R-R intervals (SDNN) as well as the minimum and maximum of the R-R intervals. The SDNN represents the short-term variability of heart rate, which is a common time-domain variable reported in neonatal studies (Rosenstock, Cassuto, & Zmora, 1999).

Through the frequency-domain analysis (or spectral analysis), the high-frequency power (HF), the low-frequency power (LF) as well as the ratio of the LF/HF were calculated. The LF power is represented by both the sympathetic and parasympathetic nervous system functions and many authors consider that an increase in its value is a marker of sympathetic activation (Akselrod et al., 1981; Cerutti, Bianchi, & Mainardi, 1995; Oberlander & Saul, 2002; Verklan & Padhye, 2004). Conversely, HF power is accepted as synchronous with respiratory rate and is recognized as a marker of parasympathetic activation and vagal activity (Akselrod et al.; Cerutti et al.; Oberlander & Saul; Verklan & Padhye). Infants who have an increased vagal tone would be healthier whereas a lower vagal tone would be associated with infants at risk (Oberlander & Saul). In addition, a withdrawal of the parasympathetic nervous system tone (shown by

lower vagal activity) in response to challenging situations may represent stress (Porges, 1992). Increased HRV is therefore an indication of adaptation and healthy functioning of the autonomic nervous system mechanisms (Verklan & Padhye). Peaks in the spectrum analysis that were higher than 0.15 Hz were classified by the Somté^{TM} software as HF power whereas LF power was characterized by peaks between 0.04 and 0.15 Hz. These values are provided in milliseconds squared (ms^2). The antagonist relationship between the LF and HF powers allows the assessment of the sympatho-vagal balance by calculating the ratio of LF/HF powers (Cerutti et al., 1995). A lower ratio indicates a decrease sympathetic modulation or an increase parasympathetic modulation of the heart, or both (Fei et al., 1994), which is reflective of a better sympatho-vagal balance.

Oxygen saturation. Oxygen saturation was assessed by calculating the mean, minimum and maximum recorded during the study periods.

Data Cleaning and Editing

All ECG tracings (n = 108) were reviewed by an experienced electrophysiology medical technician to validate the analysis made by the software and the modifications that were manually made to the tracings. A complete description of the steps followed for cleaning and editing the ECG tracings can be found in Appendix C. Following data cleaning and editing, reports were generated using the software cardiac analysis of the Somté^{TM}. The reports were generated every 5 minutes to allow the calculation of the spectral analysis of HRV which is performed on at least 200 to 500 consecutive heart beats (Cerutti et al.). A total number of 48 reports were then generated for each four hours of study periods (240 minutes / 5 = 48) providing the physiological

parameters that were used to summarize the outcome evaluated in this study. A sample of the report sheet produced by the Somté™ software where the physiological parameters used in this study are circled can be found in Appendix C. In cases where there were missing reports for infants (e.g. 45 reports instead of 48), values were not replaced since they were classified as artefacts by the Somté™ software. The range of the number of reports used in the statistical analysis for heart rate and HRV for both the intervention and control sequence varied from 36 to 48 respectively.

Data cleaning and editing were also performed for the oxygen saturation data collected through the pulse oximeter sensor. Exporting data collected through the pulse oximeter sensor from the Somté™ to Excel created a data file of 14402 data points (one data for every second) for each study period recorded. The precision of the pulse oximeter was intermittently inaccurate when the infant was moving the extremity (foot or hand) where the sensor was located creating artefacts. Missing values were not replaced mainly because they were due to artefacts or infants' movements.

Data cleaning for oxygen saturation was performed by comparing the heart rate values recorded by the ECG to heart rate readings from Somte™. If these matched, then the oxygen saturation from the pulse oximeter was considered accurate. Further screening was applied by removing all data below 50% to prevent excluding values that could be associated with bradycardia. After removing values under 50%, there were nine infants out of the 54 in the intervention period that had a minimum of 50% for the oxygen saturation and eight infants in the control period. After data cleaning and editing, the number of

values computed for the oxygen saturation for the 108 samples (including both intervention and control) varied from 3331 to 13983.

Extraneous Variables

The potential confounding variables that may have influenced the study outcome measures were compared between the study sequence allocations. Extraneous variables that were examined before data collection were: a) gestational age, b) respiratory support, c) administration of medication, and d) phototherapy treatment. Other extraneous variables that may be both associated with the intervention and the outcome variables were also evaluated. These were: a) the infants' position and b) handling of the infants. The levels of the NICU lighting and noise were also assessed to compare the environmental conditions between both study periods. The following paragraphs provide a description of the extraneous variables.

Respiratory support. The infants' proportion and duration (in hours) of respiratory support: intubation and CPAP prior to entering the study was collected through medical chart review. Respiratory support was evaluated since it provided some information about the preterm infants' physiological stability following birth.

Administration of medication. The infants' proportion and duration (in days) of medication administration were monitored. The administration of caffeine, dopamine, dobutamine, and indomethacine was evaluated since they are commonly given to preterm infants and provided information about their physiological stability. The monitoring was done through chart review of the medication administered after birth and during data collection (i.e. both study

periods and wash-out) to screen for a potential history bias to the internal validity of the study. Only the results associated with administration of caffeine is reported in the findings since only a small number of infants participating in the study received the other agents: dopamine (n = 10), dobutamine (n = 1) and indomethacine (n = 4). Caffeine stimulates the central nervous system of preterm infants to reduce the incidence of bradycardia and apnea.

Phototherapy treatment. The infants' proportion and duration (in minutes) of phototherapy treatment was noted as it meant that infants participating in the study had previously worn eye goggles for a determined period which could have influenced their habituation to the material during the study.

Position. The infant's position was evaluated by calculating the amount of time (in minutes) during which the preterm infant was in the prone, supine and lateral positions during data collection using the videotape. The prone position was of particular interest since it is particularly reported as improving physiological stability in preterm infants (Chang, Anderson, Dowling, & Lin, 2002; Monterosso, Kristjanson, & Cole, 2002).

Handling. The type and duration of handling to which the infant was exposed over the 4-hour periods were compared between the two study sequences using the videotape. The following categories were examined: a) the mean amount of time (in minutes) and number of occasions the infant was handled in total, b) the mean amount of time (in minutes) the infant was handled with the purpose of verifying, adjusting or reinstalling eye goggles and/or earmuffs, c) the mean amount of time (in minutes) the infant was handled for a painful procedure, and, d) the mean amount of time (in minutes) the infant was

provided comfort. For the total handling (a), the number of occasions was counted as one when the handling was separated by at least one minute. The identification of painful procedures made by Stevens et al. (2000), i.e. heel lances, installation of IV, insertion an orogastric or nasogastric tube, suctioning the infant, and removing tape were used to categorize the handling for painful procedures. Comfort handling was defined as anytime where the infant was sucking a pacifier, and/or was caressed by a nurse or the parents. Handling was evaluated since it may influence physiological stability in infants (Harrison, 1997; Long, Philip, & Lucey, 1980; Peters, 1999 Zahr & Balian, 1995).

Light and noise levels. Light and noise levels were recorded every hour during both study periods of the data collection to evaluate the environmental conditions in the NICU. The lighting level was measured in lux using a photometer. The photometer used for the study was the Cal-LIGHT 400 from the Cooke Corporation Company. In order to measure the light intensity as perceived by the infant, the readings were taken at the infant's eye level. The sound levels were measured using a dB A-weighted scale sound meter, which measures environmental noises as they are heard by the human ear (Gray & Philbin, 2000). The ambient noise level was measured at the infant's ear level to collect a reading reflecting as closely as possible the sound level heard by the infant (Gray & Philbin). The sound measurement was done in equivalent level (L_{eq}), which is the appropriate measure to obtain an average sound level (Gray & Philbin). Four different sound meters were used in the study due to equipment malfunction; however the same sound meter was used for both the intervention and control

periods for the same infant. Reliability of the photometer and sound meters was assured by the calibration done by each company.

For light and noise, a mean of the five hourly measurements was performed for the statistical analysis. In cases where there was some readings missing, the values were not replaced and the means were calculated over the total number of readings noted in the study period (i.e. 3 or 4).

Data Analysis

The effect of the intervention on selected outcomes of physiological stability in preterm infants was evaluated in SAS 9.1 using a proc mixed model. This is the model of choice to use for cross-over trials since it offers an analysis of variance (ANOVA) between groups and allows the comparison of both study periods by treating them as repeated measures (RM). The mixed model was conducted following a two-stage analysis procedure (Hills & Armitage, 1979) where the first step consisted of evaluating if there was a carry-over effect (between subjects) associated with the sequence of the trial on the selected outcomes. The second step consisted of evaluating if there was a significant effect associated with the period and the intervention (within subjects). Testing for carry-over effect has been criticized by Senn (2002) who argues that cross-over trials are based on the assumption that there is minimal carry-over effect possible because of the establishment of a wash-out period. However, since there is no guarantee that the wash-out period was effective in this study, and that carry-over effect is one of the main concerns of cross-over trials, an interaction between the period and intervention (i.e. sequence) was preliminarily tested to determine if there was presence or not of a significant carry-over effect. If no carry-over effect

was present, both study sequences (n = 54) were considered and compared in the statistical analysis. However, if a carry-over effect was found, only the first period of the study (intervention or control) was entered in the analysis (n = 27) to diminish bias associated with the carry-over effect of the intervention in the subsequent control period.

Repeated measures of analysis of covariance (RM-ANCOVA) were used for covariates to equalize their partial effect on the dependent variables and ensure that physiological stability was primarily associated with the intervention. Before performing the statistical analysis, all variables were examined for normal distribution and if they were not normally distributed, they were submitted to a log transformation prior to analysis.

Independent samples t test were used to compare demographic data (see appendix D): gestational age, birth weight, APGAR, postnatal age and wash-out period for preterm infants randomized in the intervention then control sequence versus the control then intervention sequence. Gender and type of delivery were compared using a Chi-square analysis. Paired samples t test were performed to compare the extraneous variables between the study sequences. All statistical analysis was performed with a significance level of 0.05 (two-sided alpha).

In four cases, data collection could not be performed accordingly to the study protocol due to clinical conditions, i.e. resumed phototherapy treatment and eye infection (n = 2) or problems with equipment (n = 2). There was no difference between the statistical analysis' results excluding or including these infants in the sample for all study variables except for the SDNN of normal R-R intervals. Therefore, the statistical analysis for the SDNN of R-R intervals were conducted

with a sample of 50 infants (i.e. the four infants were removed from the sample) whereas for all outcome measures the four infants were kept in the final study sample to maintain the sample size at 54.

Pain Response

Study Design – Randomized Control Trial

A randomized control trial (RCT) was used to evaluate the effect of the sensory minimization intervention on the pain response of preterm infants. This study design respected the three essential characteristics of an experimental design, i.e. manipulation of an intervention, randomization into groups and having a control condition to eliminate threats to inference (Buckwalter, Maas, & Wakefield, 1998). In the RCT, the same intervention and group allocations were used as in the cross-over trial. Infants were randomly assigned to wear eye goggles and earmuffs (intervention group) or standard care (control group) for a 4-hour period before undergoing a heel lance for blood procurement for clinical purposes. The infants' pain response to the procedure was therefore measured at the end of the four hours of the intervention or control period (see Figure 6, p. 79).

Participants and Settings

A convenience sample of 72 preterm infants was recruited into the study. These participants were recruited from the same four university-affiliated hospitals cited previously (see participants and settings in the cross-over trial section, p. 79).

Sample size. The sample size calculated for the pain response was 72 preterm infants. The expected effect size for the pain response was based on the

Premature Infant Pain Profile (PIPP) instrument (Stevens, Johnston, Petryshen, & Taddio, 1996). This multidimensional scale, which is composed of physiological, behavioral and modifying indicators such as gestational age and behavioral states, indicates that a significant clinical change in pain response is associated with a 2-point change in the parameters composing the scale (Stevens & Gibbins, 2002). Since the present study was measuring pain response using physiological parameters, the indicator of heart rate in the PIPP instrument was used to determine the expected effect size for pain response. To attribute a 2-point difference score for heart rate, a change of 15 to 24 beats must occur during the painful procedure. Since a 15 to 24 beat change represents an important effect size, a more conservative estimate was used for the sample size calculation, i.e. 5 to 15 beats, which corresponds to a 1-point change on the PIPP scale. The middle point of this change in heart rate, 9.5 beats (14-5 = 9 / 2 = 4.5; 5 + 4.5 = 9.5) or 1.5 on the scale was chosen as the expected effect size for the pain response since it was the significant difference found by Johnston et al. (2003) on the PIPP scale 30 seconds following heel lancing with preterm infants in kangaroo care vs. in an incubator during the procedure. The standard deviation (*SD*) used for this calculation was then based on Johnston et al.'s study (personal communication, August 24, 2003). The physiological outcomes measured in their study included the maximum heart rate throughout the procedure. The *SD* of the heart rate of the skin-to-skin group was 10 while it was 13 in the control group. The greater *SD* was used in the calculation. Using Colton's (1974, p. 145) formula with a two-sided alpha at 0.05 and a beta of 80% (.80), the sample required for the pain response was:

n per group = $\frac{2\,(Z\alpha - Z\beta)^2\,\sigma^2}{\Delta^2}$ where: $\alpha = 0.05$ and $\beta = 0.2$ (80%) then $Z\alpha =$ 1.96 and $Z\beta = -0.84$; σ is the greatest standard deviation of both groups = 13, and Δ in heart rate = 9.5 so: $\frac{2\,(1.96 + 0.84)^2 \times 13^2}{9.5^2} = 29.36$ so 30 infants in each group, 60 in total. As in the cross-over trial, an attrition rate of 20% was considered, increasing the sample size to 72 infants (60 + 12 = 72).

Inclusion and exclusion criteria. The inclusion and exclusion criteria enumerated in the cross-over trial were also employed for the preterm infants' measurement of pain response in the RCT (see inclusion and exclusion criteria in the cross-over trial section, p. 80 and 81).

Intervention

Materials and procedure. The preterm infants wore eye goggles and earmuffs (same material and installation procedure as in the cross-over trial) for four hours in their incubators between 06:00 a.m. to 12:00 p.m. before undergoing a painful procedure. Having the intervention in place with the beginning of daylight was also in keeping with the procurement of blood samples that are usually taken in the morning. In conjunction with routine nursing clinical care and at the end of the 4-hour study, a heel lance was performed on the infant. The heel lance procedure was selected as the painful stimulus because it is the most common tissue-damaging procedure that preterm infants experience, with reports ranging from three times per day to several times per week (Barker & Rutter, 1995; Johnston et al., 1997). This procedure was not performed without a prescribed order and included three phases: a) baseline which lasted one minute, preceding foot cleaning by 30 seconds, b) heel lancing ending with the

application of bandage, and c) time in minutes until the heart rate returned to baseline from the application of the bandage (specifically, until baseline heart rate was reached again or up to a maximum of five minutes). In two hospitals, a laboratory technician performed the heel lance procedure on the infant, while NICU nurses conducted the procedure in the two other hospitals.

Data Collection

Pain response was evaluated by measuring physiological parameters during the baseline (a) and blood sampling (b) phases. The parameters were heart rate, HRV, and oxygen saturation. During the painful procedure, heart rate and HRV were collected using the Somté™ device and oxygen saturation by the pulse oximeter feeding into the Somté™. At the end of the recording, the data were extracted from the device into a computer using the Somté™ software and further exported into Excel files. A marker inserted at the time of heel lancing in the Somté™ indicated the beginning of the blood sample in the ECG tracings. All painful procedures were also videotaped and recordings were uploaded into a computer. The entire procedure recorded on the video, i.e. foot disinfection, heel lance, heel squeeze, cleaning foot and applying bandage were coded by the same research assistants as in the cross-over trial. Using the videos, the research assistants coded the beginning time of the baseline (preceding foot disinfection by 30 sec.) and the duration of the painful event (from heel lancing to bandage). The coded times were then used to calculate the outcome measures for the baseline and blood sample phases with the reports generated by the Somté™ cardiac software.

Outcome Measures

Heart rate. Heart rate was assessed by calculating the mean and maximum during baseline (phase 1) and blood sample (phase 2). There were two ways to calculate heart rate during the taking of the blood sample: a) over the entire procedure signifying that calculations were made over different lengths of time; or, b) calculating it over equivalent periods of time, such as 30 seconds, from the beginning to the end of the procedure. The first option (a) was chosen as it was difficult to set up definite small segments in the cardiac software used for the analysis, thereby possibly engendering different sample sizes for each single segment. To account for differences associated with the procedures' differing lengths of time, the time to harvest the blood sample was treated as a covariate in all statistical models of the outcome measures for pain. The ECG tracings were used to summarize the results for heart rate.

HRV. Heart rate variability was assessed by the minimum and maximum R-R intervals during the baseline and blood sample phases. The time-domain analysis calculated the distance in milliseconds (ms) between the Rs of normal consecutive QRS complexes during the procedure. Same as for the heart rate, HRV was calculated over the entire time of the procedure.

Oxygen saturation. The data collected for the oxygen saturation was insufficient to report findings in relation to this variable. As noted previously, the precision of the pulse oximeter was intermittently inaccurate when the infant was moving the extremity where the sensor was placed (foot or hand), creating artefacts. Since preterm infants were moving their extremities during the heel lancing, artefacts were mostly recorded by the Somté^{TM} during the procedure.

Only six infants out of 41 had more than 50% of the oxygen saturation recorded during the blood sample and about half of the infants (19 out of 41) had 50% of the data recorded in baseline.

Return to baseline. Return to baseline (phase 3) was calculated by the time in minutes until the baseline heart rate was reached again after the application of bandage. Using the ECG tracings, blocks of 15 seconds were used in the Somté^{TM} starting from the moment where the bandage was applied to the foot of the infant to the moment where the mean heart rate calculated in baseline was reached again. Once the block of 15 seconds provided a mean heart rate close to one or two beats of the baseline heart rate, the block was further separated in 5-second blocks to determine more precisely the time where the preterm infants' heart rate had return to baseline. The start time of that block was used for the results. Return to baseline was calculated by a research assistant who was blinded to the preterm infants' group allocation.

The salivary cortisol was intended to serve as a biophysical measure for pain response, however the amount of saliva collected was insufficient for analysis and the collection was stopped after trying to collect saliva on the first nine infants recruited in the study.

Data Cleaning and Editing

Data editing and cleaning of the ECG tracings for pain response was performed using the same steps described in the Appendix C.

Extraneous Variables

To diminish possible threats to internal validity to the RCT, extraneous variables, which were identified in the literature review as having an influence on

pain response of preterm infants, were assessed. These variables were: a) gestational age at birth (Grunau et al., 2001), b) postnatal age at the time of data collection (Johnston & Stevens, 1996; Porter et al., 1999), c) the number of previous invasive procedures (Grunau et al.; Johnston & Stevens), and d) the preterm infants' handling in the four hours preceding the painful procedure (Porter et al., 1998). Other potential confounding variables that could have influenced the preterm infants' pain response were also assessed: a) respiratory support prior to data collection, b) administration of medication, c) phototherapy treatment, and d) the time it took to harvest the blood during the procedure. The levels of the NICU lighting and noise were also assessed to compare the environmental conditions between both study groups. The following paragraphs provide a description of the extraneous variables.

Number of previous invasive procedures. The number of previous invasive procedures performed on the infants since birth were noted from the medical chart. The invasive procedures noted were the number of heel lances and intravenous line (IV) installations. Suctioning (gastric, endotracheal...) was not included in the calculation due to the inaccuracy in determining their occurrence from chart review.

Handling. The duration and number of occasions that infants were handled in the 4-hour preceding the blood sample were also assessed using the videos recorded for the cross-over trial.

Respiratory support. The infants' proportion and duration (in hours) of two different types of respiratory support: intubation and CPAP were assessed through medical chart review. Respiratory support was noted since it provides

information about the severity of the infants' illness after birth, which may influence their response to painful procedures.

Administration of medication. The infants' proportion and number of doses of caffeine and dopamine administered was evaluated through chart review. The administration of these medications gives information about the physiological stability of preterm infants. Caffeine is used as a CNS stimulant to reduce bradycardia and apnea, while dopamine increases blood pressure. Only the results associated with administration of caffeine is reported in the findings since a small number of infants randomized to the intervention group (n = 5) and to the control group (n = 3), received doses of indomethacine in the first days of life.

Phototherapy treatment. The infants' proportion and duration (in minutes) of phototherapy treatment was compared between groups as it meant that infants participating in the study had already worn eye goggles for a determined period and could have influenced their habituation to the material during the study.

Time to harvest the blood sample. The time to harvest the blood sample in minutes was determined using the video recording and compared between groups since the duration of the painful event may have influenced the pain response of infants.

Light and noise levels. Light and noise levels were monitored every hour of the 4-hour study to evaluate if the environmental conditions were different between groups. The readings performed in the cross-over trial were used for the RCT. Noteworthy, four different sound meters were used in the study due to equipment malfunction creating therefore an instrumentation bias in the measurement of noise levels. Since all sound meters were submitted to

calibration before being used, a mean for the noise levels was still reported in the RCT.

Data Analysis

The effect of the intervention on the selected outcomes of pain response for the baseline, blood sample and return to baseline was evaluated with SPSS 14.0 using independent samples *t* tests. Analysis of covariance was used when extraneous variables were significantly correlated with the outcome measures in order to equalize their partial effect on the dependent variables and ensure that pain response was primarily associated with the intervention. Before performing the statistical analysis, all variables were examined for normal distribution and, if they were not normally distributed, they were submitted to a log transformation prior to analysis.

Independent samples *t* tests were also used to compare demographic data (see appendix D) between the intervention and control groups. Gender and type of delivery were compared using a Chi-square analysis. Independent samples *t* test were performed to compare the extraneous variables between the study groups. All statistical analysis was performed with a significance level of 0.05 (two-sided alpha). In two cases data collection could not be performed according to the study protocol, i.e. blood sampling was done at the end of the second study sequence of the cross-over trial instead of the first sequence. Since there was no difference in results excluding or including these infants, the two infants were kept in the final study sample to increase the sample size.

Finally, linear regressions were used to verify the third study hypothesis. Regressions were performed to quantify the association between the mean heart

rate obtained for the physiological stability and the mean heart rate evaluated during the blood sample procedure. Regressions were done for both study conditions, i.e., intervention and control, and were interpreted at a significance level of 0.05 (two-sided alpha).

Research Procedure for the Cross-Over Trial and RCT

The first day of data collection coincided with a prescribed blood procurement performed between 10:00 a.m. and 12:00 p.m., so that the infants would spend four hours in the intervention or control condition before experiencing the heel lance. Phototherapy treatment was terminated at least 24 hours before the start of data collection to comply with the wash-out period established for the cross-over trial. Figure 7 shows the research procedure that was followed for both study designs when the infant was randomized in the

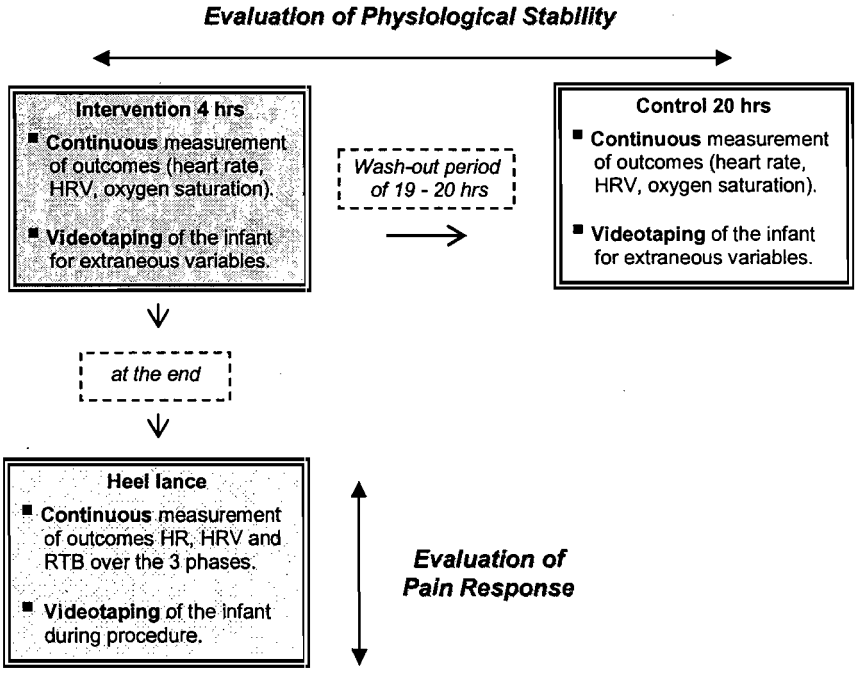

Figure 7. Research procedure for both study designs.

intervention – control sequence for the cross-over trial and in the intervention group for the RCT.

Ethical Considerations

The study was approved by the Institutional Review Board of the Faculty of Medicine of McGill University as well as by the Scientific Research Committees and Research Ethics Boards (REB) of the hospitals where data collection was performed (see appendix E). The admission book and chart review were used to screen eligible preterm infants. Parents were then approached with an information letter and consent form (see appendix F) inviting their preterm infant to participate in the study. The research's purpose and procedure was explained and a sample of the eye goggles and earmuffs was shown to them to allow a visualisation of the intervention. To give parents time to consider their choice, they were approached again one day after the initial contact to seek their decision. The signature of one parent on the consent form was obtained, and a copy of the signed form was returned to the parents. The sealed envelop with the infants group allocation was then opened by the parent. All parents were phoned and informed the day prior to the beginning of data collection and after the completion of the research, a certificate indicating the infant's participation in the study was given to them (see appendix F).

FINDINGS

This chapter presents the study findings. The chapter is divided into three sections. The first section presents the results of the physiological stability for preterm infants evaluated by the cross-over trial, while the second section presents the results of the pain response assessed by the RCT. The last section summarizes the results found for the third hypothesis evaluating if preterm infants showing greater physiological stability during the 4-hour were also showing a reduced pain response at the end of the 4-hour period.

Physiological Stability – Cross-Over Trial

The first hypothesis tested in this study was that preterm infants born between 28 and 32 weeks gestational age show *greater physiological stability* while wearing eye goggles and earmuffs over a 4-hour period than when they are exposed to light and noise in the NICU. A total of 54 preterm infants were included in the analysis of physiological stability. Figure 8 depicts the flow diagram demonstrating the recruitment phases of the preterm infants in the cross-over trial. Fourteen infants were lost after randomization: six were randomized in the intervention then control sequence and 9 in the control then intervention sequence. The reasons for losing these infants are listed in the figure 8.

Sample

Table 2 presents the sample characteristics of the preterm infants included in the analysis for physiological stability. There were no significant

differences between the infants allocated in the intervention-control sequence versus the control-intervention sequence for gestational age, birth weight,

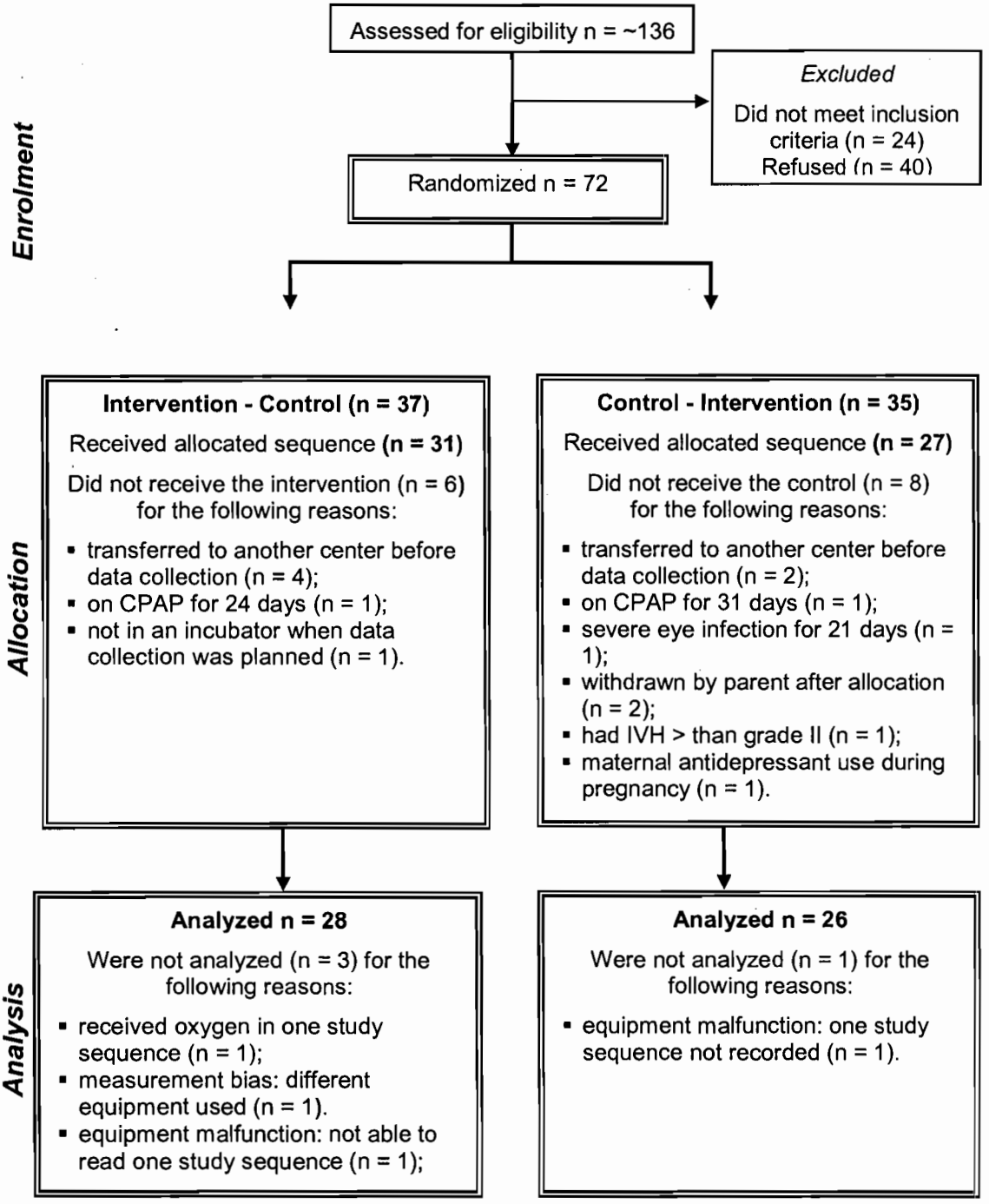

Figure 8. Flow diagram of the recruitment phases for the cross-over trial.

Table 2.

Sample Characteristics of the Cross-Over Trial (N = 54)

	Study Sequences		
	Intervention – Control	Control – Intervention	*p*
Number of preterm infants	28	26	
Gestational age (wks) *M (SD)*	30 5/7 (1.06)	31 (1.09)	.44[a]
Birth weight (gms), *M (SD)*	1465.32 (244.87)	1369.50 (272.00)	.18[a]
Gender (*n*, %)			
girl	13 (46.4)	15 (53.6)	.41[b]
boy	15 (57.7)	11 (42.3)	
Type of delivery (*n, %*)			
vaginal	11 (64.7)	6 (35.3)	.20[b]
caesarean	17 (45.9)	20 (54.1)	
APGAR, *M (SD)*			
1 min	6.64 (2.20)	6.85 (1.89)	.72[a]
5 min	8.14 (1.21)	8.46 (0.81)	.26[a]
10 min	8.46 (1.06)[c]	8.71 (0.75)[c]	.35[a]
Postnatal age (days) at the first period, *M (SD)*	12.75 (4.84)	13.35 (5.04)	.66[a]
Postnatal age (days) at the second period, *M (SD)*	13.75 (4.84)	14.50 (5.05)	.58[a]
Time between study periods (in hrs), *M (SD)*	19 hrs 45 min 7 sec (17 min 54 sec)	23 hrs 32 min 01 sec (14 hrs 25 min 13 sec)	.17[a]

[a] Independent samples *t* test, [b] Chi-Square test, [c] *n* = 24

gender, APGAR at 1, 5 and 10 minutes, type of delivery and the postnatal age of infants at the time of data collection. There were also no significant differences between the demographic characteristics of preterm infants that composed the final sample (N = 54) and those who were lost (n = 14), respectively for gestational age (30 6/7 vs. 30 2/7, $t(66) = 1.15$, $p = .26$), birth weight (1419.19 vs. 1490.43, $t(66) = -0.87$, $p = .39$),. APGAR at 1 min (6.74 vs. 7.21, $t(66) = -0.81$, $p = .42$), and APGAR at 5 min (8.30 vs. 8.43, $t(66) = -0.43$, $p = .67$) (independent samples t tests).

Extraneous Variables

In order to identify potential confounding variables in the cross-over trial, comparisons of the following variables: a) gestational age, b) respiratory support, c) administration of caffeine, d) phototherapy treatment, e) infant position, f) handling as well as g) light and noise levels are first presented to evaluate whether there were significant difference between infants randomized in the intervention – control sequence versus the control – intervention sequence.

Gestational Age

The comparison for gestational age of preterm infants presented in Table 2 shows that there was no significant difference between the study sequence allocations. However, since gestational age is a common confounding variable reported in research conducted with preterm infants, Pearson correlations were calculated between this variable and the outcome measures. In Appendix G, the Tables 3 and 4 show the correlation matrices for the intervention and control periods. Gestational age was only found to be significantly and positively correlated with the standard deviation of normal R-R intervals (SDNN) in the

control period (r = .28, p = .04) (see Table 4 in Appendix G) and was therefore treated as a covariate in the statistical analysis carried out for this outcome.

Respiratory Support

Intubation. Twenty of the 54 infants were intubated in the first days of life and there was no significant difference between the proportion of infants randomized in the intervention – control sequence versus the control – intervention sequence (p = .36) (see Table 5). In addition, there was no significant difference between the mean duration in hours of intubation between infants assigned to begin with the intervention opposed to the control period (p = .21) (see Table 5). Since the sample was composed of almost half the infants who were intubated in the first days of life, independent samples t test were conducted to compare the outcomes measures (HR, HRV, and oxygen saturation) between the 20 intubated infants versus the 34 who were not intubated. For the intervention period, the maximum heart rate was found to be significantly higher (p = .01) and the minimum R-R intervals significantly lower (p = .04) for infants who were intubated following birth versus those who were not intubated (see Table 6 in Appendix H). For the control period, only the mean R-R intervals was found to be significantly lower (p = .03) in intubated versus non-intubated infants (see Table 7 in Appendix H). Intubation, as a categorical variable (yes or no), was then treated as a covariate in the statistical analysis of these outcomes.

CPAP. Forty-two of the 54 infants received CPAP in the first days of life and they were almost equally randomized in the intervention – control sequence versus the control – intervention sequence (see Table 5). There was no

significant difference between the proportions (p = .24) of infants receiving this type of respiratory support and the mean duration in hours they spent on CPAP (p = .64) (see Table 5).

Table 5.

Comparisons of Respiratory Support, Administration of Caffeine, and Phototherapy Treatment between Study Periods in Cross-Over Trial (N = 54)

	Intervention – Control *n* = 28	Control – Intervention *n* = 26		Intervention – Control *n* = 28	Control – Intervention *n* = 26	
	M (SD)	*M (SD)*	*p*	*n (%)*	*n (%)*	*p*
Intubation (in hrs)	36.54 (67.13)	18.12 (31.24)	.21[a]	12 (42.9)	8 (30.8)	.36[b]
CPAP (in hrs)	171.54 (351.17)	130.19 (293.48)	.64[a]	20 (71.4)	22 (84.6)	.24[b]
Caffeine (n of doses)	9.46 (6.05)	10.50 (5.89)	.53[a]	25 (89.3)	23 (88.5)	.92[b]
Phototherapy (in hrs)	52.53 (48.05)	58.95 (51.80)	.64[a]	25 (89.3)	22 (84.6)	.61[b]

[a] Independent Samples *t* test, [b] Chi-square Test

Administration of Caffeine

Forty-eight of the 54 infants received caffeine before data collection and there was no significant difference between the proportions of infants who were randomized in the intervention – control versus the control – intervention sequence (p = .92) (see Table 5). There was also no significant difference found between the mean number of doses administered between both study sequences (p = .53) (see Table 5). In addition, 41 infants out of 54 received caffeine while they were participating in the study; nine infants received a dose

during both the intervention and the control periods while 32 infants received a dose between both study periods (i.e. during wash-out).

Phototherapy Treatment

Forty-seven of the 54 infants received phototherapy treatment before data collection and there was no significant difference between the proportion of infants randomized in the intervention – control sequence versus the control – intervention sequence (p = .61) nor between the mean duration in hours of the treatment between infants assigned to begin with the intervention opposed to the control period (p = .64) (see Table 5).

Position

Table 8 shows that there were no significant differences between the intervention and control periods for the amount of time that preterm infants spent in the prone position (p = .49), supine position (p = .93) and lateral position (p = .74) during the four hours.

Handling

There was no significant difference between the mean duration in minutes of preterm infants' total handling between both study periods (p = .18) (see Table 8), but the number of occasions infants were handled in the intervention period (M = 11.65, SD = 3.88) was significantly higher compared to control period (M = 8.54, SD = 3.35) ($t(53) = 4.86$, p = .00) (paired samples t test). Because of the significant difference between the number of occasions infants were handled between the study periods, Pearson correlations were calculated to evaluate to which extent this variable and the outcome measures were correlated. Since no significant correlations were found between this variable and the outcome

measures for the intervention and control periods (see Table 9 and Table 10 in Appendix G), handling was not treated as a covariate in the statistical analysis.

Table 8.

Comparisons of Position and Handling between Study Periods in Cross-Over Trial (N = 54)

	Intervention Period	Control Period	
	M (SD)	*M (SD)*	*p*
	Position		
Prone	23 min 28 sec (57 min 14 sec)	29 min 33 sec (52 min 58 sec)	.49[a]
Supine	2 hrs 43 min 09 sec (1 hr 27 min 40 sec)	2 hrs 41 min 40 sec (1 hr 21 min 02 sec)	.93[a]
Lateral	1 hr 2 min 35 sec (1 hr 18 min 38 sec)	57 min and 45 sec (1 hr 12 min 40 sec)	.74[a]
	Handling		
Total (in min)	22:48 (14:18)	19:52 (13:38)	.18[a]
Painful procedures (in min)	00:46 (01:20)	00:56 (02:22)	.64[a]
Comfort	03:07 (07:28)	03:03 (09:47)	.96[a]

[a] Paired Samples *t* Test

Of particular interest, the mean time in minutes infants were handled for verification, adjustment and reinstallation of the eye goggles and earmuffs over the 4-hour period was 4 min and 29 sec (*SD* = 06:00, *min* = 00:00, *max* = 25:25). The median time of infants' handling associated with the study material over the

4-hour period was 2 min and 1 sec. Thirteen out of 54 infants lost one or both earmuffs during the intervention and the mean time these 13 infants did not wear the earmuffs was 20 min and 56 sec (*SD* = 14:31, *min* = 0:00 and *max* = 48:50).

The mean time in minutes of handling for painful procedures (IV installation, heel lances and removing tape) was not significantly different between study periods ($p = .64$) (see Table 8). In addition, there was no significant difference between the mean time in minutes of handling for comforting purposes (giving pacifier or caressing infant) between the intervention and control periods ($p = .96$) (see Table 8).

Light and Noise Levels

The mean light intensity ($n = 51$) calculated while the infants were wearing the eye goggles and earmuffs was 50.39 lux (*SD* = 48.13) compared to 69.61 lux (*SD* = 112.53) while they were not wearing them. Although there is a difference of almost 20 lux between both periods for light intensity, the results of the paired samples *t* test analysis indicate that this difference did not reach statistical significance ($t(50) = -1.30$, $p = .20$). For the sound levels, ($n = 49$), the mean computed over the four hours in the intervention was 50.62 dBA (*SD* = 11.56) and almost identical to the mean obtained in the control period (*M* = 52.40 dBA, *SD* = 7.93) with no significant difference detected by paired samples *t* test analysis ($t(48) = -1.25$, $p = .22$).

Outcome Measures

All tables (11 to 23) presenting the descriptive statistics: mean (*M*), standard deviation (*SD*), minimum (*min*) and maximum (*max*) calculated for the outcome measures of physiological stability (heart rate, HRV, and oxygen

saturation) per study sequence (intervention – control or control – intervention) and period (1 or 2) are presented in Appendix I.

Heart Rate

Mean. The mean heart rate calculated for the 54 preterm infants while they wore the eye goggles and the earmuffs in the NICU was 157.72 (*SD* = 9.70, *min* = 141.31 and *max* = 176.19). This was similar to the mean calculated while they were in the control period (*M* = 157.50, *SD* = 9.45, *min* = 136.62 and *max* = 176.79). The results of the repeated measures of ANOVA (RM-ANOVA) showed that there was no carry-over effect ($F(1, 52) = 0.22$, $p = .64$, least-square (LS) means difference[5] = 1.18, *CI* [-6.24 – 3.88]) and there was no significant effect associated with the intervention for the mean heart rate ($F(1, 52) = 0.09$, $p = .76$, LS means difference[6] = 0.22, *CI* [-1.70 – 1.25]) (see Table 24 in Appendix J).

Minimum. The preterm infants' minimum heart rate recorded during the intervention period was 85.52 (*SD* = 19.28, *min* = 48, *max* = 131) compared to 90.07 (*SD* = 19.03, *min* = 51, *max* = 135) while they were in the control period. As indicated by the results of the RM-ANOVA, there was no carry-over effect ($F(1, 52) = 1.26$, $p = .27$, LS means difference = 5.02, *CI* [-14.00 – 3.96] as well as no significant effect associated with the intervention ($F(1, 52) = 3.02$, $p = .09$, LS means difference = -4.64, *CI* [-0.72 – 10.00]) (see Table 25 in appendix J).

Maximum. The preterm infants' maximum heart rate recorded while wearing the goggles and earmuffs was 198.06 (*SD* = 14.03, *min* = 171, *max* = 225) compared to 193.57 (*SD* = 12.76, *min* = 170, *max* = 224) while they were

[5] Least-square (LS) means difference from the model used to test the carry-over effect.
[6] LS means difference from the model used to test the intervention effect.

not wearing them. As indicated by the results of the RM-ANOVA, there was no significant carry-over effect ($F(1,52) = 0.14$, $p = .71$, LS means difference = -1.25, *CI* [-5.34 – 7.84]) (see Table 26 in Appendix J).

As described above, intubation as a categorical variable needed to be treated as a covariate in the statistical model testing the intervention's effect on maximum heart rate. According to Senn (2002), when the covariate corresponds to a single baseline measurement (as is the case with intubation), an analysis of the interaction between the treatment (intervention) and the covariate is the recommended method to verify whether the treatment (intervention) effect varies according to the baseline measurement. If a significant interaction is found, it signifies that the intervention effect varies according to the covariate, and conversely if no significant interaction effect is detected, it means that the covariate has no effect on the intervention. After adding intubation as a covariate in the model, the results of the RM-ANCOVA showed that the intervention effect was significant ($F(1,51) = 9.23$, $p = .004$, LS means difference = 5.18, *CI* [-8.60 – -1.76]) whereas the interaction between the intervention * intubation was not significant ($F(1,51) = 2.44$, $p = .12$) (Table 26 in Appendix J).

Since an intervention effect was found for maximum heart rate, the means obtained for infants who were intubated versus those who were not, were examined in both study periods. This examination was done for exploratory purposes because the interaction between intervention * intubation was statistically non-significant. Figure 9 shown in Appendix K illustrates that for preterm infants who were intubated at birth compared to those who were not, the maximum heart rate was higher in both study periods. Moreover, the mean

difference between intubated infants versus non-intubated infants was greater in the intervention period compared to the control period (see Figure 9, Appendix K).

HRV

Mean R-R intervals. The mean R-R intervals while the preterm infants were in the intervention period was 382.57 ms (*SD* = 22.71, *min* = 339.15 and *max* = 425.96) which is almost identical to the mean obtained in the control period (*M* = 381.90 ms, *SD* = 23.20, *min* = 338.71 and *max* = 437.32). No significant effect was found for carry-over (RM-ANOVA) ($F(1, 52) = 0.50$, $p = .48$, LS means difference = -4.29, *CI* [-7.84 – 16.41]) (see Table 27 in Appendix J). No significant effect was found for the intervention after adding intubation as a covariate in the model ($F(1, 51) = 0.28$, $p = .60$, LS means difference = .96, *CI* [-4.58 – 2.66]) in addition to no significant effect for the interaction between intervention and intubation ($F(1, 51) = 0.49$, $p = .49$) (RM-ANCOVA) (see Table 27 in Appendix J).

SDNN. There was a significant carry-over effect found for the standard deviation of all R-R intervals (SDNN) when the analyses were performed with the complete sample (*N* = 54) (RM-ANOVA) ($F(1, 52) = 4.66$, $p = .04$, LS means difference = -3.16, *CI* [0.22 – 6.26]) compared to when the analyses were done according to the study protocol, i.e., four infants whose data were collected not respecting the study protocol were removed from the sample (see data analysis in cross-over trial section, p. 92) ($F(1, 50) = 3.78$, $p = .06$, LS means difference = -2.93, *CI* [-0.10 – 5.95]) (see Table 28, Appendix J). Therefore, the statistical analysis testing the intervention effect for SDNN was conducted according to the

study protocol with a sample of 50 infants. The SDNN calculated for the preterm infants while they wore the eye goggles and earmuffs in the NICU was 19.59 ms (*SD* = 6.22, *min* = 9.02 and *max* = 37.19). This was similar to the mean calculated while they were in the control period (*M* = 19.47 ms, *SD* = 5.98, *min* = 8.39 and *max* = 37.01). After adding gestational age as a covariate in the statistical model, the results of the RM-ANCOVA demonstrated that there was no significant effect for the intervention ($F(1, 25) = 0.77$, $p = .39$, LS means difference = 0.65, *CI* [-2.19 – 0.88]) nor for the interaction between intervention and gestational age ($F(1, 25) = 1.06$, $p = .44$) (see Table 28, Appendix J).

Minimum R-R intervals. The minimum R-R intervals for preterm infants wearing the eye goggles and earmuffs was 338.02 ms (*SD* = 20.44, *min* = 298.79 and *max* = 377.29) while the mean obtained while they were not wearing them was 337.88 ms (*SD* = 20.00, *min* = 299.04 and *max* = 384.60). The Table 29 in Appendix J shows that there was no significant carry-over effect (RM-ANOVA) ($F(1, 52) = 0.07$, $p = .80$, LS means difference = 1.34, *CI* [-11.85 – 9.17]). Furthermore, according to the results of the RM-ANCOVA, there were no significant intervention effect $F(1, 51) = 0.02$, $p = .88$, LS means difference = -.30, *CI* [-3.58 – 4.19]) and no interaction effect (intervention * intubation) ($F(1, 51) = 0.68$, $p = .41$) (RM-ANCOVA) (see Table 29 in Appendix J).

Maximum R-R intervals. The preterm infants' maximum R-R intervals in the intervention period was 483.06 ms (*SD* = 48.71, *min* = 389.10 and *max* = 610.69) while the mean obtained in the control period was 476.64 ms (*SD* = 49.02, *min* = 390.42 and *max* = 598.67). The results of the repeated measures ANOVA demonstrates that there was no significant carry-over effect ($F(1, 52) =$

3.02, p = .09, LS means difference = -20.88, *CI* [-3.22 – 44.98]), and that there was no significant effect associated with the intervention for that variable (F(1, 52) = 1.57, p = .22, LS means difference = 6.58, *CI* [-17.12 – 3.97]) (see Table 30, Appendix J).

LF. The low-frequency did not follow a normal distribution, therefore a logarithmic transformation was performed before conducting statistical analysis for this variable. To facilitate the interpretation of the values obtained, the means and standard deviations calculated for the LF before and after the logarithmic transformation are presented in Appendix I (see Table 18).

Following the logarithmic transformation and according to the results of the RM-ANOVA, there was a significant carry-over effect for the LF (F(1, 52) = 6.97, p = .01, LS means difference = -0.44, *CI* [0.11 – 0.77]). Therefore, only the first study period (intervention or control) was included in the statistical analysis to evaluate the effect of the intervention on this variable. According to the results of the independent samples t test, there was no significant difference between the means calculated for the intervention (M = 4.46 ms^2, SD = 0.75) and the control period (M = 4.80 ms^2, SD = 0.71) for the LF (t(52) = -1.73, p = .09, means difference = -0.34, *CI* [-0.74 – 0.05) (see Table 31 in Appendix J).

HF. The high-frequency did not follow a normal distribution and was also submitted to a logarithmic transformation. The means and standard deviations calculated for the HF power before and after the logarithm transformation are shown in Table 19 in Appendix I. There was also a significant carry-over effect calculated from the first stage of the RM-ANOVA following the logarithm transformation (F(1, 52) = 6.50, p = .01, LS means difference = -0.56, *CI* [0.12 –

0.99]). The intervention effect was therefore calculated only for the first period of the study. The findings of the independent samples *t* test indicate that there was a significant difference between the means computed for the intervention and control periods ($t(52) = -2.01$, $p = .048$, means difference = -0.49, *CI* [-0.97 – 0.00]) revealing that the HF was lower in the intervention period ($M = 2.69$ ms^2, $SD = 1.00$) compared to the control period ($M = 3.17$ ms^2, $SD = 0.76$) (see Table 32 in appendix J).

Since only the first study sequence was analyzed for both LF and HF, it was worth exploring the correlations between documented HRV confounding variables such as gestational age and postnatal age (Longin, Schaible, Lenz, & König, 2005; Rosenstock et al., 1999) and these outcomes. According to the correlation matrix showed in Table 33 in Appendix G there were no significant correlations between these potential confounding variables and these outcomes. Therefore, gestational age and postnatal age were not treated as covariates in the statistical analysis of LF and HF.

LF/HF ratio. The mean LH/HF ratio computed for the 54 preterm infants while wearing the eye goggles and the earmuffs in the NICU was 8.39 (SD = 3.34, *min* = 3.77 and *max* = 17.23), and was almost the same as the mean obtained while they were in the control period ($M = 8.54$, $SD = 3.49$, *min* = 3.52 and *max* = 17.25). According to the findings of the RM-ANOVA, there was no significant carry-over effect for the LH/HF ratio ($F(1, 52) = 2.51$, $p = .12$, LS means difference = 1.40, *CI* [-3.17 – 0.37]), and no significant effect for the intervention ($F(1, 52) = -0.31$, $p = .58$, LS means difference = -0.14, *CI* [-0.37 – 0.65]) (see Table 34 in Appendix J).

Oxygen Saturation

Mean. The preterm infants' mean oxygen saturation while they were in the intervention period was $M = 94.00$ ($SD = 3.24$, *min* = 83.34 and *max* = 98.35), which is similar to the one calculated in the control period ($M = 93.57$, $SD = 3.59$, *min* = 81.34 and *max* = 98.20). Table 35 in Appendix J shows that there was no carry-over effect ($F(1, 52) = 1.53$, $p = .22$, LS means difference = -1.05, *CI* [-0.65 – 2.75]) nor any significant intervention effect ($F(1, 52) = 1.21$, $p = .28$, LS means difference = -0.41, *CI* [-1.15 – 0.34]) for the oxygen saturation.

Minimum. There was a significant carry-over effect found for the minimum oxygen saturation when the analyses were performed according to the study protocol, i.e. when four infants whose data were collected not respecting the study protocol were removed from the sample (see data analysis in cross-over trial section, p. 92). To determine if the four infants should still be included in the final sample for analysis, a comparison between the first study sequences (intervention vs. control) was performed with the two infants included and excluded from the analysis. Since there was no significant difference for this variable between the intervention and control periods when the infants were included in the independent samples *t* test analysis ($t(52) = -1.32$, $p = .19$) and excluded from the analysis ($t(48) = -1.54$, $p = .13$), repeated measures ANOVA considering all 54 infants were carried out for the minimum oxygen saturation.

The preterm infants' minimum oxygen saturation while they were wearing the eye goggles and earmuffs was 65.70 ($SD = 12.72$, *min* = 50 and *max* = 86) while the mean obtained in the control period was 64.93 ($SD = 12.71$, *min* = 50 and *max* = 92). Table 36 in Appendix J demonstrates that there was no

significant carry-over effect ($F(1, 52) = 3.23$, $p = .08$, LS means difference = -5.36, *CI* [-0.62 – 11.35]) and no significant intervention effect for the minimum oxygen saturation ($F(1, 52) = 0.25$, $p = .62$, LS means difference = 0.82, *CI* [-4.14 – 2.50]).

Maximum. The maximum oxygen saturation level computed for the 54 preterm infants while they were in the intervention period was 99.54 (*SD* = 1.13, *min* = 93 and *max* = 100), and was almost the same as the mean obtained while they were in the control period (*M* = 99.50, *SD* = 1.09, *min* = 95 and *max* = 100). According to the results of the RM-ANOVA showed in Table 37 in Appendix J, there was no significant carry-over effect for the minimum oxygen saturation ($F(1, 52) = 1.10$, $p = .30$, LS means difference = 0.30, *CI* [-0.86 – 0.27]) nor any significant effect for the intervention ($F(1, 52) = 0.07$, $p = .79$, LS means difference = 0.03, *CI* [-0.24 – 0.18]).

Summary of Findings for Physiological Stability

In summary, preterm infants wearing eye goggles and earmuffs had significantly higher maximum heart rate and lower HF compared to when they were not wearing the material. For all other outcome measures, there were no significant differences between the intervention and control periods. Table 38 presents a summary of findings for the outcomes measures of physiological stability.

Intubation was treated as a covariate in the statistical analysis of maximum heart rate, mean R-R intervals, and minimum R-R intervals and a significant

Table 38.

Summary of Findings for the Outcomes Measures of Physiological Stability (N = 54)

	Intervention Period	Control Period	
Outcome Measures	*M (SD)*	*M (SD)*	*p*
Heart Rate			
mean (bpm)	157.72 (9.70)	157.50 (9.45)	.76[a]
min (bpm)	85.82 (19.28)	90.07 (19.03)	.09[a]
max (bpm)	198.06 (14.03)	193.57 (12.76)	.004[b**]
HRV			
Mean R-R intervals (ms)	382.57 (22.71)	381.90 (23.20)	.60[b]
SDNN (ms)	19.59 (6.22)	19.47 (5.98)	.39[c]
Min R-R intervals (ms)	338.02 (20.44)	337.88 (20.00)	.88[b]
Max R-R intervals (ms)	483.06 (48.71)	476.64 (49.02)	.22[a]
Log LF[d] (ms^2)	4.46 (0.75)	4.80 (0.71)	.09[e]
Log HF[f] (ms^2)	2.69 (1.00)	3.17 (0.76)	.0498[e*]
Ratio LF/HF	8.39 (3.34)	8.54 (3.49)	.58[c]
Oxygen Saturation			
mean (%)	94.00 (3.24)	93.57 (3.59)	.28[c]
min (%)	65.70 (12.72)	64.93 (12.71)	.62[c]
max (%)	99.54 (1.13)	99.50 (1.09)	.79[c]

[a] RM-ANOVA, [b] RM-ANCOVA with intubation as covariate, [c] RM-ANCOVA with gestational age as covariate, [d] logarithm of high-frequency (HF), [e] Independent samples *t* test with only with first study sequence, [f] logarithm of low-frequency (LF), * $p < .05$, ** $p < .01$.

intervention effect was found only for maximum heart rate. Exploratory analysis revealed that infants who were intubated after birth had higher maximum heart rate in both study periods compared to those who were not intubated. Furthermore, the mean difference for maximum heart rate of intubated infants versus non-intubated infants was greater in the intervention period than in the control period. No significant effect was found for the intervention after adding gestational age as a covariate in the statistical model of SDNN. The number of occasions preterm infants were handled during data collection was significantly higher in the intervention period compared to the control period. This variable was not treated as a covariate in the statistical analysis as it was not found to be significantly correlated with any of the outcome measures. In addition, gestational age and postnatal age, which are identified as potential confounders for HRV, were not significantly correlated with LF and HF and were not treated as covariates in the statistical analysis for these variables.

Pain Response – RCT

The second hypothesis tested in this study was that preterm infants born between 28 and 32 weeks of gestational age would show a *reduced pain response* during heel lance at the end of a 4-hour period where they wore eye goggles and earmuffs, compared to when they are not protected from light and noise. A total number of 44 preterm infants were analyzed for the pain response. A diagram on the recruitment phases of the RCT can be seen in Figure 10. The same fourteen infants as in the cross-over trial were lost after randomization. In addition, fourteen infants were not included in the final analysis for the pain response: eight were randomized in the intervention group and six in the control

group. The reasons for excluding these infants from the analysis are listed in Figure 10.

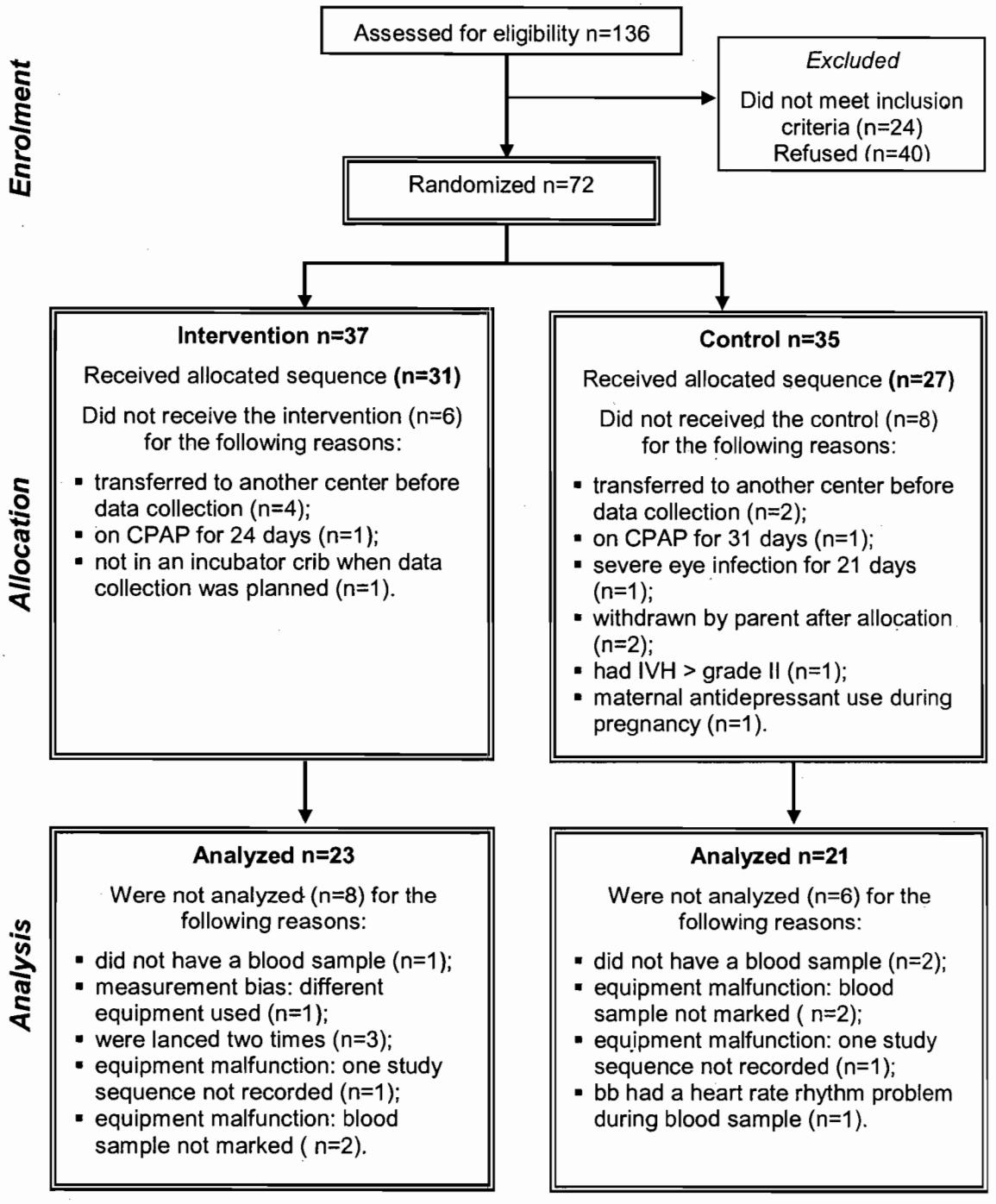

Figure 10. Flow diagram of the recruitment phases for the RCT.

Sample

The sample characteristics of the preterm infants analyzed for pain response in the RCT are seen in Table 39. There were no significant differences between the preterm infants randomized to the intervention versus the control group for gestational age, birth weight, gender, APGAR at 1, 5 and 10 minutes, type of delivery, and postnatal age at the time of data collection.

Table 39.

Sample Characteristics of the RCT (N = 44)

	Groups		
	Intervention	Control	*p*
Number of preterm infants	23	21	
Gestational age (wks) at birth, *M (SD)*	30 4/7 (1.05)	31 1/7 (1.12)	.26[a]
Birth weight (gms), *M (SD)*	1496.96 (229.34)	1390.38 (280.82)	.17[a]
Gender (*n*, %)			
girl	11 (47.8)	13 (61.9)	.35[b]
boy	12 (52.2)	8 (38.1)	
Type of delivery (*n, %*)			
vaginal	7 (30.4)	5 (23.8)	
caesarean	16 (69.6)	16 (76.2)	.62[b]
APGAR, *M (SD)*			
1 min	6.35 (2.21)	7.05 (1.72)	.25[b]
5 min	8.04 (1.26)	8.57 (0.75)	.10[b]
10 min	8.41 (1.10)[c]	8.84 (0.69)[c]	.15[b]
Postnatal age (days) at data collection, *M (SD)*	12.13 (4.53)	12.81 (5.37)	.65[a]

[a] Independent samples *t* test, [b] Chi-Square test, [c] *n* = 41

There were also no significant differences between the sample characteristics of preterm infants who composed the final sample (n = 44) and those who were lost (n =14), respectively for gestational age (30 6/7 vs. 30 2/7, $t(56) = 1.22$, $p = .23$), birth weight (1446.09 vs. 1490.43, $t(56) = -.53$, $p = .60$), APGAR at 1 min (6.68 vs. 7.21, $t(56) = -.91$, $p = .37$) and 5 min (8.30 vs. 8.43, $t(56) = -.41$, $p = .68$) (independent samples t test).

For preterm infants randomized in the intervention group (n = 23), the mean time they spent wearing the eye goggles and earmuffs before undergoing the painful procedure was 4:04:00 (*SD* = 00:09:35, *min* = 03:51:22, *max* = 04:34:13). Six infants out of the 23 lost one or both earmuffs during the 4-hour of intervention and the mean time these six infants did not wear the earmuffs was 00:24:49 (*SD* = 00:18:13, *min* = 00:04:19, *max* = 00:48:50).

Extraneous Variables

The first part of this section presents the findings of potentially confounding variables that were examined in the RCT: a) gestational age, b) postnatal age, c) previous number of invasive procedures, d) handling, e) respiratory support, f) administration of caffeine, g) phototherapy treatment, h) time to harvest the blood sample, and i) light and noise levels by comparing them between the intervention and control groups. The second part presents the correlation between these extraneous variables in order to evaluate the presence of multicollinearity and identify redundant variables. The last part presents the correlations between the extraneous variables and the outcomes measures to identify which ones should be treated as covariates for the second phase of the painful procedure, i.e. blood sample.

Comparisons of Extraneous Variables between Groups

The results for gestational age and postnatal age are presented in the sample characteristics (see Table 39). There were no significant differences between the intervention and the control group for these variables.

Previous invasive procedures. There was no significant difference between the total number of previous invasive procedures (IV installations and heel lances) performed from birth to the day of data collection for infants randomized to the intervention versus the control group (p = .60) (see Table 40).

Handling. There was no significant difference for the mean duration of handling in the 4-hours preceding the blood sample between both study groups (p = .99) (see Table 40). However, results of independent samples t test analysis showed that infants randomized in the intervention group were handled more frequently (or on more occasions) (M = 12.26, SD = 4.04) compared to infants in the control group (M = 9.24, SD = 2.34) ($t(42) = 2.99$, p = .01).

Respiratory support. Sixteen infants out of 44 were intubated in the first days of life and the proportion of infants randomized to the intervention group was significantly higher than the proportion of infants randomized to the control group (p = .02) (see Table 40). In addition, there was a significant difference in the mean duration in hours of intubation where infants assigned to the intervention had more hours of intubation than infants in the control condition (p = .03) (see Table 40).

Thirty-five of the 44 infants received CPAP before data collection and these were equally distributed between the intervention and control groups with

Table 40.

Comparisons of Extraneous Variables between Groups in RCT (N = 44)

	Interventio n (*n* = 23)	Control (*n* = 21)		Intervention (*n* = 23)	Control (*n* = 21)	
	M (SD)	*M (SD)*	*p*	*n (%)*	*n (%)*	*p*
Previous painful procedures (n)	28.57 (11.34)	26.71 (11.92)	.60[a]	---	---	---
Total handling (in min)	23:16 (16:19)	23:19 (18:02)	.99[a]	---	---	---
Intubation (in hrs)	44.48 (71.84)	8.71 (20.76)	.03*[a]	12 (52.2)	4 (19.0)	.02*[b]
CPAP (in hrs)	138.17 (335.19)	54.95 (62.86)	.27[a]	17 (73.9)	18 (85.7)	.33[b]
Caffeine (n of doses)	10.30 (7.15)	9.81 (5.62)	.80[a]	20 (87.0)	18 (85.7)	.91[b]
Phototherapy (in hrs)	48.99 (45.00)	57.31 (55.89)	.59[a]	20 (87.0)	17 (81.0)	.59[b]
Time to harvest the blood sample (in min)	02:43 (01:14)	02:14 (00:54)	.15[a]	---	---	---

[a] Independent Samples *t* test, [b] Chi-square Test

no significant differences between the proportions (p = .33) and the mean time in hours they spent on CPAP (p = .27) (see Table 40).

Administration of caffeine. Thirty-eight of the 44 infants received a dose of caffeine before data collection and there was no significant difference between the proportion of infants in the intervention versus the control condition (p = .91)

(see Table 40). There was also no significant difference found between the mean number of doses administered between both study groups (p = .80) (see Table 40).

Phototherapy treatment. Thirty-seven of the 44 infants received phototherapy treatment before data collection and there was no significant difference between the proportion of infants randomized in the intervention group versus the control group (p = .59). In addition there was no significant difference between the mean duration in hours of the treatment between infants assigned to the intervention group as opposed to the control group (p = .59) (see Table 40).

Time to harvest the blood sample. Table 40 shows that the mean time to harvest the blood was longer in the intervention group compared to the control group but the findings of the independent samples t test indicate that this difference was not significant (p = .15).

Light and noise levels. The light and noise levels in the NICU were compared between groups to evaluate if preterm infants were exposed to the same environmental conditions in both groups. The mean for the light intensity calculated in the NICU for infants wearing the eye goggles and earmuffs (n = 22) was 46.86 lux (SD = 44.53) compared to 54.33 lux (SD = 41.59) for the infants in the control group (n = 21). The results of the independent samples t test showed that there was no significant difference for light intensity between both groups ($t(41) = -0.57$, p = .57). For the sound levels, the means computed over the four hours was almost identical in the intervention (n = 21) (M = 48.34 dBA, SD = 2.85) and the control condition (n = 20) (M = 51.89 dBA, SD = 8.68) with no

significant difference detected by independent samples *t* test analysis ($t(39) = -1.78$, $p = .08$).

Correlations between Extraneous Variables

Pearson's correlations were calculated between the extraneous variables to further evaluate their potential effect on the outcome measures during the blood sample. The continuous variables included in the correlation matrix were: a) gestational age, b) postnatal age, c) previous painful procedures, d) number of occasions of preterm infants' handling preceding heel lance, e) mean duration of intubation and f) time to harvest the blood. The mean duration in hours of intubation was included in the correlation matrix instead of the number of infants receiving this type of respiratory support as this is more indicative of physiological stability. The correlation matrix of the extraneous variables can be found in Table 41 in Appendix G. Since gestational age was highly correlated with postnatal age ($r = .77$, $p = .00$) and number of previous painful procedures ($r = -.38$, $p = .01$), only gestational age was kept as a potential confound among the three variables since it has been commonly reported as a covariate in pain research with preterm infants, making comparison among other studies possible.

Correlations between Extraneous Variables and Outcome Measures

The remaining potential confounders, i.e. gestational age, number of occasions of handling, duration of intubation, and time to harvest the blood were examined in relation to the outcome measures of the blood sample as well as return to baseline. The Table 42, showing the correlation matrix of these variables, can be found in Appendix G. Since the number of occasions the preterm infants were handled was negatively correlated with mean heart rate ($r =$

-.38, p = .01) and maximum heart rate (r = -.33, p = .03), and positively correlated with minimum R-R intervals (r = .31, p = .046) during the blood harvesting, this variable was treated as a covariate in the statistical analysis of these outcome measures. In addition, even if time to harvest the blood sample was only significantly correlated with maximum R-R intervals (r = .33, p = .03), it was still treated as a covariate in the statistical model of all outcome measures for pain. The goal of this statistical procedure was to account for differences associated with the procedures' differing lengths of time.

Outcome Measures

The findings are presented for each outcome measures of pain response according to the three phases of the painful procedure: baseline, blood sample, and return to baseline.

Baseline – Heart Rate

Mean. The mean heart rate during baseline for the infants randomized to the intervention group was 162.30 (*SD* = 14.59, *min* = 131, *max* = 194) compared to 156.57 (*SD* = 12.84, *min* = 132, *max* = 180) for the ones in the control group. The results of the independent samples *t* test indicated that there was no significant difference for the mean heart rate between groups ($t(42) = 1.38$, p = .18, mean difference = 5.73, *CI* [-2.66– 14.13]).

Maximum. The maximum heart rate computed for infants wearing the eye goggles and earmuffs in baseline was 171.61 ms (*SD* = 16.86, *min* = 135, *max* = 204) and was almost similar to the mean calculated for infants in the control group (*M* = 171.38 ms, *SD* = 13.77, *min* = 149, *max* = 202). According to independent *t* test analysis, there was no significant difference between groups

for the mean maximum heart rate ($t(42) = 0.05$, p = .96, mean difference = 0.23, *CI* [-9.19 – 9.65]).

Baseline – HRV

Minimum R-R intervals. The minimum R-R intervals during baseline for infants in the intervention group was 350.91 ms (*SD* = 36.07, *min* = 292, *max* = 441), which is almost identical to the mean computed in the control group (*M* = 354.84 ms, *SD* = 25.48, *min* = 316, *max* = 402). The findings of independent samples *t* test revealed that there was no significant difference between groups for minimum R-R intervals ($t(40) = -.40$, p = .69, mean difference = -3.93, *CI* [-23.82 – 15.96]).

Maximum R-R intervals. The maximum R-R intervals calculated during baseline for the infants wearing the eye goggles and earmuffs was 408.78 ms (*SD* = 39.34, *min* = 324, *max* = 484) compared to 439.81 ms (*SD* = 63.83, *min* = 367, *max* = 601) for the ones in the control group. The results of the independent samples t test indicated that there was no significant difference for the maximum R-R intervals between groups ($t(42) = -1.96$, p = .06, mean difference = -31.03, CI [-62.97 - 0.92]).

Overall, there were no significant differences between the groups for any of the outcome measures at baseline. However, for infants wearing eye goggles and earmuffs, baseline measures were taken at the end of the four-hour period and just before the painful procedure so the extent to which these outcomes were influenced by the intervention is unknown. In addition, there were 16 preterm infants (nine in intervention group and seven in control group) who were handled within ten minutes prior to the beginning of the procedure. Comparisons between

infants who were handled and not handled in each group revealed that there were significant differences for heart rate and maximum R-R intervals in baseline for the control group (see Table 43 and 44 in appendix L). For these two reasons, baseline measures were treated as covariates in the statistical analysis for the blood sample to equalize their potential effect on the outcome measures.

Blood Sample – Heart Rate

Mean. The mean heart rate calculated during the blood sample for the preterm infants wearing the eye goggles and earmuffs was 181.82 (*SD* = 16.03, *min* = 152, *max* = 216) compared to 181.19 (*SD* = 13.80, *min* = 146, *max* = 204) in the control condition. According to the analysis of covariance (ANCOVA), there was no significant difference between groups after adjusting for mean heart rate at baseline, number of occasions of handling, and time to harvest the blood ($F(1, 38) = 0.23$, $p = .63$, LS means difference[7] = 2.23, *CI* [-7.15 – 11.61]) (see Table 45 in Appendix M).

Maximum. The maximum heart rate calculated during the blood sampling for the infants wearing the eye goggles and the earmuffs was 195.09 (*SD* = 18.94, *min* = 172, *max* = 243) compared to 198.52 (*SD* = 19.86, *min* = 158, *max* = 247) for the ones in the control group. The results of the ANCOVA indicate that there was no significant difference between groups after adjusting for maximum heart rate at baseline, number of occasions of handling, and time to harvest the blood ($F(1, 38) = 0.14$, $p = .72$, LS means difference = -2.04, *CI* [-13.25 – 9.17]) (see Table 46 in Appendix M).

---

[7] LS means difference from the model used to test the intervention effect with covariates.

Blood Sample – HRV

Minimum R-R intervals. The minimum R-R intervals computed during the blood sampling for the preterm infants in the intervention group was 306.95 ms (*SD* = 27.12, *min* = 246, *max* = 347) compared to 303.81 ms (*SD* = 30.25, *min* = 242, *max* = 378) for the infants in the control group. According to the ANCOVA, there was no significant difference between groups after adjusting for minimum R-R intervals at baseline, number of occasions of handling, and time to harvest the blood ($F(1, 35) = 0.04$, $p = .85$, LS means difference = 1.68, *CI* [-16.01 – 19.36]) (see Table 47 in Appendix M).

Maximum R-R intervals. The maximum R-R intervals did not follow a normal distribution; therefore a logarithm transformation was performed before analyzing this variable. The means and standard deviations calculated for the maximum R-R intervals before and after the logarithm transformation are shown in Table 48 in Appendix N. Following the logarithm transformation, the maximum R-R intervals calculated during the blood sample for the preterm infants in the intervention group was 5.94 (*SD* = 0.17, *min* = 5.77, *max* = 6.57) compared to 5.95 (*SD* = 0.10, *min* = 5.79, *max* = 6.12) for the control group. The results of the ANCOVA indicate that there was no significant difference between groups after adjusting for maximum R-R intervals at baseline and time to harvest the blood ($F(1, 38) = 0.23$, $p = .64$, LS means difference = -0.02, *CI* [-0.11 – 0.07]) (see Table 49 in Appendix M).

Return to Baseline

The mean time to return to baseline for infants in the intervention group was 00:01:23 (*SD* = 00:01:09, *min* = 00:00:01, *max* = 00:04:12), which was 19

seconds longer than the mean computed in the control group (*M* = 00:01:04, *SD* = 00:00:47, *min* = 00:00:11, *max* = 00:03:12). The findings of the ANCOVA indicate that there was no significant difference between groups after adjusting for time to harvest the blood ($F(1, 38) = 0.97$, $p = .33$, LS means difference = -00:00:19 [-00:00:20 – 00:00:59]) (see Table 50 in Appendix M).

Summary of Findings for Pain Response

In summary, there was no significant difference for any of the outcomes measuring pain response (heart rate, HRV, and return to baseline) between infants who wore eye goggles and earmuffs for a 4-hour period preceding the blood sample as compared to those who were exposed to light and noise in the NICU (see summary of findings in Table 51). All baseline measures were treated as covariates in the statistical analysis of the outcomes evaluated during the blood sample procedure.

Some variables that were identified as potential confounders, i.e., gestational age, postnatal age, previous painful procedures, mean duration of intubation, and number of doses of caffeine, were not treated as covariates in the statistical analysis because they were not found to be significantly correlated with any of the outcome measures.

However, the number of occasions the preterm infants were handled was treated as a covariate in the statistical analysis of mean heart rate, maximum heart rate, and minimum R-R intervals during the blood sample procedure. In addition, time to harvest the blood sample was treated as a covariate in the analysis of all outcome measures to account for differences associated with the

Table 51.

Summary of Findings for the Outcomes Measures of Pain Response during Blood Sample and Return to Baseline (N = 44)

	Intervention (n = 23)	Control (n = 21)	
Outcome Measures	*M (SD)*	*M (SD)*	*p*
Blood sample			
Heart Rate			
mean (bpm)	181.82 (16.03)	181.19 (13.80)	.63[a]
max (bpm)	195.09 (18.94)	198.52 (19.86)	.72[a]
HRV			
Min R-R intervals (ms)	306.95 (27.12)	303.81 (30.25)	.85[a]
Max R-R intervals (ms)[b]	5.94 (0.17)	5.95 (0.10)	.64[c]
Return to baseline (min)	00:01:23 (00:01:09)	00:01:04 (00:00:47)	.33[d]

[a] ANCOVA with baseline, number of occasions of handling, and time to harvest the blood as covariates, [b] logarithm of maximum of R-R intervals, [c] ANCOVA with baseline and time to harvest the blood as covariates, [d] ANCOVA with time to harvest the blood as covariate.

procedures' differing lengths of time. No significant difference was found between the study groups for these variables after adding these covariates in the statistical analysis.

Physiological Stability and Pain Response

The third hypothesis tested in this study was that preterm infants born between 28 and 32 weeks of gestational age who showed *greater physiological*

stability while wearing eye goggles and earmuffs for a 4-hour period, would also show a *reduced pain response* during the painful procedure at the end of the 4-hour period. Linear regressions were performed to quantify the relationship between the mean heart rate obtained for physiological stability and the mean heart rate evaluated during the blood sample for both study conditions, i.e., intervention and control.

For the intervention condition (n = 16), results of the linear regression analysis revealed that mean heart rate measured during the 4-hour period of the cross-over trial was a significant predictor of mean heart rate calculated during the blood sample in the RCT (b = .99, SE = .36, p = .02), accounting for 34.4% of the variance in mean heart rate. Conversely, under the control condition (n = 17), linear regression indicated that mean heart rate of physiological stability was not a significant predictor of mean heart rate during the painful procedure (b = .33, SE = .44, R^2 = .04, p = .47). These findings revealed that the mean heart rate of physiological stability did predict the mean heart rate during the blood sample procedure only when the preterm infants were wearing eye goggles and earmuffs.

DISCUSSION

This chapter presents a discussion of the research findings and is separated into six sections. The first section presents the discussion related to the findings of physiological stability measured by the cross-over trial of eye goggles and earmuffs over a 4-hour period, while the second section discusses the findings for pain response evaluated by the RCT of those sensory interventions. The third section links the components of the study framework with the findings including results of the relationship between physiological stability and pain response outcomes which is the third hypothesis. Research strengths and limitations are presented in the fourth section. Implications for clinical practice and research conclude the chapter in the fifth and sixth sections.

Physiological Stability

Results of this study show that physiological stability of preterm infants was not improved when they were wearing eye goggles and earmuffs for a 4-hour period in the NICU. The first study hypothesis was therefore not supported. There were no significant differences between the study periods for outcomes measuring physiological stability except for maximum heart rate and high-frequency (HF), and those were not in the expected direction. The findings revealed that preterm infants had higher maximum heart rate and lower HF power while they were wearing the eye goggles and earmuffs whereas it had been predicted that their maximum heart rate would be lower and their HF power higher in the intervention period in comparison with the control period.

The findings of this study suggest that preterm infants had more stress responses when they were wearing eye goggles and earmuffs than when they

were not wearing the material. Indeed, an increase in heart rate reflects a stress response by the autonomic nervous system (Anand, 1993; Bowden et al., 2000; Modrcin-McCarthy et al., 1997) while a lower HF power signifies a withdrawal of the parasympathetic nervous system on the heart and may also represent stress (Porges, 1992). There are several possible explanations for these findings related to physiological stability which can be associated with preterm infants handling, sensory stimulation, implications of other sensory systems, cardiac autonomic responses, and other potential confounders of HRV. In addition, the timing of the intervention and the measurement of other outcomes are subsequently discussed in relation to the study design.

Factors Related to Physiological Stability

Handling

The higher maximum heart rate and lowered HF power may be associated with the effect of handling the preterm infants during the 4-hour of the study sequences. Handling was permitted during the study periods so as not to modify the care environment. There was no significant difference between the duration of handling between the study periods, but the number of instances when infants were handled in the intervention period was found to be significantly higher than during the control period. Even if handling was not significantly correlated with any of the outcome measures, it is still possible that it has created more stress in preterm infants wearing the eye goggles and earmuffs.

The purpose of this study was to promote the physiological stability of 28 to 32 week gestational age preterm infants by reducing their exposure to light and noise in the NICU acknowledged as a developmental care intervention (Bowden

et al. 2000; Byers, 2003; Holditch-Davis et al., 2003; Pressler, Turnage-Carrier, & Kenner, 2004; Symington & Pinelli, 2006). However, controlling tactile stimulation by reducing disruptions and handling in the NICU is also identified as a stress reducing developmental care intervention (Bowden et al., 2000; Byers, 2003; Symington & Pinelli; Peters, 1999). As the sensory tactile system is the first to develop in fetal life (Graven, 2000; Vanhatalo & van Nieuwenhuizen, 2000; White-Traut et al., 1994), this system is functional and reactive to tactile stimulation following preterm birth. Handling is identified as creating adverse physiological reactions in preterm infants (Harrison, 1997; Long, Philip, & Lucey, 1980; Peters) and, more precisely, handling associated with nursing interventions has been found to clinically provoke acute heart rate increases in 16% of preterm infants (Zahr & Balian, 1995). Consequently, handling in the intervention period could have created physiological instability in infants which was counterproductive to the potentially beneficial effect of reducing their exposure to light and noise in the NICU environment.

The findings of this study also indicate that handling might have interfered with the physiological stability of preterm infants who were intubated in the first days following birth, particularly if they were wearing eye goggles and earmuffs. Accordingly, it was found that infants intubated at birth had higher maximum heart rates in both intervention and control periods. More importantly, the difference between the maximum heart rate of infants who had been intubated versus those who had not was greater when they were wearing the eye goggles and earmuffs compared to when they were not wearing the material. An explanation for this finding could be that preterm infants necessitating mechanical

ventilation at birth are generally more physiologically unstable and consequently were probably more vulnerable to the effect of handling during the intervention period.

Compared to the control period, the higher incidence of preterm infant handling in the intervention period may be associated with the material used in this study. Eye goggles needed to be repositioned or replaced on infants during the 4-hour study period, and because of strapping around the head, they were not easy to properly put back on without disturbing the infant. The displacement of eye goggles may have resulted from infants' head movements and/or while they were handled for nursing care. Earmuffs also needed to be replaced on the infants' ears during the study. Even if replacing them implicated minimal handling, it still disturbed the infants by touching them.

It is noteworthy that the number of times infants were handled in this study is similar to results reported by Horton, Waldenström, and Bowman (1998) who evaluated the frequency of preterm infant handling over a 24-hour period. Their average number of times of handling per hour was 2.9 times which was identical to that found in this study, 2.9 times (11.65 / 4 = 2.9 in the intervention period). In comparison, Zahr & Balian (1995) reported an average number of 8.5 episodes of handling for nursing procedures over four hours of observation which is less but still similar to the findings of this study.

There appears to be no concrete recommendation about the frequency and amount of time that preterm infants should be handled in the NICU, but minimal handling is strongly suggested (Bowden et al., 2000; Byers, 2003; Peters, 1999; Symington & Pinelli, 2006). Even if the actual study results are

comparable to previous research, it is difficult not to relate the higher incidence of handling in the intervention period with the replacement of eye goggles and earmuffs. This suggests at the same time that their use is inappropriate for reducing preterm infants' exposure to light and noise.

Sensory Stimulation

Of particular interest, one recent study reported that environmental sensory stimulation seems to influence infants' heart rate and HRV (Richard & Mosko, 2004). The researchers reported that infants born over 38 weeks of gestation who slept with their mothers at the age of 11 to 15 weeks demonstrated a higher heart rate and a reduction in HRV than when sleeping alone (measured by the interquartile range of R-R intervals). They explained these findings as sensory stimulation that might have been created by the mother's presence. Extrapolating this possible explanation to the present study, it is then plausible to think that preterm infants in the intervention period may have shown higher maximum heart rate and lower HF power because of the sensory stimulation associated with handling or the tactile irritation of wearing the eye goggles and earmuffs.

Indeed, the tactile irritation created by the material could have negatively influenced the infants' heart rate and HF power. It might be possible that preterm infants handled for nursing care while wearing the eye goggles and earmuffs may have reacted differently than when they were handled without wearing them. It seems touching the infants wearing the material might have exacerbated the effect of handling. It is interesting to note that it appears that the sensory experiences of one system will also influence the other sensory systems

(Lickliter, 2000). Therefore, reducing the visual and auditory systems sensory experience of preterm infants with eye goggles and earmuffs may have influenced the tactile sensory system and thereby, amplified the effect of handling.

The extent to which preterm infants were comfortable while wearing the material is unknown and is also open for discussion. The question of comfort for preterm infants wearing earmuffs was also mentioned by other researchers using this equipment (Zahr & de Traversay, 1995). Although it was not systematically coded, a video coder noted that preterm infants in the intervention period were startling more when they were touched as compared to when they were in the control period. Whether this observation reflects that the infants felt more tactile irritation when they were handled wearing the eye goggles and earmuffs, or that they were in active sleep while resting in darkness with muffled sounds, is unknown. It is a possibility that this factor has influenced the findings of this study.

Implications of Other Sensory Systems

As all sensory systems play an important role in AIs' theory, the lack of significant findings for the physiological stability of preterm infants receiving an intervention focusing on the most immature sensory systems, such as the visual and auditory, may suggest that it is the more mature systems, for example the tactile and vestibular, that are most responsible for the infant's ability to self-regulate. These sensory systems would perhaps be the ones contributing to the positive results found in studies evaluating the effectiveness of the Neurobehavioral Individualized Developmental Care Assessment Program

(NIDCAP) based on Als' theory. These studies indicate that preterm infants receiving developmental care interventions as defined in the NIDCAP showed better neurobehavioral development and brain function (Als et al., 2003), as well as improved medical, neurobehavioral outcomes, and family functioning (Als et al., 2004) compared to infants receiving standard care. Based on the results of these studies, an intervention combining the preterm infants' reduction to light and noise as well as to minimal handling, such as clustering care, could then have created different findings for this study.

Cardiac Autonomic Responses

It is not surprising that preterm infants in the intervention period who had a higher maximum heart rate also had a significantly reduced HF power as compared to the control period. This is explained by the fact that an increased HF power would have meant an activation of the parasympathetic system responsible for slowing the heart rate (Hainsworth, 1995). As a result, the higher maximum heart rate calculated in this study was probably due to a withdrawal of the parasympathetic system on the heart. It is worth mentioning that in a neonatal care manual (Wechsler & Wernovsky, 2008), the ECG standard for mean heart rate in preterm infants aged between seven and 30 days is reported to be 170, with a variation between 133 and 200 beats. Thus, the mean for maximum heart rate calculated for the infants in the intervention period (M = 198.06) was within the normal range.

Based on studies examining the developmental trajectory of the PNS in preterm infants, the lower HF component found in this study could also be explained by an undeveloped PNS at birth. It is reported that preterm infants at

theoretical term age have an immature PNS compared to full-term infants as shown by a significantly lower HF component (Patural et al., 2004), and that the vagal tone (parasympathetic tone) experiences a rapid increase in its development towards the 37th to 38th week PCA (Clairambault et al., 1992). However, in this study, as the HF was significantly lower in the intervention period, the immaturity of the PNS in preterm infants was observed only while they were wearing the eye goggles and earmuffs. As an activation of the parasympathetic system reflects a calm and recuperative state where energy is being preserved (Verklan & Padhye, 2004), promoting the maturity of the PNS in preterm infants is desirable. The intervention tested in this study failed to demonstrate such an effect and furthermore seemed to promote stressful cardiac autonomic responses in infants. This is shown by a withdrawal of the parasympathetic system on their heart rate.

An important observation is that preterm infants' stress responses were expressed only by the cardiac system, as oxygen saturation remained the same with or without eye goggles and earmuffs. It is difficult to explain this discordance. These findings are both comparable to and paradoxical to the results of previous studies evaluating the effects of procedures on physiological parameters of preterm infants. For instance, infants who were weighed without any environmental or behavioral intervention had a higher mean heart rate but no significant alteration was found for their oxygen saturation (Catelin, Tordjman, Morin, Oger, & Sizun, 2005). Conversely, Peters (1998) reported that bathing preterm infants had the effect of increasing heart rate and reducing oxygen

saturation and these physiological responses are also observed during heel lancing (Stevens et al., 1993; Stevens & Johnston, 1994).

It is unclear what physiological mechanisms underlie the physiological responses of infants born preterm, but it could be that the cardiac system is more reactive and sensitive to stress than oxygen saturation. Also, when there was a response in both heart rate and oxygen saturation, perhaps the procedure was more stressful for preterm infants, as could be the case with bathing and heel lance versus weighing. The latest explanation would imply, in the context of this study, that the infants' experience while wearing the eye goggles and earmuffs was disturbing enough to create autonomic cardiac responses but not to the point of perturbing oxygen saturation.

Confounders for HRV

Among potential confounders influencing HRV, gestational age and postnatal age (Longin et al., 2005; Rosenstock et al., 1999) have been reported, but were not found to be significantly correlated with the HF component of the HRV measured for the physiological stability. Body temperature (Davidson, Reina, Shefi, Hai-Tov, & Akselrod, 1997) and sleep states (Porges, Doussard-Roosevelt, Stifter, McClenny, & Riniolo, 1999) are other documented confounders for HRV. More specifically, a higher HF power resulting from a parasympathetic activity, as observed in infants in the control period compared to the intervention period, is reported to be associated with a body temperature of 36°C in preterm infants with a mean gestational age of 32 weeks (Davidson et al.). As preterm infants were all in their incubators with ambient heat set around 36.5°C in servo-control mode (i.e., incubator heat adjusting to the infant's

temperature) at the time of data collection, there is no reason to believe that preterm infants' body temperature could have been different between the intervention and control periods.

Porges et al. (1999) also report that there seems to be a withdrawal of vagal activity in full-term newborns in active sleep compared to quiet sleep. As observed with the lower HF power results in the intervention period, infants might have been in active sleep more frequently during the 4-hour period of wearing the eye goggles and earmuffs as compared to the control period. Since the assessment of sleep-wake states is particularly based on eye opening and eye movements (Holditch-Davis, Scher, Schwartz, & Hudson–Barr, 2004), it could not be measured in this study because the preterm infants' eyes were covered by goggles. Again, it might be possible that infants in the intervention period could have been in active sleep more because of the tactile irritation created by the material or by the instances of handling which were significantly higher in the intervention period.

Factors Related to Study Design

Timing of the Intervention

Besides maximum heart rate and HF power, the lack of significant findings may be related to the time when the intervention was performed. As explained in the methods chapter, the intervention was conducted between 06:00 a.m. and 12:00 p.m. to coincide with the beginning of daylight and a period when noise intensity and peaks were reported to be significantly higher (Chang et al., 2001; Krueger et al., 2005). Previous studies using physiological indicators such as heart rate, respiratory rate, and variability of respiratory rate have reported that

preterm infants' physiological stability was improved with lower light levels in the evening and night (Blackburn & Patteson, 1991; Shiroiwa et al., 1986). This practice is consistent with light cycling in NICU environments which is recognized as a strategy to produce the mimicking of diurnal differences in preterm infants.

According to Jorgensen et al. (2004), the cycled lighting studies provide suggestive but non-conclusive results about benefits for physiological stability, weight gain, sleep state and activity level of 29 weeks gestational age infants or older. If indeed, further research demonstrates that cycled lighting is beneficial for preterm infants of 29 weeks gestational age and more, the study findings might have been different if light exposure would have been reduced in the evening instead of the morning, as was done in this study. In addition, Zahr and de Traversay (1995) reported improved oxygen saturation levels in infants who wore earmuffs two hours in the morning as well as two hours in the evening. Noteworthy, even if only light or noise was controlled in these previous studies, comparisons of their results with the actual study findings provide guidance for clinical practice and further intervention research. Similar findings were reported in Mann et al.'s (1986) study where both light and noise levels were controlled. These researchers reported that after discharge from the hospital, preterm infants assigned to a nursery where both light and noise levels were reduced in the evening and night (from 19:00 p.m. to 07:00 a.m.), spent less time awake, took less time for feeding per day, and had greater weight gain than infants assigned to a standard nursery.

Measuring Additional Outcomes

Besides the outcomes measured in this study, measuring other physiological outcomes, such as respiration, as well as behavioral outcomes and sleep state, might have contributed to the study's findings. Shiroiwa et al. (1986) reported lower respiration rates and less variability in respiration with a reduction of sustained movements in blindfolded preterm infants, but no significant differences were found for heart rate and variability of heart rate. Blackburn and Patteson (1991) have also reported, in addition to a significant difference for heart rate, a lower level of activity in preterm infants exposed to cycle lighting versus continuous lighting versus over a 24-hour period. Zahr and de Traversay (1995) have reported that infants wearing earmuffs had significantly improved oxygen saturation levels and spent more time in quiet sleep than when they were not wearing the earmuffs.

Pain Response

Pain response in preterm infants was not improved when they were undergoing a heel lance following the 4-hour period in which they had worn the eye goggles and earmuffs in the NICU. Hence, the second hypothesis was not supported. There were no significant differences between the heart rate, R-R intervals and return to baseline of preterm infants assigned to the intervention group compared to the control group. There are several elements which are worth discussing related to the findings on pain response, including comparison with the effect of other non-pharmacological interventions, the preterm infants' handling, the return to baseline, and the measurement of behavioral outcomes.

Comparison with Other Non-Pharmacological Interventions

The findings of this study are consistent with a previous study evaluating the effect of a non-pharmacological intervention on physiological parameters of pain response in preterm infants, even though there are some contradictory results in the literature. For instance, Johnston et al. (2003) evaluated the effect of kangaroo care during heel lance and reported that preterm infants' heart rate and oxygen saturation were similar in both intervention and control sequences throughout the procedure. At the same time, kangaroo care (Lundington-Hoe et al., 2005), non-nutritive sucking (Corbo et al., 2000; Field & Goldson, 1984), and facilitated tucking (Corff et al., 1995) are all reported as significantly reducing preterm infants' heart rates during or after the heel lancing procedure.

More importantly, developmental care interventions, such as decreasing environmental light and noise, positioning, and grasping have previously been reported to decrease preterm infants' pain scores and hypoxic events during a nursing procedure (Sizun et al., 2002). The reduction of light and noise was made by covering the infants' incubators and closing the NICU room door. It should be noted that pain response in that study was measured during a diaper change, which is not a painful procedure, and has been used in other studies as a contrast to painful procedures (Gibbins et al., 2008; Holsti, Grunau, Oberlander, & Osiovich, 2008). So whether Sizun et al. were measuring responses to a painful or a stressful procedure is not clear, however the developmental care interventions were still effective in reducing physiological instability, such as the number of hypoxic events and oxygen desaturation.

Handling

It is important to note that even if preterm infants in the intervention group were handled more frequently in the four hours preceding the taking of the blood sample as compared to the control group, they did not show an increase in their pain response during the heel lance. An unexpected result was the negative relationship obtained between the number of occasions infants were handled in the four hours prior to the heel lancing and their mean and maximum heart rate during the procedure. It is difficult to explain these findings as they are paradoxical to the documented effect of handling on preterm infants' pain response. These results contradict Porter et al. (1998) who report a higher pain response (e.g. higher mean heart rate) in preterm infants who were handled before a painful procedure. In addition, the empirical work of Anand & Scalzo (2000) support that previous exposure to stressful stimulation could increase the preterm infants' responses to painful procedures. In this study, even after controlling for the number of times infants were handled during the blood sample procedure, these empirical data were not supported.

The length of observation time during and after the painful procedure might be a factor worth exploring. Indeed, in Porter et al.'s study, infants' previous handling was continuous for about 11 minutes and preceded the painful procedure by 10 minutes. In this study, the total time of handling for infants in the intervention group was closer to 23 minutes but spread over four hours. Perhaps, the stressful stimulation created by the handling manoeuvres did not influence the infants' pain responses because there was enough time between events for them to recuperate appropriately. More precisely, the mean time between the last

handling and the beginning of the heel lancing procedure was 26 minutes and 22 seconds in the intervention group which represents almost two and half times the length of time in Porter's study. There is again a possibility that preterm infants handling in the intervention group might have counterbalanced the potentially beneficial effect of reducing light and noise over the 4-hour period and thereby not significantly decrease their pain response during the heel lances at the end of the study period. Yet, these hypotheses offer an explanation as to why there was no significant difference found for pain response between the study groups, but not to the negative relationships observed between the number of handling episodes and heart rate outcomes. Future research evaluating the effect of handling on the pain response of preterm infants would therefore contribute to a better understanding of its effect.

Return to Baseline

Reducing exposure to light and noise in the NICU did not significantly reduce the time required to return to baseline heart rate (mean heart rate measured five minutes preceding the painful procedure). Even if there was no significant difference for the return to baseline between the two groups, the time needed to recuperate following the painful event was somewhat longer by 20 seconds for the infants in the intervention group. This finding may be explained by the effect of handling infants and/or the time it took for the professionals to draw the blood. While handling was not significantly correlated with the time to return to baseline, it still may have played a role in the time infants took to recuperate from the procedure. As infants were handled more frequently in the intervention group in the four hours preceding the painful procedure, this might

have affected their capacity to return more rapidly to a homeostasis state following the challenging event. In addition, the time it took to harvest the blood was longer by 30 seconds in the intervention group, which could also have had the effect of extending the time they took to return to baseline. It is unclear why it took more time to draw the blood in the intervention group but perhaps the professionals doing the procedure were influenced by seeing the preterm infants wearing eye goggles and earmuffs. Even if the time to harvest the blood sample was treated as a covariate in the statistical analysis model of the time to return to baseline, it may still have left out some variability related to this outcome. Conversely to other non-pharmacological interventions such as swaddling (Huang, Tung, Kuo, & Chang, 2004), facilitated tucking (Corff et al., 1995), and kangaroo care (Johnston et al., 2008) reducing the preterm infants' exposure to light and noise was not effective in reducing the time they needed to return to a baseline heart rate.

Behavioral Outcomes

Finally, measuring behavioral outcomes of pain in addition to those physiological outcomes measured in this research could have significantly contributed to the findings of this study. In a recent paper, Ranger, Johnston, and Anand (2007) discuss the issue of dissociation between physiological and behavioral responses of infants to painful procedures. Hence, even if no significant difference was found for any of the physiological outcomes between the groups studied, dissimilar findings could have been observed for behavioral outcomes. For example, Johnston et al. (2003) report that facial actions (brow bulge, eye squeeze, and naso-labial furrow) of infants in kangaroo care while

experiencing a heel lance were the parameters contributing significantly to the total pain score and were observed less than when the infants were in their incubators. However, no significant difference for the physiological parameters was reported between the study conditions. Additionally, in evaluating the structure of acute pain responses, Stevens et al. (2007) reported that the group of three facial actions, as per the behavioral outcomes of the Premature Infant Pain Profile (PIPP), was the factor accounting for the greatest variance of infants' pain responses, while the group of physiological factors was the factor explaining the smaller remaining variance.

Other studies evaluating the effect of non-pharmacological interventions have reported significant differences for behavioral outcomes in relation to pain response. Swaddling following the heel lance also had the effect of significantly reducing facial activity of preterm infants (Fearon, Kisilevsky, Hains, Muir, & Tranmer, 1997). Likewise, infants spent less time crying and fussing during a painful heel lancing procedure while experiencing non-nutritive sucking (Corbo et al., 2000; Field & Goldson, 1984), kangaroo care (Lundington-Hoe et al., 2005), and facilitated tucking (Corff et al., 1995). Thus, differences may have been found if behavioral indicators had been used, but the eye goggles obscured facial actions.

Implications for Study Framework

Als' Synactive Theory of Development

The study findings do not support the links that were proposed in the study framework. Inspired from Als' (1982) Synactive Theory of Development, it was hypothesized that preventing stress in preterm infants by having them wear eye

goggles and earmuffs in the NICU would promote their self-regulation and, in return, increase their physiological stability. Not only did this intervention fail to improve the preterm infants' physiological stability, but seems to have been more stressful for them according to the findings of higher maximum heart rate and lower HF power. The link between the light and noise sensory minimization intervention and the physiological stability was therefore not supported. It was discussed that handling of the preterm infants during the 4-hour period could be a plausible explanation of these study findings as minimal handling is identified as a developmental care intervention.

Anand and Scalzo's Empirical Work

It was also hypothesized that preterm infants wearing eye goggles and earmuffs would have a reduced pain response during a heel lance procedure. Inspired by Anand and Scalzo's (2000) work, it was presumed that reducing non-painful sensory stimulation such as light and noise could influence the preterm infants' pain responses to noxious procedures. However, the findings of this study indicate that reducing preterm infants' exposure to these sensory stimuli in the NICU did not influence their pain response to the heel lance procedure. The link between the tested intervention and pain response was not supported. Evaluating how exposure to light and noise influence pain response therefore remains an avenue to explore.

Finally, it was hypothesized that preterm infants showing *greater physiological stability* while wearing the eye goggles and earmuffs for a 4-hour period would also show a *reduced pain response* during a painful procedure at the end of the 4-hour period. Preterm infants' mean heart rate measured during

the intervention period did predict their mean heart rate during the blood sample procedure, but only when they were wearing eye goggles and earmuffs.

There are two elements worth discussing in relation to this finding. First, it is difficult to explain why similar results were not observed when infants were not wearing the material. Mean heart rate measured during the control period did not predict mean heart rate during the painful procedure. Second, the presence of prediction between these two outcomes in the intervention condition is somewhat surprising. Due to the signs of stress shown by infants during the intervention period of the cross-over trial (higher maximum heart rate and lower HF), the infants should have exhibited more physiological instability in response to pain. However, during the heel lance, there was no significant difference for the mean heart rate of infants in the intervention group compared with the control group. As previously discussed, this may be explained by infants being able to recuperate properly before undergoing the painful procedure. Links made in the study framework and in the discussion related to the study hypothesis guide the clinical and research recommendations made in the last parts of this chapter.

Study Strengths and Limitations

Strengths

This multifaceted research is innovative for different reasons. It is one of the first studies evaluating the effects of reducing the exposure of preterm infants to both light and noise by having them wearing eye goggles and earmuffs. It is also one of the first studies to measure pain response of preterm infants after they had spent four hours in the NICU environment with reduced exposure to these stimuli. Both research hypotheses have been examined with a creative

combination of two experimental designs: a cross-over trial and a RCT representing research designs with strong internal validity (Brink & Wood, 1998).

Limitations

On the other hand, this research had several limitations that need to be taken into consideration when assessing its contribution. Limitations related to both study designs, such as the material used for the study, the extent of light and noise reduction, the blinding of the intervention, the time of data collection, and the apparatus used will first be discussed. Then, limits related to the establishment of a habituation period and repeating the intervention in relation to the cross-over trial measuring physiological stability will be explained followed by the limitations of the RCT measuring pain response, such as the selection of outcome measures, as well as sample size.

Limitations Related to Both Study Designs

Intervention fidelity. Most important, a limitation may be the materials available for the study. As already discussed, the eye goggles and earmuffs were not remaining in place on the preterm infants and needed to be adjusted during the study period. Not only was there an increase in the handling of infants for adjustments, it also meant that the intervention was not consistent for all infants participating in this study. The type of eye goggles chosen for this research was based on clinical and research purposes; although using another type of goggles could have created other study conditions and produced different research results. Indeed, selecting eye goggles that hold in place would probably have decreased the number of handling occasions and consequently could have reduced the preterm infants' stress responses. In addition, the earmuffs did not

always adhere well around the ears and were occasionally found lying beside the infants in their incubators. During that time, the infants were not protected from the noise in the neonatal environment indicating again that the intervention was not consistent for all infants participating in the study. Nevertheless, if similar material is used in future studies, it should be pilot tested to ensure that any material stays in place and seems comfortable to the preterm infants.

Extent of light and noise reduction. It is difficult to estimate exactly how much luminescent and auditory stimulation infants were actually exposed to during the study. According to manufacturers' reports, the eye goggles blocked 100% of the light perceived by the infant, while the earmuffs were only reducing the noise levels by seven dB. The noise levels to which preterm infants were exposed during the study was therefore dependent on the level of ambient noise in the NICU. Moreover, sound measurements were taken every hour without monitoring the occurrence of peak noise in between measurements, suggesting that noise levels might have been underestimated. It also means that the extent of light and noise reduction was not equivalent for all infants participating in the study.

Blinding of the intervention. Neonatal nurses caring for preterm infants during the 4-hour period of the study were not blinded to the intervention since it was obvious when the infants were wearing eye goggles and earmuffs in their incubators. Therefore, it might be possible that the nurses differentially influenced the physiological stability of infants either by handling or by controlling light and noise levels in the NICU environment. Similarly, the laboratory technician or nurse who performed the heel lances on preterm infants was not blinded to the

intervention and could have influenced the procedure in an immeasurable way. The video coders were also not blinded to the intervention, although they did not code any of the primary outcome measures.

Timing of data collection. Data collection was delayed until preterm infants did not need respiratory support, such as mechanical ventilation (intubation and high-frequency) or CPAP. The main reasons for this delay were to maintain a more homogeneous study sample, and to take into consideration the possibility that internal noise created by respiratory devices could be amplified in infants wearing earmuffs. Data were then collected about 13 days postnatal when infants were in the intermediate care nursery where noise levels are reported to be significantly lower than in the intensive care nursery (Levy et al., 2003). Therefore, preterm infants who participated in this research may have been already acclimated to the light and noise in the NICU environment limiting the potential benefits of reducing their exposure to these stimuli on their physiological stability and pain response.

Apparatus. There are significant limitations associated with the apparatus used in the study. The cardiac analysis software of the Somté™ provided a high percentage of artefacts in the initial analysis of the ECG tracings. Even if the tracings were manually corrected and then validated by an expert (see appendix C), it was arbitrarily decided that only tracings with artefacts of more than 20% were altered. After correcting for artefacts, both study periods were comparable; however, there were a remaining percentage of artefacts in some tracings that created missing values for heart rate and HRV. It was especially not possible to perform frequency-domain analysis of HRV for pain responses using the Somté™

cardiac software as the window calculation for the beginning and ending times of the heel lance could not be set precisely to the second. Similarly, the precision of the pulse oximeter was intermittently inaccurate when the infant was moving the extremity, so it was not possible to evaluate the incidence of either oxygen desaturations for the physiological stability or the oxygen saturation for the pain response.

Limitations Related to the Cross-Over Trial

Habituation period. No habituation period was planned in the cross-over trial. Thus, recording of physiological parameters was initiated a few minutes following the installation of eye goggles and earmuffs on preterm infants not allowing them to habituate or adapt to the material. In their study, Shiroiwa et al. (1986) started to collect data one hour after blindfolding the preterm infants with the objective of allowing a habituation period. If there was an actual period of adaptation in the preterm infants in the intervention period, it is hard to estimate how long it lasted and how it might have influenced the study findings. Examination of previous phototherapy treatment revealed that almost all infants recruited in the cross-over trial (47 out of 54) and the RCT (37 out of 44) received this type of treatment before data collection. This means that for the majority of infants participating in the study it was not the first time they were wearing eye goggles, and consequently this might have influenced their habituation to the material. Even so, planning a period of habituation period before starting data collection could have reduced some of the potential associated effects.

Repeating the intervention. As there were some carry-over effects associated with the LF and HF power, repeating the intervention a second time

would have provided more strength to the study's findings by increasing the sample size and the probability that the outcome effects were associated with the intervention. The repetition of the intervention in this research for preterm infants randomized in the A – B sequence was initially planned but the idea was abandoned to increase the study's feasibility in the clinical setting (see Appendix B for further explanation). Nonetheless, replicating effects in a design provide a better control over potential threats to the study's internal validity than when the intervention is not repeated (Portney & Watkins, 2000).

Limitations Related to the RCT

Selection of outcome measures. The measurement of pain response was solely according to physiological parameters. Expert researchers in infant pain studies confirm that physiological parameters alone are not sufficient to measure pain response and that behavioral outcomes must also be considered (Franck & Miaskowski, 1987; Stevens & Johnston, 1994). The coding of any eye movements was not possible in this study because the goggles were hiding the infants' eyes, thus limiting the evaluation of some facial actions. However, other behavioral outcomes such as body movements from the NIDCAP® program (for example, flex legs, hand on face, finger splay, salute, and frown) have been found to be reactive with heel lance (Holsti, Grunau, Oberlander, Whitfield, & Weinberg, 2005) and could have been measured in this study in combination with physiological outcomes. The hormonal sign of salivary cortisol was intended to be measured in this study; however collecting saliva was unsuccessful. This measure is often unsuccessful among preterm infants as discussed in other

studies (Herrington, Olomu, & Geller, 2004; Neu, Goldstein, Gao, & Laudenslager, 2007).

As pain is a multidimensional concept, the use of multidimensional instruments with well-established reliability and validity may be preferable to use for the measurement of pain (Duhn & Medves, 2004). Measuring pain without a combination of physiological and behavioral and/or hormonal outcomes or a validated pain instrument brings a limitation to the measurement of this variable in this study. Another methodological limitation to the measurement of pain is associated with the different length of procedure time that was used for calculation of the main outcomes. Even if the time to harvest the blood sample was treated as a covariate in the statistical model of the outcomes, it may have left out some variability related to heart rate and HRV that consequently requires further evaluation in studies.

Sample size. The final sample of infants in the RCT for pain response was 44 whereas the estimated sample size was 60. The calculation of the estimated sample size was based on a power of 80%, therefore the sample size of 44 created an underpowered study at 65%. It is noteworthy that the mean heart rate calculated during the blood sample for the intervention and control groups were very close (means difference of 0.63 beat).This suggests that even with a sample size of 60, significant differences between the outcome measures would not have been detected for pain response as a much bigger difference in heart rate, specifically 9.5 beats was expected.

Implications for Clinical Practice

Based on the findings of this research, having preterm infants wear eye goggles and earmuffs to reduce their exposure to NICU lighting and noise is not a recommended intervention for neonatal clinical practice. However, the lack of significant findings and the presence of findings in an unexpected direction should not be an indication that light and noise should not be controlled in the NICU. Reducing preterm infants' exposure to light and noise, which is an important part of developmental care, is advocated by different experts in neonatology as supportive and safe care in the NICU (Bowden et al., 2000; Byers, 2003, Holditch-Davis et al., 2003; Pressler et al., 2004), and developmental care is a suggested non-pharmacological intervention to manage pain in preterm infants (AAP & CPS 2006).

Reducing light and noise in the environment is therefore an interesting alternative to reduce the exposure of preterm infants to these stimuli in the NICU, for both their physiological stability and pain response. Implications for clinical practice are then suggested in terms of reducing environmental light and noise with the objective of respecting NICU recommended standards. Innovative ideas encouraging light and noise control in the NICU, as well as the implementation of developmental care and non-pharmacological interventions promoting physiological stability and pain management of preterm infants is also discussed.

Reducing Environmental Light and Noise

Light

Different environmental strategies to reduce light and noise have been suggested by proponents of developmentally sensitive care and should be

performed until further research establishes the effectiveness of other interventions. For example, neonatal nurses and other NICU professionals have been encouraged to reduce the preterm infant's exposure to environmental light by covering the incubator with a blanket and avoiding direct exposure of the preterm infant's eyes to procedural light (Blackburn, 1998). Lotas (1992) also suggests turning off ceiling lights and unnecessary lights, as well as shading windows to prevent the entry of daylight or sunlight. For covering the incubator, Lee et al. (2005) bring to our attention the importance of choosing dark-colored covers as they provide greater light reduction, and to control the ambient lighting as the cover's effectiveness depends on it. White (2004) also suggests that when an ambient lighting of 20 fc cannot be reached in the night, covering incubators with a blanket or shielding preterm infants' heads and eyes are acceptable strategies to reduce their exposure to light. According to the implications of this study, eye goggles used for that purpose should be selected based on their ability to hold on securely and for their increased comfort for preterm infants.

Noise

NICU professionals are also encouraged to reduce noise levels by different interventions. Actions such as, covering the incubator with a blanket (Levy et al., 2003; Saunders, 1995), closing incubators doors with care (AAP, 1997), avoiding writing or placing equipment on top of incubators (AAP; Nzama et al., 1995; Purdy, 2000; Sparshott, 1995), speaking softly close to the infant's bedside (Blackburn, 1998; Sparshott), removing water from ventilator tubes to avoid bubbling (Blackburn; Levy et al.), and responding quickly to monitor alarms (Nzama et al.; Purdy) have been suggested. Other proposed interventions are to

use padded plastic garbage cans instead of metal ones (Levy et al.), placing radios and telephones outside the unit (Blackburn; Levy et al.), and professionals wearing soft shoes (AAP). Finally, quiet and appropriate individual equipment could also reduce ambient noise levels in NICU environments.

Recommended Standards

According to the Committee on Recommended Standards for Newborn ICU Design (2006), ambient lighting in infants' spaces should be adjustable between approximately one and 60 fc as measured at each bedside. For NICU noise levels, the Committee' recommendation is to respect hourly levels of 45 dBA. Noise intensity should not exceed 50 dBA 10% of the measurement time, and should never go beyond 65 dBA.

Optimal practice for the reduction of lighting in NICUs is still discussed by different experts in neonatology. Some authors suggest cycled lighting for preterm infants of 28 weeks of PCA (White, 2004) while others recommend it only for infants older than 32 weeks (Figueiro, Appleman, Bullough, & Rea, 2006). The AAP and ACOG (2007) state that the benefits associated with diurnal variation are uncertain but still seem to be an acceptable approach for NICUs lighting. Others make the interesting comment that there is little research supporting the practice of continuous dimmed lighting in the NICU as there are no beneficial or detrimental clinical effects reported with this practice (AAP & ACOG; Harrison et al., 2004). At the same time, until further research indicates clear guidelines for lighting in NICUs, Harrison et al. recommend dimming the light for some parts of the 24-hour cycle.

Innovative Ideas for Controlling Light and Noise

There is an important clinical implication arising from the implementation of this study in clinical units. NICU lighting and noise levels were not found to be significantly different between the study sequences but lighting intensity while the infants were wearing eye goggles and earmuffs was lower by 20 lux than when they were not wearing these protectors. Seeing infants wearing eye goggles and earmuffs might have influenced nurses to exert better control over the NICU lighting by covering the incubators, turning off ceiling lights and closing window shades.

Innovative ways should therefore be designed and implemented to continuously sensitize and encourage NICU professionals to control light and noise in the environment. For example, Chang, Pan, Lin, Chang, & Lin (2006) report that a noise-sensor light alarm in the NICU that lights up when noise reaches 65 dB was successful in reducing noise levels inside incubators of preterm infants. Brandon, Ryan, and Barnes (2007) bring to our attention the importance of monitoring all NICU environmental changes made with the objective of reducing noise levels to confirm that they are in effect reduced and not increased. Other ideas could be: a) to routinely post signs in the NICU reminding professionals of light and noise control strategies, b) to organize educational rounds discussing recent evidence on interventions controlling light and noise, and c) to have a suggestion box where professionals could submit ideas for controlling these stimuli in the NICU.

Physiological Stability – Developmental Care Interventions

Previous research supports that some benefits exist for the physiological stability of preterm infants when their exposure to light (Blackburn & Patterson, 1991; Shiroiwa et al., 1986) and noise (Zahr & de Traversay, 1995) is reduced. Hence, interventions reducing light and noise exposure as described above should be performed by NICU professionals. The findings of this study also suggest that minimizing the handling of preterm infants should also be performed in addition to reducing light and noise in the NICU. More precisely, Browne (2000) suggests modifying handling in the NICU to be supportive and in conjunction with preterm infants' physiologic and behavioral responses.

Earlier research has confirmed that a combination of developmental care interventions has some benefits for preterm infants' physiological and behavioral outcomes. For example, Slevin, Farrington, Duffy, Daly, and Murphy (2000) report that preterm infants resting in a period of quiet time with reduced lighting, noise, staff activity, and handling, had lower blood pressure and exhibited less movement than when they were in a standard NICU environment. A quiet time period where different developmental interventions are performed seems therefore to be beneficial for infants and could easily be implemented in NICUs. In addition, performing developmental care interventions on a regular basis in NICUs also appears to be beneficial for preterm infants. Accordingly, Stevens, Petryshen, Hawkins, Smith, and Taylor (1996) report that infants receiving developmental care interventions, emphasizing the reduction of light, noise and handling, were more physiologically stable over time compared to infants receiving standard care.

Pain Response – Non-pharmacological Interventions

Until research confirms the effectiveness of reducing light and noise exposure to reduce the pain response of preterm infants, other non-pharmacological interventions that have been shown to be effective in reducing infants' pain should be done in the NICU while they are undergoing painful procedures. In recent literature reviews, non-nutritive sucking, kangaroo care, swaddling, and facilitated tucking have been identified as effective pain management interventions in neonates (Cignacco et al., 2007; Paquette, Le May, & Aita, 2007) and should be routinely performed. According to the AAP and CPS (2006), non-pharmacological interventions should be used in combination with sucrose for pain management during minor routine procedures in preterm infants.

Implications for Neonatal Research

Among the neonatal research implications, it is essential to mention that there were some difficulties in recruitment of preterm infants for this study. In the original research project, the intervention was designed to last 24 hours (see appendix B) and was modified in part because there was a high percentage of refusal by parents (approximately 78%). This high refusal rate may have been related to the nature of the study or to parents coping with preterm delivery or prematurity, but reflects at the same time that recruiting preterm infants in intervention studies is not easy. Conducting a pilot study prior to carrying out the research project to evaluate the study's feasibility, as suggested by Buckwalter et al. (1998), perhaps would have contributed to an easier implementation in the clinical field. For example, once the intervention time was reduced to four hours,

the percentage of refusals dropped to 29%. Following this important modification in the research design, the research project was successfully conducted.

Future studies should be aimed at evaluating the effects of other developmental care interventions on the physiological stability and pain response of preterm infants, including other outcomes besides physiological parameters. Recommendations regarding future research samples will be made, whereas conducting research exploring the link between the light, noise and pain exposure will be suggested for pain response.

Evaluation of Developmental Care Interventions

Further research evaluating the effect of developmental care interventions such as reducing light and noise exposure on physiological stability and procedural pain in preterm infants is needed. According to the study's findings, designing interventions for these purposes should be aimed at controlling light and noise in the NICU environment instead of having infants wear individual material.

As handling may have been an important confounder in this research, future intervention studies should consider including minimal handling in addition to light and noise reduction, or planning an intervention when the handling of the preterm infants would not be allowed, such as a quiet period. At the same time, it would then be interesting to evaluate the relative contribution of reducing light, noise, and handling in relation to both the physiological stability and pain response of preterm infants. For example, reducing light exposure might be more beneficial than decreasing noise, given that the womb is totally dark, but not totally silent. Or it could be that minimizing handling would be more beneficial

than reducing light and/or noise because it would allow appropriate periods of rest between care activities. Conducting research with the purpose of separately evaluating these components would not only contribute to neonatal research knowledge, but also to a more focused implementation of developmental care interventions in neonatal units.

Measuring Additional Outcomes

Studies evaluating the effect of interventions in relation to light and noise exposure of preterm infants in the NICU environment should include behavioral parameters in addition to physiological parameters. Measuring both physiological and behavioral signs is congruent with Als's (1982; 1986) Synactive Theory of Development guiding this study. According to this theory, the neurobehavioral assessment of preterm infants is partially achieved through the observation of behaviors related to the autonomic and motor systems that are interrelated and are evolving side by side. Future studies should also consider measuring HRV, as it offers an interesting asset to the evaluation of preterm infants' responses to stress. Actually, HRV is a non-invasive method of evaluating preterm infants' CNS integrity (Oberlander & Saul, 2002) as well as the balance and imbalance of the autonomic nervous system (Cowan, 1995). Research measuring the effect of handling of preterm infants on their HRV is still an avenue to explore and would contribute to a better understanding of the effects associated with nursing interventions.

A similar recommendation is made for studies evaluating pain and the measurement of pain responses, such as behavioral parameters of facial actions (Franck & Miaskowski, 1987; Johnston & Stevens, 1996), body movements

(Holsti et al., 2005), and HRV (Lindh et al., 1997; Oberlander & Saul, 2002). Including behavioral in addition to heart rate parameters in future studies offers a more systemic evaluation of the interventions' effects on the physiological stability and pain response of preterm infants.

Research Samples

It would also be interesting to conduct intervention research with preterm infants born earlier than 28 weeks of gestation, as they are more likely to be physiologically vulnerable with their greater neurological immaturity. Indeed, Gressens et al. (2002) state that the critical steps of human cortical brain development begins at 24 weeks PCA and, according to Holditch-Davis et al. (2003), preterm infants only start showing physiological homeostasis by controlling sympathetic function between 28 and 32 weeks of gestational age. Preterm infants born between 24 and 28 weeks of gestation could therefore benefit as well and perhaps even more than 28 to 32 week infants, from interventions reducing the sensory stimulation of light, noise, and pain in the NICU. Finally, as mechanical ventilation (intubation) was found to be an important extraneous variable in this research, it is recommended to either stratify future research samples according to this variable, or to consider it as an inclusion or exclusion criteria.

Pain Response

More research is needed to evaluate the link between how reducing the preterm infant's exposure to sensory stimulation, such as light and noise, influence their response to painful procedures. On the other hand, it would be essential to evaluate if the preterm infant's previous exposure to a painful

procedure influences their response when they are subsequently exposed to light and/or noise. Accordingly, Holsti, Grunau, Oberlander, & Whitfield (2005) report that infants exposed to pain before tactile procedures (clustering care) showed increased facial, body, and heart rate responses compared to when tactile stimulation was not preceded by a painful procedure. Research conducted with one of these purposes would contribute to the empirical work of Anand and Scalzo (2000) where it is believed that abnormal stimulation, such as light, noise, and pain, caused hyperexcitability in the central nervous system.

SUMMARY STATEMENT

This study is a first effort to evaluate the physiological stability of preterm infants of 28 to 32 weeks gestational age who wear eye goggles and earmuffs for a 4-hour period in the NICU; and to evaluate their pain response to a heel lance procedure following this 4-hour period. It should be noted that this study was not conducted to refute cycled lighting, or to support continuous dimmed lighting, in the NICU for preterm infants aged between 28 to 32 weeks gestational age. The objective was to evaluate if reducing light and noise exposure of preterm infants during an identified period of increased light and noise in the NICU would reduce their stress and consequently improve their physiological stability and pain response. Based on the results of this study, wearing eye goggles and earmuffs did not show any significant benefit either for physiological stability or for pain response of the preterm infants.

The findings of this research contribute to the body of knowledge on developmental care and pain management of preterm infants by providing direction and incentive toward the implementation of neonatal care interventions

in NICUs. Interventions leading to the control of NICU environmental light and noise, as opposed to minimizing the preterm infant's sensory perceptions to these stimuli, are recommended for clinical practice. Still, collaborative efforts of all neonatal care professionals are required to successfully attain the goal of a developmentally supportive care environment.

This research has also offered direction for future studies with the objective of evaluating preterm infants' physiological and behavioral outcomes in relation to developmental care and pain management interventions. Ultimately, the goal is to allow preterm neonates to grow in an environment supporting physiological and motor systems and encouraging periods of rest long enough to allow recovery and restoration following painful procedures.

REFERENCES

Aita, M., & Goulet, C. (2003). Assessment of neonatal nurses' behaviors that prevent overstimulation in preterm infants. *Intensive and Critical Care Nursing, 19*(2), 109-118.

Akselrod, S., Gordon, D., Ubel, F. A., Shannon, D. C., Barger, A. C., & Cohen, R. J. (1981). Power spectrum analysis of heart rate fluctuation: A quantitative probe of beat-to-beat cardiovascular control. *Science, 213*(4504), 220-222.

Allen, A-M. (1995). Stressors to neonates in the neonatal unit. *Midwives 108*(1288), 139–140.

Als, H. (1982). Toward a synactive theory of development: Promise for the assessment and support of infant individuality. *Infant Mental Health Journal, 3*(4), 229-243.

Als, H. (1986). A synactive model of neonatal behavioral organization: Framework for the assessment of neurobehavioral development in the premature infant and for support of infants and parents in the neonatal intensive care environment. *Physical and Occupational Therapy in Pediatrics, 6*(3-4), 3-53.

Als, H., Duffy, F. H., McAnulty, G. B., Rivkin, M. J., Vajapeyam, S., Mulkern, R. V., Warfield, S. K., Huppi, P. S., Butler, S. C., Conneman, N., Fischer, C., & Eichenwald, E. C. (2004). Early experience alters brain function and structure. *Pediatrics, 113*(4), 846-857.

Als, H., Gilkerson, L., Duffy, F. H., McAnulty, G. B., Buehler, D. M., Vandenberg, K., Sweet, N., Sell, E., Parad, R. B., Ringer, S. A., Butler, S. C., Blickman, J. G., & Jones, K. J. (2003). A three-center, randomized, controlled trial of individualized developmental care for very low birth weight preterm infants: Medical, neurodevelopmental, parenting, and caregiving effects. *Developmental and Behavioral Pediatrics, 24*(6), 399-408.

American Academy of Pediatrics [AAP]. (1997). Noise: A hazard for the fetus and newborn. *Pediatrics 100*(4), 724–727.

American Academy of Pediatrics [AAP], & American College of Obstetricians and Gynecologists [ACOG] (2007). *Guidelines for Perinatal Care (*6th ed.) Elk Grove Village: American Academy of Pediatrics and Washington: American College of Obstetricians and Gynecologists.

American Academy of Pediatrics [AAP], & Canadian Paediatric Society [CPS]. (2000). Prevention and management of pain and stress in the neonate. *Pediatrics, 105*(2), 454-461.

American Academy of Pediatrics [AAP], & Canadian Paediatric Society [CPS] (2006). Prevention and management of pain in the neonate: An update. *Pediatrics, 118*(5), 2231-2241.

Anand, K. S. (1993). Relationships between stress responses and clinical outcome in newborns, infants, and children. *Critical Care Medicine, 21*(9), S358- S359.

Anand, K. J. S. (1998). Clinical importance of pain and stress in preterm neonates. *Biology of the Neonate, 73*(1), 1-9.

Anand, K. J. S., & Carr, D. B. (1989). The neuroanatomy, neurophysiology, and neurochemistry of pain, stress, and analgesia in newborns and children. *Pediatric Clinics of North America, 36*(4), 795-822.

Anand, K. J. S., & Hickey, P. R. (1987). Pain and its effects in the human neonate and fetus. *The New England Journal of Medicine, 317*(21), 1321-1329.

Anand, K. J. S., & International Evidence-Based Group for Neonatal Pain. (2001). Consensus statement for the prevention and management of pain in the newborn. *Archives of Pediatrics and Adolescent Medicine, 155*(2), 173-180.

Anand, K. J. S., & Scalzo, F. M. (2000). Can adverse neonatal experiences alter brain development and subsequent behavior? *Biology of the Neonate, 77*(2), 69-82.

Barker, D. P., & Rutter, N. (1995). Exposure to invasive procedures in neonatal intensive care unit admissions. *Archives of Disease in Childhood. Fetal and Neonatal Edition, 72*(1), F47-F48.

Bartocci, M., Bergqvist, L. L., Lagercrantz, H., & Anand, K. J. S. (2006). Pain activates cortical areas in the preterm newborn brain. *Pain, 122*(1-2), 109-117.

Birch, E. E., & O'Connor, A. R. (2001). Preterm birth and visual development. *Seminars in Neonatology, 6*(6), 487-497.

Blackburn, S. T. (1996). Research utilization: Modifying the NICU light environment. *Neonatal Network, 15*(4), 63–66.

Blackburn, S. (1998). Environmental impact of the NICU on developmental outcomes. *Journal of Pediatric Nursing, 13*(5), 279–289.

Blackburn, S., & Patteson, D. (1991). Effects of cycled light on activity state and cardiorespiratory function in preterm infants. *Journal of Perinatal and Neonatal Nursing, 4*(4), 47–54.

Bo, L. K., & Callaghan, P. (2000). Soothing pain-elicited distress in chinese neonates. *Pediatrics, 105*(4), 1-5.

Boo, N-Y., Chee, S-C., & Rohana, J. (2002). Randomized controlled study of the effects of different durations of light exposure on weight gain by preterm infants in a neonatal intensive care unit. *Acta Paediatrica, 91*(6), 674-679.

Borg, E. (1997). Perinatal asphyxia, hypoxia, ischemia and hearing loss. An overview. *Scandinavian Audiology, 26*(2), 77-91.

Bowden, V. R., Greenberg, C. S., & Donaldson, N. E. (2000). Developmental care of the newborn. *Online Journal of Clinical Innovations, 3*(7), 1-77.

Brandon, D. H., Holditch-Davis, D., & Belyea, M. (2002). Preterm infants born at less than 31 weeks' gestation have improved growth in cycled light compared with continuous near darkness. *The Journal of Pediatrics, 140*(2), 192-199.

Brandon, D. H., Ryan, D. J., & Barnes, A. H. (2007). Effect of environmental changes on noise in the NICU. *Neonatal Network, 26*(4), 213-218.

Brink, P. J., & Wood, M. J. (1998). Introduction. In P. J. Brink, & M. L. Wood (Eds.), *Advanced Design in Nursing Research* (2nd ed., pp. 3-18). Thousand Oaks: SAGE Publications, Inc.

Browne, J. V. (2000). Considerations for touch and massage in the neonatal intensive care unit. *Neonatal Network, 19*(1), 61-64.

Buckwalter, K. C., Maas, M. L., & Wakefield, B. (1998). Classical experimental designs. In P. J. Brink, & M. L. Wood (Eds.), *Advanced Design in Nursing Research* (2nd ed., pp. 21-62). Thousand Oaks: SAGE Publications, Inc.

Byers, J. F. (2003). Components of developmental care and the evidence for their use in the NICU. *MCN The American Journal of Maternal/Child Nursing, 28*(3), 175-180.

Campbell, L. A. (1985). The very low birth weight infant: sensory experience and development. *Topics in Clinical Nursing, 6*(4), 19-33.

Catelin, C., Tordjman, S., Morin, V., Oger, E., & Sizun, J. (2005). Clinical, physiologic, and biologic impact of environmental and behavioral interventions in neonates during a routine nursing procedure. *The Journal of Pain, 6*(12), 791-797.

Cerutti, S., Bianchi, A. M., & Mainardi, L. T. (1995). Spectral analysis of the heart rate variability signal. In M. Malik, & A. J. Camm (Eds.), *Heart Rate Variability* (pp. 63-74). New York: Futura Publishing Company Inc.

Chang, Y-J., Anderson, G. G., Dowling, D., & Lin, C-H. (2002). Decreased activity and oxygen desaturation in prone ventilated preterm infants during the first postnatal week. *Heart & Lung, 31*(1), 34-42.

Chang, Y-J., Lin, C-H., & Lin, L-H. (2001). Noise and related events in a neonatal intensive care unit. *Acta Paediatrica Taiwanica, 42*(4), 212-217.

Chang, Y-J., Pan, Y-J., Lin, Y-J., Chang, Y-Z., & Lin, C-H. (2006). A noise-sensor light alarm reduces noise in the newborn intensive care unit. *American Journal of Perinatology, 23*(5), 265-271.

Cheng, C. M., & Chapman, J. S. (1997). Assessment of reliability and validity of the behavioral observation record for developmental care. *Nursing Research, 46*(1), 40-45.

Cignacco, E., Hamers, J. P. H., Stoffel, L., van Lingen, R. A., Gessler, P., McDougall, J., & Nelle, M. (2007). The efficacy of non-pharmacological interventions in the management of procedural pain in preterm and term neonates. A systematic literature review. *European Journal of Pain, 11*(2), 139-152.

Clairambault, J., Curzi-Dascalova, L., Kauffmann, F., Médigue, C., & Leffler, C. (1992). Heart rate variability in normal sleeping full-term and preterm neonates. *Early Human Development, 28*(2), 169-183.

Colton, T. (1974). *Statistics in Medicine*. Boston: Little, Brown and Company.

Committee on Fetus and Newborn (2004). Age terminology during the perinatal period. *Pediatrics, 114*(5), 1362-1364.

Committee on Recommended Standards for Newborn ICU Design. (2006). Recommended Standards for Newborn ICU Design. Retrieved November 22nd, 2007, from http://www.nd.edu/~nicudes/

Corbo, M. G., Mansi, G., Stagni, A., Romano, A., Van den Heuvel, J., Capasso, L., Raffio, T., Zoccali, S., & Paludetto, R. (2000). Nonnutritive sucking during heelstick procedures decreases behavioral distress in the newborn infant. *Biology of the Neonate, 77*(3), 162-167.

Corff, K. E., Seideman, R., Venkataraman, P. S., Lutes, L., & Yates, B. (1995). Facilitated tucking: A non pharmacological comfort measure for pain in preterm neonates. *Journal of Obstetric, Gynecologic and Neonatal Nursing, 24*(2), 143-147.

Cowan, M. J. (1995). Measurement of heart rate variability. *Western Journal of Nursing Research, 17*(1), 32-48.

Craig, K. D., Whitfield, M. F., Grunau, R. V. E., Linton, J., & Hadjistavropoulos, H. D. (1993). Pain in the preterm neonate: behavioural and physiological indices. *Pain, 52*(3), 287-299.

D'Agostino, J. A., & Clifford, P. (1998). Neurodevelopmental consequences associated with the premature neonate. *AACN Clinical Issues 9*(1), 11–24.

Davidson, S., Reina, N., Shefi, O., Hai-Tov, U., & Akselrod, S. (1997). Spectral analysis of heart rate fluctuations and optimum thermal management for low birth weight infants. *Medical & Biological Engineering & Computing, 35*(6), 619-623.

Davis, A., & Wood, S. (1992). The epidemiology of childhood hearing impairment: factors relevant to planning of services. *British Journal of Audiology, 26*(2), 77-90.

DePaul, D., & Chambers, S. E. (1995). Environmental noise in the neonatal intensive care unit: Implications for nursing practice. *Journal of Perinatal and Neonatal Nursing, 8*(4), 71–76.

Duhn, L. J., & Medves, J. M. (2004). A systematic integrative review of infant pain assessment tools. *Advances in Neonatal Care, 4*(3), 126-140.

Elander, G., & Hellström, G. (1995). Reduction of noise levels in intensive care units for infants: Evaluation of an intervention program. *Heart & Lung 24*(5): 376–379.

Fearon, I., Kisilevsky, B. S., Hains, S. M.,Muir, D. W., & Tranmer, J. (1997). Swaddling after heel lance: Age-specific effects on behavioral recovery in preterm infants. *Journal of Developmental and Behavioral Pediatrics, 18*(4), 222-232.

Fei, L., Statters, D. J., Hnatkova, K., Poloniecki, J., Malik, M., & Camm, A. J. (1994). Change of autonomic influence on the heart immediately before the onset of spontaneous idiopathic ventricular tachycardia. *Journal of the American College of Cardiology, 24*(6), 1515-1522.

Field, T., & Goldson, E. (1984). Pacifying effects of nonnutritive sucking on term and preterm neonates during heelstick procedures. *Pediatrics, 74*(6), 1012-1015.

Fielder, A. R., & Moseley, M. J. (2000). Environmental light and the preterm infant. *Seminars in Perinatology, 24*(4), 291-298.

Figueiro, M. G., Appleman, K., Bullough, J. D., & Rea, M. S. (2006). A discussion of recommended standards for lighting in the newborn intensive care unit. *Journal of Perinatology, 26*(S3), S19-S26.

Fitzgerald, M., Millard, C., & McIntosh, N. (1989). Cutaneous hypersensitivity following peripheral tissue damage in newborn infants and its reversal with topical anaesthesia. *Pain, 39*(1), 31-36.

Fitzgerald, M., Shaw, A., & McIntosh, N. (1988). The postnatal development of the cutaneous flexor reflex: a comparative study in premature infants and newborn rat pups. *Developmental Medicine & Child Neurology, 30*(4), 520-526.

Franck, L. S. (1998). Identification, management, and prevention of pain in the neonate. In C. Kenner, J. W. Lott, & A. A. Flandermeyer (Eds.), *Comprehensive Neonatal Nursing: A Physiologic Perspective* (2nd ed., pp. 788-803). Montreal: W.B. Saunders Company.

Franck, L. S., & Lawhon, G. (1998). Environmental and behavioral strategies to prevent and manage neonatal pain. *Seminars in Perinatology, 22*(5) 434-443.

Franck, L. S., & Miaskowski, C. (1997). Measurement of neonatal responses to painful stimuli: A research review. *Journal of Pain and Symptom Management, 14*(6), 343-378.

Gibbins, S., Stevens, B., Beyene, J., Chan, P. C., Bagg, M., & Asztalos, E. (2008). Pain behaviours in extremely low gestational age infants. *Early Human Development, 84*(7), 451-458.

Glass, P. (1993). Development of visual function in preterm infants: Implications for early intervention. *Infants and Young Children 6*(1), 11–20.

Glass, P. (1999). The vulnerable neonate and the neonatal intensive care environment. In G. B. Avery, M. A. Fletcher, & M. G. MacDonald (Eds.) *Neonatology: Pathophysiology and management of the newborn* (5th ed., pp. 91-108). New York: Lippincott Williams and Wilkins.

Glass, P., Avery, G. B., Siva Subramanian, K. N., Keys, M. P., Sostek, A. M., & Friendly, D. S. (1985). Effect of bright light in the hospital nursery on the incidence of retinopathy of prematurity. *The New England Journal of Medicine, 313*(7), 401-404.

Glover, V., & Fisk, N. M. (2007). Pain and the human fetus. In K. J. S. Anand, B. J. Stevens, & P. J. McGrath (Eds.), *Pain in neonates and infants* (3rd ed., pp. 191-199). Toronto: Elsevier.

Goldson, E. (1999). The environment of the neonatal intensive care unit. In E. Goldson (Ed.), *Nurturing the premature infant. Developmental interventions in the neonatal intensive care nursery* (pp. 3-17). New York: Oxford University Press.

Gonzalez, L., & Dweck, H. S. (1994). Eye of the newborn: A neonatologist's perspective. In S.J. Isenberg (Ed.), *The eye in infancy* (2nd ed., pp. 1-8). Toronto: Mosby.

Graven, S. N. (2000). Sound and the developing infant in the NICU: Conclusions and recommendations for care. *Journal of Perinatology, 20*(8s), S88-S93.

Graven, S. N. (2004). Early neurosensory visual development of the fetus and newborn. *Clinics in Perinatology, 31*(2) 199-216.

Gray, K., Dostal, S., Ternullo-Retta, C., & Armstrong, M. A. (1998). Developmentally supportive care in a neonatal intensive care unit: A research utilization project. *Neonatal Network, 17*(2), 33–37.

Gray, L., & Philbin, M. K. (2000). Measuring sound in hospital nurseries. *Journal of Perinatology, 20*(8s), S99-S103.

Gressens, P., Rodigo, M., Paindaveine, B., & Sola, A. (2002). The impact of neonatal intensive care practices on the developing brain. *The Journal of Pediatrics, 140*(6), 646-653.

Grunau, R. E., Linhares, M. B. M., Holsti, L., Oberlander, T. F., & Whitfield, M. F. (2004). Does prone or supine position influence pain responses in preterm infants at 32 weeks gestational age? *Clinical Journal of Pain, 20*(2), 76-82.

Grunau, R. E., Oberlander, T. F., Whitfield, M. F., Fitzgerald, C., & Lee, S. K. (2001). Demographic and therapeutic determinants of pain reactivity in very low birth weight neonates at 32 weeks' postconceptional age. *Pediatrics, 107*(1), 105-112.

Grunau, R. E., & Tu, M. T. (2007). Long-term consequences of pain in human neonates. In K. J. S. Anand, B. J. Stevens, & P. J. McGrath (Eds.), *Pain in neonates and infants* (3rd ed., pp. 45-55). Toronto: Elsevier.

Grunau, R. V. E., Whitfield, M. F., & Petrie, J. H. (1994). Pain sensitivity and temperament in extremely low-birth-weight premature toddlers and preterm and full-term controls. *Pain, 58*(3), 341-346.

Grunau, R. V. E., Whitfield, M. F., Petrie, J. H., & Fryer, E. L. (1994). Early pain experience, child and family factors, as precursors of somatization: a prospective study of extremely premature and fullterm children. *Pain, 56*(3), 353-359.

Hack, M. (1983). The sensorimotor development of the preterm infant. In A. A. Faranoff, & R. J. Martin (Eds.) *Behrman's Neonatal-perinatal medicine. Diseases of the fetus and infant* (3rd ed., pp.328-346). Toronto: The C.V. Mosby Company.

Hainsworth, R. (1995). The control and physiological importance of heart rate. In M. Malik, & A. J. Camm (Eds.), *Heart Rate Variability* (pp. 3-19). New York: Futura Publishing Company Inc.

Halimaa, S-L. (2003). Pain management in nursing procedures on premature babies. *Journal of Advanced Nursing, 42*(6), 587-597.

Hamer, R. D., Dobson, V., & Mayer, M. J. (1984). Absolute thresholds in human infants exposed to continuous illumination. *Investigative Ophthalmology & Visual Science, 25* (4), 381-388.

Harrison, L. (1997). Research utilization: Handling preterm infants in the NICU. *Neonatal Network, 16*(3), 65-69.

Harrison, D., Evans, C., Johnston, L., & Loughnan, P. (2002). Bedside assessment of heel lance pain in the hospitalized infant. *Journal of Obstetric, Gynecologic and Neonatal Nursing, 31*(5), 551-557.

Harrison, L. L., Lotas, M. J., & Jorgensen, K. M. (2004). Environmental issues. In C. Kenner & J. M. McGrath (Eds.), *Developmental Care of Newborns & Infants. A Guide for Health Professionals* (pp. 229-269). St-Louis: Mosby.

Hellström-Westas, L., Inghammar, M., Isaksson, K., Rosén, I., & Stjernqvist, K. (2001). Short-term effects of incubator covers on quiet sleep in stable premature infants. *Acta Paediatrica, 90*(9), 1004-1008.

Herrington, C. J., Olomu, I. N., & Geller, S. M. (2004). Salivary cortisol as indicators of pain in preterm infants. *Clinical Nursing Research, 13*(1), 53-68.

Hills, M., & Armitage, P. (1979). The two-period cross-over clinical trial. *British Journal of Clinical Pharmacology, 8*(1), 7-20.

Holditch-Davis, D., Blackburn, S. T., & Vandenberg, K. (2003). Newborn and infant neurobehavioral development. In C. Kenner, & J. W. Lott (Eds.), *Comprehensive Neonatal Nursing: A Physiologic Perspective* (3rd ed., pp. 236-284). Toronto: Saunders.

Holditch-Davis, D., Scher, M., Schwartz, T., & Hudson-Barr, D. (2004). Sleeping and waking state development in preterm infants. *Early Human Development, 80*(1), 43-64.

Holsti, L., Grunau, R. E., Oberlander, T. F., & Osiovich, H. (2008). Is it painful or not? Discriminant validity of the behavioral indicators of infant pain (BIIP) scale. *Clinical Journal of Pain, 24*(1), 83-88.

Holsti, L., Grunau, R. E., Oberlander, T. F., & Whitfield, M. F. (2005). Prior pain induces heightened motor responses during clustered care in preterm infants in the NICU. *Early Human Development, 81*(3), 293-302.

Holsti, L., Grunau, R. E., Oberlander, T. F., Whitfield, M. F., & Weinberg, J. (2005). Body movements: An important additional factor in discriminating pain from stress in preterm infants. *Clinical Journal of Pain, 21*(6), 491-498.

Holsti, L., Grunau, R. E., Whitfield, M. F., Oberlander, T. F., & Lindh, V. (2006). Behavioral responses to pain are heightened after clustered care in preterm infants born between 30 and 32 weeks gestational age. *Clinical Journal of Pain, 22*(9), 757-764.

Horton, J., Waldenström, U., & Bowman, E. (1998). Touch of low birth weight babies in the neonatal intensive care: Observations over a 24 hour period. *Journal of Neonatal Nursing, march*, 24-28.

Huang, C-M., Tung, W-S., Kuo, L-L., & Chang, Y-L. (2004). Comparison of pain responses of premature infants to the heelstick between containment and swaddling. *Journal of Nursing Research, 12*(1), 31-39.

Humphrey, T. (1964). Some correlations between the appearance of human fetal reflexes and the development of the nervous system. *Progress in Brain Research, 4*, 93-135.

International Association for the Study of Pain (2001). Pain terms. Retrieved December 5th, 2003, from http://www.iasp-pain.org/AM/Template.cfm?Section=Home&template=/CM/HTMLDisplay.cfm&ContentID=5144#Pain

Johnson, A. N. (2001). Neonatal response to control of noise inside the incubator. *Pediatric Nursing, 27*(6), 600-605.

Johnson, E. O., Kamilaris, T. C., Chrousos, G. P., & Gold, P. W. (1992). Mechanisms of stress: A dynamic overview of hormonal and behavioral homeostasis. *Neuroscience and Biobehavioral Reviews, 16*(2), 115-130.

Johnston, C. C., Collinge, J. M., Henderson, S. J., & Anand, K. J. S. (1997). A cross-sectional survey of pain and pharmacological analgesia in Canadian neonatal intensive care units. *Clinical Journal of Pain, 13*(4), 308-312.

Johnston, C. C., Filion, F., Campbell-Yeo, M., Goulet, C., Bell, L., McNaughton, K. J., Byron, J., Aita, M., Finley, G. A., & Walker, C-D. (2008). Kangaroo mother care diminishes pain from heel lance in very preterm neonates: A crossover trial. *BMC Pediatrics, 8*:13.

Johnston, C. C., Filion, F., Snider, L., Majnemer, A., Limperopoulos, C., Walker, C-D., Veilleux, A., Pelausa, E., Cake, H., Stone, S., Sherrard, A., & Boyer, K. (2002). Routine sucrose analgesia during the first week of life in neonates younger than 31 weeks' postconceptional age. *Pediatrics, 110*(3), 523-528.

Johnston, C. C., & Stevens, B. J. (1996). Experience in a neonatal intensive care unit affects pain response. *Pediatrics, 98*(5), 925-930.

Johnston, C. C., Stevens, B., Craig, K. D., & Grunau, R. V. E. (1993). Developmental changes in pain expression in premature, full-term, two- and four-month-old infants. *Pain, 52*(2), 201-208.

Johnston, C. C., Stevens, B., Pinelli, J., Gibbins, S., Filion, F., Jack, A., Steele, S., Boyer, K., & Veilleux, A. (2003). Kangaroo care is effective in diminishing pain response in preterm neonates. *Archives of Pediatrics & Adolescent Medicine, 157*(11), 1084-1088.

Jones, B., & Kenward, M. G. (1989). *Design and analysis of cross-over trials*. India: Chapman and Hall.

Kennedy, K. A., Fielder, A. R., Hardy, R. J., Tung, B., Gordon, D. C., Reynolds, J. D. (2001). Reduced lighting does not improve medical outcomes in very low birth weight infants. *The Journal of Pediatrics, 139*(4), 527-531.

Kennedy, K. A., Ipson, M. A., Birch, D. G., Tyson, J. E., Anderson, J. L., Nusinowitz, S., West, L, Spencer, R., & Birch, E. E. (1997). Light reduction and the electroretinogram of preterm infants. *Archives of Disease in Childhood. Fetal and Neonatal Edition, 76*(3), F168-F173.

Kent, W. D. T., Tan, A. K. W., Clarke, M. C., & Bardell, T. (2002). Excessive noise levels in the neonatal ICU: Potential effects on auditory system development. *The Journal of Otolaryngology, 31*(6), 355- 360.

Kleiger, R. E., Stein, P. K., Bosner, M. S., & Rottman, J. N. (1995). Time-domain measurements of heart rate variability. In M. Malik, & A. J. Camm (Eds.), *Heart Rate Variability* (pp. 33-45). New York: Futura Publishing Company Inc.

Krueger, C., Wall, S., Parker, L., & Nealis, R. (2005). Elevated sound levels within a busy NICU. *Neonatal Network, 24*(6), 33-37.

Lee, Y-H., Malakooti, N., & Lotas, M. (2005). A comparison of the light-reduction capacity of commonly used incubators covers. *Neonatal Network, 24*(2), 37-44.

Levy, G. D., Woolston, D. J., & Browne, J. V. (2003). Mean noise amounts in level II vs level III neonatal intensive care units. *Neonatal Network, 22*(2), 33-38.

Lickliter, R. (2000). Atypical perinatal sensory stimulation and early perceptual development: Insights from developmental psychobiology. *Journal of Perinatology, 20*(8s), S45-S54.

Lindh, V., Wiklund, U., Sandman, P-O., & Håkansson, S. (1997). Assessment of acute pain in preterm infants by evaluation of facial expression and frequency domain analysis of heart rate variability. *Early Human Development, 48*(1-2), 131-142.

Long, J. G., Lucey, J. F., & Philip, A. G. S. (1980). Noise and hypoxemia in the intensive care nursery. *Pediatrics, 65*(1), 143-145.

Long, J. C., Philip, A. G. S., Lucey, J. F. (1980). Excessive handling as a cause of hypoxemia. *Pediatrics, 65*(2), 203-207.

Longin, E., Schaible, T., Lenz, T., & König, S. (2005). Short term heart rate variability in healthy neonates : Normative data and physiological observations. *Early Human Development, 81*(8), 663-671.

Lotas, M. J. (1992). Effects of light and sound in the neonatal intensive care unit environment on the low-birth-weight infant. *NAACOG's Clinical Issues 3*(1), 34–44.

Ludington-Hoe, S. M., Hosseini, R., & Torowicz, D. L. (2005). Skin-to-skin contact (kangaroo care) analgesia for preterm infant heel stick. *AACN Clinical Issues, 16*(3), 373-387.

Malik, M. (1995). Geometrical methods for heart rate variability assessment. In M. Malik, & A. J. Camm (Eds.), *Heart Rate Variability* (pp. 47-61). New York: Futura Publishing Company Inc.

Mann, N. P., Haddow, R., Stokes, L., Goodley, S., & Rutter, N. (1986). Effect of night and day on preterm infants in a newborn nursery: randomized trial. *British Medical Journal, 293*(6557), 1265-1267.

McIntosh, N., Van Veen, L., & Brameyer, H. (1993). The pain of heel prick and its measurement in preterm infants. *Pain, 52*(1), 71-74.

Mendell, L. M., & Wall, P. D. (1965). Responses of single dorsal cord cells to peripheral cutaneous unmyelinated fibres. *Nature, 206*(4979), 97-99.

Miller, C. L., White, R., Whitman, T. L., O'Callaghan, M. F., & Maxwell, S. E. (1995). The effects of cycled versus noncycled lighting on growth and development in preterm infants. *Infant Behavior and Development, 18*(1), 87-95.

Mirmiran, M., Baldwin, R. B., & Ariagno, R. L. (2003). Circadian and sleep development in preterm infants occurs independently from the influences of environmental lighting. *Pediatric Research, 53*(6), 933-938.

Modrcin-McCarthy, M., McSue, S., & Walker, J. (1997). Preterm infants and STRESS: a tool for the neonatal nurse. *Journal of Perinatal and Neonatal Nursing, 10*(4), 62-71.

Monterosso, L., Kristjanson, L., & Cole, J. (2002). Neuromotor development and the physiologic effects of positioning in very low birth weight infants. *Journal of Obstetric, Gynecologic, & Neonatal Nursing, 31*(2), 138-146.

Morris, B. H., Philbin, M. K., & Bose, C. (2000). Physiological effects of sound on the newborn. *Journal of Perinatology, 20*(8s), S54-S59.

Neu, M., Goldstein, M., Gao, D., & Laudenslager, M. L. (2007). Salivary cortisol in preterm infants: Validation of a simple method for collecting saliva for cortisol determination. *Early Human Development, 83*(1), 47-54.

Nzama, N. P. B., Nolte, A. G. W., & Dörfling, C. S. (1995). Noise in a neonatal unit: Guidelines for the reduction or prevention of noise. *Curationis, 18*(2), 16-21.

Oberlander, T., & Saul, J. P. (2002). Methodological considerations for the use of heart rate variability as a measure of pain reactivity in vulnerable infants. *Clinics in Perinatology, 29*(3), 427-443.

Paquette, G., Le May, S., & Aita, M. (2007). Revue systématique des méthodes non pharmacologiques de soulagement de la douleur chez les prématurés. *Douleur et analgésie*, *20*(3), 180-192.

Patural, H., Barthelemy, J. C., Pichot, V., Mazzocchi, C., Teyssier, G., Damon, G., & Roche, E. (2004). Birth prematurity determines prolonged autonomic nervous system immaturity. *Clinical Autonomic Research, 14*(6), 391-395.

Perlman, J. M. (2001). Neurobehavioral deficits in premature graduates of intensive care - Potential medical and neonatal environmental risk factors. *Pediatrics, 108*(6), 1339-1348.

Peters, K. L. (1998). Bathing premature infants: Physiological and behavioral consequences. *American Journal of Critical Care, 7*(2), 90-100.

Peters, K. L. (1999). Infant handling in the NICU: Does developmental care make a difference? An evaluative review of the literature. *Journal of Perinatal and Neonatal Nursing, 13*(3), 83-109.

Phelps, D. L., & Watts, J. L. (2001). Early light reduction for preventing retinopathy of prematurity in very low birth weight infants. *Cochrane Database of Systematic Reviews, 2001*(1), CD000122.

Philbin, M. K. (1996). Some implications of early auditory development for the environment of hospitalized preterm infants. *Neonatal Network, 15*(8), 71-73.

Philbin, M. K. (2000). The influence of auditory experience on the behavior of preterm newborns. *Journal of Perinatology, 20*(8s), S76-S86.

Philbin, M. K., Roberston, A., & Hall III, J. W. (1999). Recommended permissible noise criteria for occupied, newly constructed or renovated hospital nurseries. *Journal of Perinatology, 19*(8), 559-563.

Pinelli, J., Symington, A., & Ciliska, D. (2002). Nonnutritive sucking in high-risk infants: Benign intervention or legitimate therapy? *Journal of Obstetric, Gynecologic, & Neonatal Nursing, 31*(5), 582-591.

Polit, D. F., & Hungler, B. P. (1999). *Nursing Research. Principles and Methods* (6^{th} ed). New York: Lippincott.

Pomeroy, S. L., & Ullrich, N. J. (2004). Development of the nervous system. In R. A. Polin, W. W. Fox, & Abman, S. H. (Eds.), Fetal and Neonatal Physiology. Volume 2 (3^{rd} ed., pp. 1675-1698). Philadelphia: Saunders.

Porges, S. W. (1992). Vagal tone: A physiologic marker of stress vulnerability. *Pediatrics, 90*(3), 498-504.

Porges, S. W., Doussard-Roosevelt, J. A., Stifter, C. A., McClenny, B. D., & Riniolo, T. C. (1999). Sleep state and vagal regulation of heart period patterns in the human newborn: An extension of the polyvagal theory. *Psychophysiology, 36*(1), 14-21.

Porter, F. L., Wolf, C. M., & Miller, J. P. (1998). The effect of handling and immobilization on the response to acute pain in newborn infants. *Pediatrics, 102*(6), 1383-1389.

Porter, F. L., Wolf, C. M., & Miller, J. P. (1999). Procedural pain in newborn infants: The influence of intensity and development. *Pediatrics, 104*(1), 1-10.

Portney, L. G., & Watkins, M. P. (2000). *Foundations of clinical research. Applications to practice* (2nd ed.). New Jersey: Prentice Hall Health.

Pressler, J. L., Turnage-Carrier, C. S., & Kenner, C. (2004). Developmental Care: An overview. In C. Kenner, & J. M. McGrath (Eds.), *Developmental Care of Newborns & Infants. A Guide for Health Professionals* (pp. 1-34). St-Louis: Mosby.

Purdy, I. B. (2000). Newborn auditory follow-up. *Neonatal Network, 19*(2), 25-33.

Ranger, M., Johnston, C. C., & Anand, K. J. S. (2007). Current controversies regarding pain assessment in neonates. *Seminars in Perinatology, 31*(5), 283-288.

Richard, C. A., & Mosko, S. S. (2004). Mother-infant bedsharing is associated with an increase in infant heart rate. *Sleep, 27*(3), 507-511.

Rivkees, S. A., Mayes, L., Jacobs, H., & Gross, I. (2004). Rest-activity patterns of premature infants are regulated by cycled lighting. *Pediatrics, 113*(4), 833-839.

Robertson, A., Cooper-Peel, C., & Vos, P. (1999). Contribution of heating, ventilation, and air conditioning airflow and conversation to the ambient sound in a neonatal intensive care unit. *Journal of Perinatology, 19*(5), 362-366.

Robinson, J., & Fielder, A. R. (1990). Pupillary diameter and reaction to light in preterm neonates. *Archives of Disease in Childhood*, *65*(1), 35-38.

Robinson, J., & Fielder, A. R. (1992). Light and the neonatal eye. *Behavioural Brain Research, 49*(1), 51-55.

Robinson, J., Moseley, M. J., & Fielder, A. R. (1990). Illuminance of neonatal units. *Archives of Disease in Childhood, 65*(7), 679–682.

Robinson, J., Moseley, M. J., Thompson, J. R., & Fielder, A. R. (1989). Eyelid opening in preterm neonates. *Archives of Disease in Childhood, 64*(7), 943-948.

Rosenstock, E. G., Cassuto, Y., & Zmora, E. (1999). Heart rate variability in the neonate and infant: analytical methods, physiological and clinical observations. *Acta Paediatrica, 88*(5), 477-482.

Roy, M-S., Caramelli, C., Orquin, J., Uleckas, J., Hardy, P., & Chemtob, S. (1999). Effects of early reduced light exposure on central visual development in preterm infants. *Acta Paediatrica, 88*(4), 459-461.

Sammons, W. A. H., & Lewis, J. M. (1985). *Premature babies. A different beginning*. Toronto: The C.V. Mosby Company.

Saunders, A. N. (1995). Incubator noise: A method to decrease decibels. *Pediatric Nursing, 21*(3), 265–268.

Senn, S. (2002). *Cross-over Trials in Clinical Research* (2nd ed.). Ontario: John Wiley and Sons, Ltd.

Shiroiwa, Y., Kamiya, Y., Uchibori, S., Inukai, K, Kito, H., Shibata, T., & Ogawa, J. (1986). Activity, cardiac and respiratory responses of blindfold preterm infants in a neonatal intensive care unit. *Early Human Development, 14*(3-4), 259-265.

Shogan, M. G., & Schumann, L. L. (1993). The effect of environmental lighting on the oxygen saturation of preterm infants in the NICU. *Neonatal Network, 12*(5), 7-13.

Silverman, W. A. (1999). Effects of premature exposure to light: a credibility struggle. *Paediatric and Perinatal Epidemiology, 13*(2), 128-130.

Simons, S. H. P., & Tibboel, D. (2006). Pain perception development and maturation. *Seminars in Fetal & Neonatal Medicine, 2006*(11), 227-231.

Simons, S. H. P., van Dick, M., Anand, K. S., Roofthooft, D., van Lingen, R. A., Tibboel, D. (2003). Do we still hurt newborn babies? A prospective study of procedural pain and analgesia in neonates. *Archives of Pediatrics and Adolescent Medicine, 157*(11), 1058-1064.

Sizun, J., Ansquer, H., Browne, J., Tordjman, S., & Morin, J-F. (2002). Developmental care decreases physiologic and behavioral pain expression in preterm neonates. *The Journal of Pain, 3*(6), 446-450.

Slater, R., Cantarella, A., Gallella, S., Worley, A., Boyd, S., Meek, J., & Fitzgerald, M. (2006). Cortical pain responses in human infants. *The Journal of Neuroscience, 26*(14), 3662-3666.

Slevin, M., Farrington, N., Duffy, G., Daly, L., & Murphy, J. F. A. (2000). Altering the NICU and measuring infants' responses. *Acta Paediatrica, 89*(5), 577-581.

Sparshott, M. M. (1995). 'The sound' – of neonatal intensive care. Effect of noise levels in the neonatal unit on the sleep patterns of sick preterm infants. *Journal of Neonatal Nursing, jan*, 7-9.

Stevens, B., Franck, L., Gibbins, S., McGrath, P. J., Dupuis, A., & Yamada, J., & Beyene, J., Camfield, C., Finley, G. A., Johnston, C., O'Brien, K., & Ohlsson, A. (2007). Determining the structure of acute pain responses in vulnerable neonates. *Canadian Journal of Nursing Research, 39*(2), 32-47.

Stevens, B. J., & Franck, L. (1995). Special needs of preterm infants in the management of pain and discomfort. *Journal of Obstetric, Gynecologic and Neonatal Nursing, 24*(9), 856-862.

Stevens, B., & Gibbins, S. (2002). Clinical utility and clinical significance in the assessment and management of pain in vulnerable infants. *Clinics in Perinatology, 29*(3), 1-10.

Stevens, B., Gibbins, S., & Franck, L. S. (2000). Treatment of pain in the neonatal intensive care unit. *Pediatric Clinics of North America. 47*(3), 633-50.

Stevens, B. J., & Johnston, C. C. (1994). Physiological responses of premature infants to a painful stimulus. *Nursing Research, 43*(4), 226-231.

Stevens, B., Johnston, C., Franck, L., Petryshen, P., Jack, A., & Foster, G. (1999). The efficacy of developmentally sensitive interventions and sucrose for relieving procedural pain in very low birth weight neonates, *Nursing Research, 48*(1), 35-43.

Stevens, B. J., Johnston, C. C., & Grunau, R. V. E. (1995). Issues of assessment of pain and discomfort in neonates, *Journal of Obstetric, Gynecologic, and Neonatal Nursing, 24*(9), 849-855.

Stevens, B. J., Johnston, C. C., & Horton, L. (1993). Multidimensional pain assessment in premature neonates: A pilot study. *Journal of Obstetric, Gynecologic, and Neonatal Nursing, 22*(6), 531-541.

Stevens, B. J., Johnston, C. C., & Horton, L. (1994). Factors that influence the behavioral pain responses of premature infants. *Pain, 59*(1), 101-109.

Stevens, B., Johnston, C., Petryshen, P., & Taddio, A. (1996). Premature infant pain profile: Development and initial validation. *Clinical Journal of Pain, 12*(1), 13-22.

Stevens, B., Petryshen, P., Hawkins, J., Smith, B., & Taylor, P. (1996). Developmental care versus conventional care: A comparison of clinical outcomes for very low birth weight infants. *Canadian Journal of Nursing Research, 28*(4), 97-113.

Stevens, B., Yamada, J., & Ohlsson, A. (2004). Sucrose for analgesia in newborn infants undergoing painful procedures. *Cochrane Database of Systematic Reviews, Issue 3*. Art. No.: CD001069. DOI:1002/14651858.CD001069.pub2.

Strauch, C., Brandt, S., & Edwards-Beckett, J. (1993). Implementation of a quiet hour: Effects on noise levels and infant sleep states. *Neonatal Network, 12*(2), 31-35.

Surenthiran, S. S., Wilbraham, K., May, J., Chant, T., Emmerson, A. J. B., & Newton, V. E. (2003). Noise levels within the ear and post-nasal space in neonates in intensive care. *Archives of Disease in Childhood. Fetal and Neonatal Edition, 88*(4), F315-F318.

Symington, A., & Pinelli, J. (2006). Developmental care for promoting development and preventing morbidity in preterm infants. *Cochrane Database of Systematic Reviews*, Issue 2. Art. No.: CD001814. DOI: 10.1002/14651858.CD001814.pub2.

Thiringer, K., Kankkunen, A., Lidén, G., & Niklasson, A. (1984). Perinatal risk factors in the aetiology of hearing loss in preschool children. *Developmental Medicine & Child Neurology, 26*(6), 799-807.

Thomas, K. A., & Martin,. P. A. (2000). NICU sound environment and the potential problems for caregivers. *Journal of Perinatology, 20*(8s), S93-S98.

Thomas, K. A., & Uran, A. (2007). How the environment sounds to a preterm infant: Update. *MCN The American Journal of Maternal/Child Nursing, 32*(4), 250-253.

Turkewitz, G., & Kenny, P. A. (1982). Limitations on input as a basis for neural organization and perceptual development: A preliminary theoretical statement. *Developmental Psychobiology, 15*(4), 357-368.

Vanhatalo, S., & van Nieuwenhuizen, O. (2000). Fetal pain? *Brain & Development, 22*(3), 145-150.

Verklan, M. T., & Padhye, N. S. (2004). Spectral analysis of heart rate variability: An emerging tool for assessing stability during transition to extrauterine life. *Journal of Obstetric, Gynecology and Neonatal Nurses, 33*(2), 256-265.

Volpe, J. J. (2000). Overview: Normal and abnormal human brain development. *Mental Retardation and Developmental Disabilities Research Reviews, 6*(1), 1-5.

Warren, I. (2002). Facilitating infant adaptation: The nursery environment. *Seminars in Neonatology, 7*(6), 459-467.

Wechsler, S. B., & Wernovsky, G. (2008). Cardiac disorders. In J. P. Cloherty, E. C. Eichenwald, & A. R. Stark (Eds.), *Manual of Neonatal Care* (6th ed., pp. 388-435). New York: Lippincott Williams & Wilkins.

Wharrad, H. J., & Davis, A. C. (1997). Behavioural and autonomic responses to sound in pre-term and full-term babies. *British Journal of Audiology, 31*, 315-329.

White, R. D. (2004). Lighting design in the neonatal intensive care unit: Practical applications of scientific principles. *Clinics in Perinatology, 31*(2), 323-330.

White-Traut, R. C, Nelson, M. N., Burns, K., Cunningham, N. (1994). Environmental influences on the developing premature infant: Theoretical issues and applications to practice. *Journal of Obstetric, Gynecologic, and Neonatal Nursing, 23*(5), 393–401.

Woolf, C. J., & Mannion, R. J. (1999). Neuropathic pain: aetiology, symptoms, mechanisms, and management. *The Lancet, 353*(9168), 1959-1964.

Woolf, C. J., & Salter, M. W. (2000). Neuronal plasticity: Increasing the gain in pain. *Science, 288*(9), 1765-1768.

Zahr., L. K., Balian, S. (1995). Responses of premature infants to routine nursing interventions and noise in the NICU. *Nursing Research, 44*(3), 179–185.

Zahr, L. K., & de Traversay, J. (1995). Premature infant responses to noise reduction by earmuffs: Effects on behavioral and physiologic measures. *Journal of Perinatology, 15*(6), 448-455.

Zwick, M. B. (1993). Decreasing environmental noise in the NICU through staff education. *Neonatal Intensive Care, 6*(2), 16-19.

APPENDIX G

Correlation Matrices

Table 3.

Intercorrelations between Gestational Age and Outcome Measures for the Intervention Period in Cross-Over Trial (N = 54)

	GA[a]	HR[b]	HR min	HR max	R-R[c]	SDNN[d]	R-R min	R-R max	Log LF[e]	Log HF[f]	Ratio LF/HF	O_2[g]	O_2 min	O_2 max
GA[a]	---	-.12	.11	.12	.15	.25	-.01	.03	.15	.16	-.12	.14	-.05	.19
HR[b]	-.12	---	.18	.61**	-.99**	-.36*	-.86**	-.58**	-.35*	.34*	.21	-.11	-.22	.08
HR min	.11	.18	---	.16	-.19	.18	-.12	-.61**	-.68**	-.57**	.15	.12	.14	.10
HR max	.12	.61**	.16	---	-.58**	.61**	-.75**	-.21	-.04	.02	-.04	.12	-.20	.02
R-R[c]	.15	-.99**	-.19	-.58**	---	.41**	.84**	.61**	.38**	.39**	-.24	.12	.22	-.09
SDNN[d]	.25	-.36*	.18	61**	.41**	--	-.01	.76**	.75**	.76**	-.52**	.16	-.01	-.08
R-R min	-.01	-.86**	-.12	-.75**	.84**	-.01	---	.39**	.14	.11	.00	-.02	.20	-.08
R-R max	.03	-.58**	-.61**	-.21	.61**	.76**	.39**	---	.85**	.85**	-.48**	-.03	-.03	-.22
Log LF[e]	.15	-.35*	-.68**	-.04	.38**	.75**	.14	.85**	---	.92**	-.40**	.09	-.05	-.07
Log HF[f]	.16	-.34*	-.57**	.02	.39**	.76**	.11	.85**	.92**	---	-.65**	.04	-.01	-.12
Ratio LF/HF	-.12	.21	.15	-.04	-.24	-.52**	.00	-.48**	-.40**	-.65**	---	.02	-.09	.10
O_2[g]	.14	-.11	.12	.12	.12	.16	-.02	-.03	.09	.04	.02	---	.32*	.33*
O_2 min	-.05	-.22	.14	-.20	.22	-.01	.20	-.03	-.05	-.01	-.09	.32*	---	-.31*
O_2 max	.19	.08	.10	.02	-.09	-.08	-.08	-.22	-.07	-.12	.10	.33*	-.31*	---

[a] gestational age, [b] heart rate, [c] R-R intervals, [d] standard deviation of normal R-R intervals, [e] logarithm of low-frequency (LF) power, [f] logarithm of high-frequency (HF) power, [g] oxygen saturation, * $p < .05$, ** $p < .01$

Table 4.
Intercorrelations between Gestational Age and Outcome Measures for the Control Period in Cross-Over Trial (N = 54)

	GA[a]	HR[b]	HR min	HR max	R-R[c]	SDNN[d]	R-R min	R-R max	Log LF[e]	Log HF[f]	Ratio LF/HF	O_2[g]	O_2 min	O_2 max
GA[a]	---	-.10	.19	.12	.10	.28*	-.00	.11	.14	.18	-.04	.19	-.18	.27
HR[b]	-.10	---	.34*	.70**	-.99**	-.48**	-.86**	-.78**	-.41**	-.40**	.06	.01	-.02	.15
HR min	.19	.34*	---	.14	-.34*	-.38**	-.29*	-.59**	-.69**	-.48**	.16	.15	.10	.01
HR max	.12	.70**	.14	---	-.67**	.05	-.79**	-.40**	-.05	-.01	-.20	.25	.04	.23
R-R[c]	.10	-.99**	-.34*	-.67**	---	.52**	.83**	.78**	.42**	.41**	-.10	-.01	.04	-.14
SDNN[d]	.28	-.48**	-.38**	.05	.52**	---	.08	.77**	.73**	.74**	-.54**	.14	.02	.02
R-R min	-.00	-.86**	-.29*	-.79**	.83**	.08	---	.55**	.18	.11	.20	-.21	-.14	-.20
R-R max	.11	-.78**	-.59**	-.40**	.78**	.77**	.55**	---	.77**	.78**	-.35**	-.04	.02	-.14
Log LF[e]	.14	-.41**	-.69**	-.05	.42**	.73**	.18	.77**	---	.84**	-.35*	.10	.01	.10
Log HF[f]	.18	-.40**	-.48**	-.01	.41**	.74**	.11	.78**	.84**	---	-.58**	.11	.11	.00
Ratio LF/HF	-.04	.06	.16	-.20	-.10	-.54**	.20	-.35**	-.35*	-.58**	---	-.08	-.04	.10
O_2[g]	.19	.01	.15	.25	-.01	.14	.-21	-.04	.10	.11	-.08	---	.47*	.30*
O_2 min	-.18	-.02	.10	.04	.04	.02	-.14	.02	.01	.11	-.04	.47*	---	-.13
O_2 max	.27	.15	.01	.23	-.14	.02	-.20	-.14	.10	.00	.10	.30*	-.13	---

[a] gestational age, [b] heart rate, [c] R-R intervals, [d] standard deviation of normal R-R intervals, [e] logarithm of low-frequency (LF) power, [f] logarithm of high-frequency (HF) power, [g] oxygen saturation, * $p < .05$, ** $p < .01$

Table 9.

Intercorrelations between Handling and Outcomes Measures for the Intervention Period in Cross-Over Trial (N = 54)

	Hand-ling[a]	HR[b]	HR min	HR max	R-R[c]	SDNN[d]	R-R min	R-R max	Log LF[e]	Log HF[f]	Ratio LF/HF	O_2[g]	O_2 min	O_2 max
Handling[a]	---	.04	.06	-.01	-.01	-.08	-.01	.00	.00	.04	-.10	-.26	-.18	.02
HR[b]	.04	---	.18	.61**	-.99**	-.36*	-.86**	-.58**	-.35*	.34*	.21	-.11	-.22	.08
HR min	.06	.18	---	.16	-.19	.18	-.12	-.61**	-.68**	-.57**	.15	.12	.14	.10
HR max	-.01	.61**	.16	---	-.58**	.61**	-.75**	-.21	-.04	.02	-.04	.12	-.20	.02
R-R[c]	-.01	-.99**	-.19	-.58**	---	.41**	.84**	.61**	.38**	.39**	-.24	.12	.22	-.09
SDNN[d]	-.08	-.36*	.18	61**	.41**	--	-.01	.76**	.75**	.76**	-.52**	.16	-.01	-.08
R-R min	-.01	-.86**	-.12	-.75**	.84**	-.01	---	.39**	.14	.11	.00	-.02	.20	-.08
R-R max	.00	-.58**	-.61**	-.21	.61**	.76**	.39**	---	.85**	.85**	-.48**	-.03	-.03	-.22
Log LF[e]	.00	-.35*	-.68**	-.04	.38**	.75**	.14	.85**	---	.92**	-.40**	.09	-.05	-.07
Log HF[f]	.04	-.34*	-.57**	.02	.39**	.76**	.11	.85**	.92**	---	-.65**	.04	-.01	-.12
Ratio LF/HF	-.10	.21	.15	-.04	-.24	-.52**	.00	-.48**	-.40**	-.65**	---	.02	-.09	.10
O_2[g]	-.26	-.11	.12	.12	.12	.16	-.02	-.03	.09	.04	.02	---	.32*	.33*
O_2 min	-.18	-.22	.14	-.20	.22	-.01	.20	-.03	-.05	-.01	-.09	.32*	---	-.31*
O_2 max	.02	.08	.10	.02	-.09	-.08	-.08	-.22	-.07	-.12	.10	.33*	-.31*	---

[a] mean number of occasions of infants' handling, [b] heart rate, [c] R-R intervals, [d] standard deviation of normal R-R intervals, [e] logarithm of low-frequency (LF) power, [f] logarithm of high-frequency (HF) power, [g] oxygen saturation, * $p < .05$, ** $p < .01$

Table 10.

Intercorrelations between Handling and Outcome Measures for the Control Period in Cross-Over Trial (N = 54)

	Hand-ling[a]	HR[b]	HR min	HR max	R-R[c]	SDNN[d]	R-R min	R-R max	Log LF[e]	Log HF[f]	Ratio LF/HF	O_2[g]	O_2 min	O_2 max
Handling[a]	---	.05	.05	.21	-.03	.05	-.09	.01	.06	.04	-.06	.05	.13	.10
HR[b]	.05	---	.34*	.70**	-.99**	-.48**	-.86**	-.78**	-.41**	-.40**	.06	.01	-.02	.15
HR min	.05	.34*	---	.14	-.34*	-.38**	-.29*	-.59**	-.69**	-.48**	.16	.15	.10	.01
HR max	.21	.70**	.14	---	-.67**	.05	-.79**	-.40**	-.05	-.01	-.20	.25	.04	.23
R-R[c]	-.03	-.99**	-.34*	-.67**	---	.52**	.83**	.78**	.42**	.41**	-.10	-.01	.04	-.14
SDNN[d]	.05	-.48**	-.38**	.05	.52**	---	.08	.77**	.73**	.74 **	-.54**	.14	.02	.02
R-R min	-.09	-.86**	-.29*	-.79**	.83**	.08	---	.55**	.18	.11	.20	-.21	-.14	-.20
R-R max	.01	-.78**	-.59**	-.40**	.78**	.77**	.55**	---	.77**	.78**	-.35**	-.04	.02	-.14
Log LF[e]	.06	-.41**	-.69**	-.05	.42**	.73**	.18	.77**	---	.84**	-.35*	.10	.01	.10
Log HF[f]	.04	-.40**	-.48**	-.01	.41**	.74**	.11	.78**	.84**	---	-.58**	.11	.11	.00
Ratio LF/HF	-.06	.06	.16	-.20	-.10	-.54**	.20	-.35**	-.35*	-.58**	---	-.08	-.04	.10
O_2[g]	.05	.01	.15	.25	-.01	.14	-.21	-.04	.10	.11	-.08	---	.47*	.30*
O_2 min	.13	-.02	.10	.04	.04	.02	-.14	.02	.01	.11	-.04	.47*	---	-.13
O_2 max	.10	.15	.01	.23	-.14	.02	-.20	-.14	.10	.00	.10	.30*	-.13	---

[a] mean number of occasions of infants' handling, [b] heart rate, [c] R-R intervals, [d] standard deviation of normal R-R intervals, [e] logarithm of low-frequency (LF) power, [f] logarithm of high-frequency (HF) power, [g] oxygen saturation, * $p < .05$, ** $p < .01$

Table 33.

Intercorrelations between Confounding Variables and Logarithm transformations of LF and HF Power in Cross-Over Trial (N = 54)[a]

	Gestational Age	Postnatal Age	Log LF[b]	Log HF[c]
Gestational Age	---	.76**	.05	.07
Postnatal Age	.76**	---	.02	.03
Log LF[b]	.05	.02	---	.87**
Log HF[c]	.07	.03	.87**	---

[a] only first study sequence, [b] logarithm of low-frequency (LF) power, [c] logarithm of high-frequency (HF) power, ** $p < 0.01$

Table 41.

Intercorrelations between Extraneous Variables in RCT (N = 44)

	GA[a]	Postnatal Age	Painful procedures[b]	Handling[c]	Intubation (hrs)	Time to harvest blood (min)
GA[a]	---	.77**	-.38*	.03	-.03	-.12
Postnatal Age	.77**	---	-.05	-.04	.08	-.04
Painful procedures[b]	-.38*	-.05	---	.05	.43**	.15
Handling[c]	.03	-.04	.05	---	.14	.08
Intubation (hrs)	-.03	.08	.43**	.14	---	-.01
Time to harvest blood (min)	-.12	-.04	.15	.08	-.01	---

[a] gestational age, [b] previous painful procedures, [c] mean number of occasions of infants' handling, * $p < .05$, ** $p < .01$

Table 42.

Intercorrelations between Extraneous Variables and Outcome Measures in RCT (N = 44)

	GA[a]	Handling[b]	Intubation (hrs)	Time to harvest blood (min)	HR[c]	HR max[c]	R-R[cd] min	Log R-R[ce] max	RTB[f]
GA[a]	---	.03	-.03	-.12	.02	.03	-.07	-.02	.15
Handling[b]	.03	---	.14	.08	-.38*	-.33*	.31*	.14	.03
Intubation (hrs)	-.03	.14	---	-.01	.29	.24	-.22	-.12	.16
Time to harvest blood (min)	-.12	.08	-.01	---	-.06	.004	.03	.33*	-.01
HR[c]	.02	-.38*	.29	-.06	---	.92**	-.93**	-.36*	.26
HR max[c]	.03	-.33*	.24	.004	.92**	---	-.99	-.24	.18
R-R[cd] min	-.07	.31*	-.22	.03	-.93**	-.99**	---	.25	-.22
Log R-R[ce] max	-.02	.14	-.12	.33*	-.36*	-.24	.25	---	.15
RTB[f]	.15	.03	.16	-.01	.26	.18	-.22	.15	---

[a] gestational age, [b] mean number of occasions of infants' handling, [c] outcomes measured during the blood sample phase, [d] R-R intervals, [e] logarithm of maximum R-R intervals, [f] return to baseline, * $p < .05$, ** $p < .01$

APPENDIX H

Tables of Comparisons between Intubated and Non-Intubated Infants in Cross-Over Trial

Table 6.

Comparisons of Outcome Measures between Intubated and Non-Intubated Infants for the Intervention Period in Cross-Over Trial (N = 54)

	Intubated (n = 20)	Not Intubated (n = 34)	
Outcome Measures	*M (SD)*	*M (SD)*	*p*
HR (bpm) [a] (*M*)	160.40 (7.91)	156.13 (10.40)	.12[f]
HR (bpm) (*min*)	85.80 (20.31)	85.35 (18.96)	.94[f]
HR (bpm) (*max*)	204.75 (11.98)	194.12 (13.79)	.006[f]**
R-R intervals (ms)	376.55 (18.10)	386.10 (24.59)	.14[f]
SDNN (ms)[b]	19.83 (5.98)	19.69 (6.33)	.93[f]
R-R intervals (ms) (*min*)	330.67 (20.02)	342.34 (19.71)	.04[f]*
R-R intervals (ms) (*max*)	480.96 (51.14)	484.30 (47.97)	.81[f]
Log LF[c] (ms^2)	4.68 (0.79)	4.75 (0.79)	.74[f]
Log HF[d] (ms^2)	2.93 (1.05)	3.03 (0.92)	.71[f]
Ratio LF/HF	8.68 (3.44)	8.21 (3.31)	.62[f]
O_2[e] (%) (*M*)	93.51 (2.34)	94.28 (3.68)	.40[f]
O_2 (%) (*min*)	62.45 (12.39)	67.62 (12.70)	.15[f]
O_2 (%) (*max*)	99.30 (1.66)	99.68 (0.64)	.24[f]

[a] heart rate in beats per minute, [b] standard deviation of normal R-R intervals, [c] logarithm of low-frequency (LF), [d] logarithm of high-frequency (HF), [e] oxygen saturation in percentage, [f] Independent samples *t* test, * $p < .05$, ** $p < .01$

Table 7.

Comparisons of Outcome Measures between Intubated and Non-Intubated Infants for the Control Period in Cross-Over Trial (N = 54)

	Intubated (n = 20)	Not Intubated (n = 34)	
Outcome Measures	*M (SD)*	*M (SD)*	*p*
HR (bpm) [a] (*M*)	160.66 (8.56)	155.74 (9.62)	.07[f]
HR (bpm) (*min*)	89.35 (19.05)	91.02 (19.35)	.76[f]
HR (bpm) (*max*)	197.05 (11.06)	191.97 (13.73)	.17[f]
R-R intervals (ms)	373.10 (19.72)	386.60 (24.01)	.04[f*]
SDNN (ms)[b]	18.09 (4.49)	19.84 (6.54)	.30[f]
R-R intervals (ms) (*min*)	332.54 (20.54)	340.71 (19.38)	.15[f]
R-R intervals (ms) (*max*)	464.52 (41.82)	482.83 (52.17)	.19[f]
Log LF[c] (ms^2)	4.58 (0.66)	4.66 (0.73)	.68[f]
Log HF[d] (ms^2)	2.85 (0.93)	2.97 (0.89)	.64[f]
Ratio LF/HF	8.72 (3.56)	8.47 (3.51)	.80[f]
O_2 (%)[e] (*M*)	92.73 (3.66)	94.06 (3.51)	.19[f]
O_2 (%) (*min*)	62.80 (12.10)	66.18 (13.07)	.35[f]
O_2 (%) (*max*)	99.55 (1.15)	99.47 (1.08)	.80[f]

[a] heart rate in beats per minute, [b] standard deviation of normal R-R intervals, [c] logarithm of low-frequency (LF), [d] logarithm of high-frequency (HF), [e] oxygen saturation in percentage, [f] Independent samples *t* test, * $p < .05$

APPENDIX I

Tables of Descriptive Statistics for the Outcome Measures of Physiological Stability

Table 11.

Descriptive Statistics for Mean Heart Rate (bpm) per Study Sequence and Period in Cross-Over Trial (N = 54)

	Intervention – Control (*n* = 28)			Control - Intervention (*n* = 26)		
	M (SD)	min	max	*M (SD)*	min	max
1st period	158.21 (10.28)	141.31	176.19	156.80 (8.83)	144.27	173.08
2nd period	158.14 (10.11)	136.62	176.79	157.19 (9.21)	141.92	174.27

Table 12.

Descriptive Statistics for Minimum Heart Rate (bpm) per Study Sequence and Period in Cross-Over Trial (N = 54)

	Intervention – Control (n = 28)			Control – Intervention (n = 26)		
	M (SD)	min	max	*M (SD)*	min	max
1st period	89.04 (20.69)	49	131	88.65 (16.80)	51	123
2nd period	91.39 (21.11)	57	135	81.73 (17.23)	48	110

Table 13.

Descriptive Statistics for Maximum Heart Rate (bpm) per Study Sequence and Period in Cross-Over Trial (N = 54)

	Intervention – Control (n = 28)			Control – Intervention (n = 26)		
	M (SD)	min	max	*M (SD)*	min	max
1st period	197.61 (15.69)	171	225	194.38 (11.55)	173	225
2nd period	192.62 (13.96)	170	224	198.54 (12.28)	175	221

Table 14.

Descriptive Statistics for Mean R-R Intervals (ms) per Study Sequence and Period in Cross-Over Trial (N = 54)

	Intervention – Control (n = 28)			Control – Intervention (n = 26)		
	M (SD)	min	max	*M (SD)*	min	max
1st period	380.70 (23.80)	339.15	425.96	384.34 (22.27)	344.80	420.97
2nd period	379.64 (24.20)	338.71	437.32	384.58 (21.77)	343.16	422.09

Table 15.

Descriptive Statistics for SDNN (ms) per Study Sequence and Period in Cross-Over Trial (N = 50)

	Intervention – Control (*n* = 28)			Control – Intervention (*n* = 26)		
	M (SD)	min	max	*M (SD)*	min	max
1st period	18.43 (5.96)	9.02	35.04	21.33 (5.70)	11.98	33.53
2nd period	17.88 (5.84)	8.39	37.01	20.95 (6.37)	11.06	37.19

Table 16.

Descriptive Statistics for Minimum R-R Intervals (ms) per Study Sequence and Period in Cross-Over Trial (N = 54)

	Intervention – Control (n = 28)			Control – Intervention (n = 26)		
	M (SD)	min	max	*M (SD)*	min	max
1st period	338.39 (23.28)	298.79	377.29	336.89 (17.52)	305.23	366.25
2nd period	338.80 (22.35)	299.04	384.60	337.61 (17.31)	299.38	369.90

Table 17.

Descriptive Statistics for Maximum R-R intervals (ms) per Study Sequence and Period in Cross-Over Trial (N = 54)

	Intervention – Control (n=28)			Control – Intervention (n=26)		
	M (SD)	min	max	*M (SD)*	min	max
1st period	471.00 (51.09)	389.10	602.76	485.30 (44.49)	413.75	581.80
2nd period	468.60 (52.38)	390.42	598.67	496.06 (43.28)	409.62	610.69

Table 18.

Descriptive Statistics for LF Power (ms^2) in Cross-Over Trial before and after Logarithm Transformation (N = 54)

	LF Power before logarithm transformation			LF Power after logarithm transformation		
	M (SD)	min	max	*M (SD)*	min	max
Intervention (*n* = 28)	115.23 (105.73)	19.85	550.87	4.46 (0.75)	2.99	6.31
Control (*n* = 26)	153.40 (97.65)	32.77	378.76	4.80 (0.71)	3.49	5.94

Table 19.

Descriptive Statistics for HF Power (ms^2) in Cross-Over Trial before and after Logarithm Transformation (N = 54)

	HF Power before logarithm transformation			HF Power after logarithm transformation		
	M (SD)	min	max	*M (SD)*	min	max
Intervention (*n* = 28)	22.59 (21.88)	1.26	98.49	2.69 (1.00)	0.23	4.59
Control (*n* = 26)	31.93 (25.81)	4.26	127.21	3.17 (0.76)	1.45	4.85

Table 20.

Descriptive Statistics for LH/HF Ratio per Study Sequence and Period in Cross-Over Trial (N = 54)

	Intervention – Control (n = 28)			Control – Intervention (n = 26)		
	M (SD)	min	max	*M (SD)*	min	max
1st period	8.91 (3.78)	3.77	17.23	7.65 (3.02)	3.52	14.35
2nd period	9.37 (3.75)	4.11	17.25	7.83 (2.76)	4.37	13.65

Table 21.

Descriptive Statistics for Mean Oxygen Saturation (%) per Study Sequence and Period in Cross-Over Trial (N = 54)

	Intervention – Control (n = 28)			Control – Intervention (n = 26)		
	M (SD)	min	max	*M (SD)*	min	max
1st period	93.81 (2.62)	86.72	97.92	94.45 (3.25)	87.77	98.20
2nd period	92.74 (3.75)	81.34	98.12	94.20 (3.85)	83.34	98.35

Table 22.

Descriptive Statistics for Minimum Oxygen Saturation (%) per Study Sequence and Period in Cross-Over Trial (N = 54)

	Intervention – Control (*n* = 28)			Control – Intervention (*n* = 26)		
	M (SD)	min	max	*M (SD)*	min	max
1st period	62.54 (12.91)	50	85	67.08 (12.41)	50	92
2nd period	62.93 (12.89)	50	83	69.12 (11.81)	50	86

Table 23.

Descriptive Statistics for Maximum Oxygen Saturation (%) per Study Sequence and Period in Cross-Over Trial (N = 54)

	Intervention – Control (*n* = 28)			Control – Intervention (*n* = 26)		
	M (SD)	min	max	*M (SD)*	min	max
1st period	99.79 (0.42)	99	100	99.46 (1.36)	95	100
2nd period	99.54 (0.79)	97	100	99.27 (1.54)	93	100

APPENDIX J

RM-ANOVA and RM-ANCOVA for the Outcome Measures of Physiological Stability

Table 24.

Repeated Measures of ANOVA for Mean Heart Rate in Cross-Over Trial (N = 54)[1]

Source	*df*	*F*	η^2	*p*
Between subjects (from the model of the 1st stage analysis)				
Period * Intervention (carry-over)	1	0.22	---	.64
Between subjects (from the model of the 2nd stage analysis)				
Period	1	0.05	---	.83
Within subjects (from the model of the 2nd stage analysis)				
Intervention	1	0.09	0.06	.76

[1] All ANOVA tables show a combination of the results obtained from the first stage analysis testing for a carry-over effect and the second stage analysis testing for the period and intervention effects.

Table 25.

Repeated Measures of ANOVA for Minimum Heart Rate in Cross-Over Trial (N = 54)

Source	*df*	*F*	η^2	*p*
Between subjects (from the model of the 1st stage analysis)				
Period * Intervention (carry-over)	1	1.26	---	.27
Between subjects (from the model of the 2nd stage analysis)				
Period	1	1.02	---	.32
Within subjects (from the model of the 2nd stage analysis)				
Intervention	1	0.06	0.34	.81

Table 26.

Repeated Measures of ANCOVA for Maximum Heart Rate in Cross-Over Trial (N = 54)

Source	*df*	*F*	η^2	*p*
Between subjects (from the model of the 1st stage analysis)				
Period * Intervention (carry-over)	1	0.14	---	.71
Between subjects (from the model of the 2nd stage analysis)				
Period	1	0.00	---	1.00
Intubation	1	5.71	---	.02*
Intervention * intubation	1	2.44	---	.12
Within subjects (from the model of the 2nd stage analysis)				
Intervention	1	9.23	0.53	.004**

*$p < .05$, ** $p < .01$

Table 27.

Repeated Measures of ANCOVA for Mean R-R Intervals in Cross-Over Trial (N = 54)

Source	*df*	*F*	η^2	*p*
Between subjects (from the model of the 1st stage analysis)				
Period * Intervention (carry-over)	1	0.50	---	.48
Between subjects (from the model of the 2nd stage analysis)				
Period	1	0.03	---	.87
Intubation	1	2.37	---	.13
Intervention * intubation	1	0.49	---	.49
Within subjects (from the model of the 2nd stage analysis)				
Intervention	1	0.28	0.07	.60

Table 28.

Repeated Measures of ANCOVA for SDNN in Cross-Over Trial (N = 52)

Source	*df*	*F*	η^2	*p*
Between subjects (from the model of the 1st stage analysis)				
Period * Intervention (carry-over)	1	3.78	---	.06
Between subjects (from the model of the 2nd stage analysis)				
Period	1	0.21	---	.65
Gestational age	1	2.55	---	.01*
Intervention * gest. age	1	1.06	---	.44
Within subjects (from the model of the 2nd stage analysis)				
Intervention	1	0.77	0.04	.39

* $p < .05$

Table 29.

Repeated Measures of ANCOVA for Minimum R-R Intervals in Cross-Over Trial (N = 54)

Source	*df*	*F*	η^2	*p*
Between subjects (from the model of the 1st stage analysis)				
Period * Intervention (carry-over)	1	0.07	---	.80
Between subjects (from the model of the 2nd stage analysis)				
Period	1	0.03	---	.86
Intubation	1	2.96	---	.09
Intervention * intubation	1	0.68	---	.41
Within subjects (from the model of the 2nd stage analysis)				
Intervention	1	0.02	0.01	.88

Table 30.

Repeated Measures of ANOVA for Maximum R-R Intervals in Cross-Over Trial (N = 54)

Source	*df*	*F*	η^2	*p*
Between subjects (from the model of the 1st stage analysis)				
Period * Intervention (carry-over)	1	3.02	---	.09
Between subjects (from the model of the 2nd stage analysis)				
Period	1	0.63	---	.43
Within subjects (from the model of the 2nd stage analysis)				
Intervention	1	1.57	0.24	.22

Table 31.

Independent Samples t Test for LF Power (ms^2) in Cross-Over Trial (N = 54)

	Low-Frequency Power (ms^2)			
	M (SE)	*df*	*t*	*p*
Intervention (*n* = 28)	4.46 (0.75)	52	1.73	.09
Control (*n* = 26)	4.80 (0.71)			

Table 32.

Independent Samples t test for HF Power (ms^2) in Cross-Over Trial (N = 54)

	High-Frequency Power (ms^2)			
	M (SE)	*df*	*t*	*p*
Intervention (*n* = 28)	2.69 (1.00)	52	2.01	.0498[a]*
Control (*n* = 26)	3.17 (0.76)			

* $p < .05$

Table 34.

Repeated Measures of ANOVA for LF/HF Ratio in Cross-Over Trial (N = 54)

Source	*df*	*F*	η^2	*p*
Between subjects (from the model of the 1st stage analysis)				
Period * Intervention (carry-over)	1	2.51	---	.12
Between subjects (from the model of the 2nd stage analysis)				
Period	1	1.57	---	.22
Within subjects (from the model of the 2nd stage analysis)				
Intervention	1	0.21	0.11	.65

Table 35.

Repeated Measures of ANOVA for Oxygen Saturation in Cross-Over Trial (N = 54)

Source	*df*	*F*	η^2	*p*
Between subjects (from the model of the 1st stage analysis)				
Period * Intervention (carry-over)	1	1.53	---	.22
Between subjects (from the model of the 2nd stage analysis)				
Period	1	3.15	---	.08
Within subjects (from the model of the 2nd stage analysis)				
Intervention	1	1.21	0.22	.28

Table 36.

Repeated Measures of ANOVA for Minimum Oxygen Saturation in Cross-Over Trial (N = 54)

Source	*df*	*F*	η^2	*p*
Between subjects (from the model of the 1st stage analysis)				
Period * Intervention (carry-over)	1	3.23	---	.08
Between subjects (from the model of the 2nd stage analysis)				
Period	1	0.54	---	.46
Within subjects (from the model of the 2nd stage analysis)				
Intervention	1	0.25	0.09	.62

Table 37.

Repeated Measures of ANOVA for Maximum Oxygen Saturation in Cross-Over Trial (N = 54)

Source	*df*	*F*	η^2	*p*
Between subjects (from the model of the 1st stage analysis)				
Period * Intervention (carry-over)	1	1.10	---	.30
Between subjects (from the model of the 2nd stage analysis)				
Period	1	4.38	---	.04
Within subjects (from the model of the 2nd stage analysis)				
Intervention	1	0.07	0.07	.79

APPENDIX K

Figure Comparing Maximum Heart Rate of Intubated and Non-intubated Infants per Study Periods

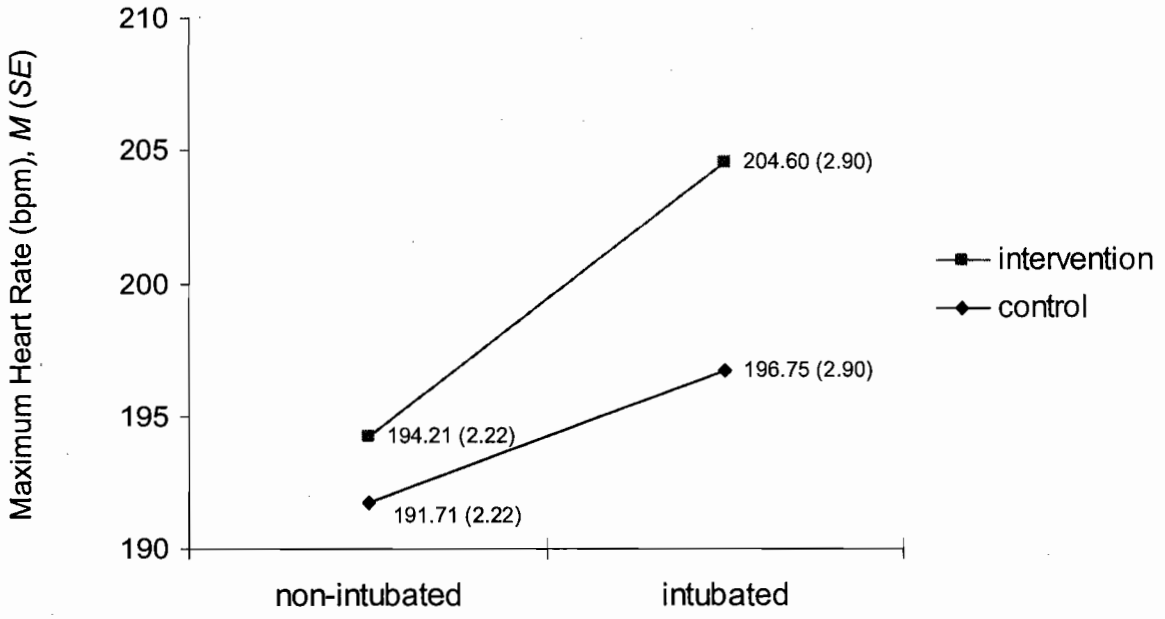

Figure 9. Comparison of maximum heart rate of intubated and non-intubated infants per study periods.

APPENDIX L

Tables of Comparisons between Handled and Non-Handled Infants before Heel Lance in RCT

Table 43.

Comparisons of Baseline Outcome Measures between Handled and Non-Handled Infants before the Painful Procedure in the Intervention Group (N = 23)

	Handled (n = 7)	Not Handled (n = 16)	
Outcomes Measures	*M (SD)*	*M (SD)*	*p*
HR (bpm)[a] (*M*)	166.29 (13.96)	160.56 (14.95)	.40[b]
HR (bpm) (*max*)	177.43 (16.35)	169.06 (16.94)	.28[b]
R-R Intervals (ms) (*min*)	339.29 (33.46)	356.00 (37.00)	.32[b]
R-R Intervals (ms) (*max*)	407.71 (45.94)	409.25 (37.75)	.93[b]

[a] heart rate, [b] independent samples *t* test

Table 44.

Comparisons of Baseline Outcome Measures between Handled and Non-Handled Infants before the Painful Procedure in the Control Group (N = 21)

	Handled (n = 5)	Not Handled (n = 16)	
Outcomes Measures	*M (SD)*	*M (SD)*	*p*
HR (bpm)[a] (*M*)	165.00 (8.34)	153.94 (13.05)	.048*[bc]
HR (bpm) (*max*)	175.20 (12.36)	170.19 (14.34)	.49[b]
R-R Intervals (ms) (*min*)	342.40 (24.38)	359.29 (25.21)	.21[b]
R-R Intervals (ms) (*max*)	395.60 (20.91)	453.63 (66.79)	.01*[bc]

[a] heart rate, [b] independent samples *t* test, [c] equal variance not assumed, * $p < .05$

APPENDIX M

ANCOVA for the Outcome Measures of Pain Response

Table 45.

ANCOVA for Mean Heart Rate during Blood Sample (N = 43)

Source	*df*	*F*	η^2	*p*
		Between subjects		
Intercept	1	20.33	0.35	.00**
Heart rate baseline	1	7.6	0.17	.01*
Handling	1	3.86	0.09	.06
Time to harvest blood sample	1	0.14	0.004	.71
Intervention	1	0.23	0.01	.63

* $p < .05$, ** $p < .01$

Table 46.

ANCOVA for Maximum Heart Rate during Blood Sample (N = 43)

Source	*df*	*F*	η^2	*p*
		Between subjects		
Intercept	1	5.04	0.12	.03*
Maximum heart rate baseline	1	15.63	0.29	.00**
Handling	1	0.51	0.01	.48
Time to harvest the blood sample	1	0.24	.01	.63
Intervention	1	0.14	0.04	.72

* $p < .05$, ** $p < .01$

Table 47.

ANCOVA for Minimum R-R Intervals during Blood Sample (N = 40)

Source	*df*	*F*	η^2	*p*
		Between subjects		
Intercept	1	7.99	0.19	.01*
Minimum R-R intervals baseline	1	11.64	0.25	.002**
Handling	1	0.85	0.02	.36
Time to harvest the blood sample	1	0.20	.01	.66
Intervention	1	0.04	0.001	.85

* $p < .05$, ** $p < .01$

Table 49.

ANCOVA for Logarithm of Maximum R-R Intervals during Blood Sample (N = 42)

Source	*df*	*F*	η^2	*p*
		Between subjects		
Intercept	1	22.59	0.37	.00**
Logarithm of maximum R-R intervals baseline	1	0.60	0.02	.44
Time to harvest blood sample	1	5.24	0.12	.03*
Intervention	1	0.23	0.01	.64

* $p < .05$, ** $p < .01$

Table 50.

ANCOVA for Return to Baseline following Blood Sample (N = 41)

Source	*df*	*F*	η^2	*p*
		Between subjects		
Intercept	1	10.13	0.21	.00**
Time to harvest blood sample	1	0.06	0.002	.80
Intervention	1	0.97	0.03	.33

** $p < .01$

APPENDIX N

Descriptive Statistics for Maximum R-R Intervals during Blood Sample before and after Logarithm Transformation

Table 48.

Descriptive Statistics for Maximum R-R Intervals (ms) during Blood Sample before and after Logarithm Transformation (N = 44)

	Max R-R intervals (ms) before logarithm transformation	Max R-R intervals (ms) after logarithm transformation
	M (SD)	*M (SD)*
Intervention (*n* = 28)	387.18 (80.79)	5.94 (0.17)
Control (*n* = 26)	384.85 (37.22)	5.95 (0.10)