Validation of the Synactive Theory of Development.

Are Body Movements In Preterm Infants Signs Of Stress?

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR

THE DEGREE OF

DOCTOR OF PHILOSOPHY

In

THE FACULTY OF GRADUATE STUDIES

Individual Interdisciplinary Graduate Studies Program

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

April, 2004

Abstract

The synactive theory of development, a widely used theory which has revolutionalized the assessment and treatment of preterm infants in the NICU, consists of five principles which are applied through a model of care. This model, the Newborn Individualized Developmental Care and Assessment Program® (NIDCAP) directs developmental specialists to interpret preterm infant movements either as stress or stability cues. However, limited empirical validation of this dualistic classification system has been reported.

The primary aim of this dissertation is to examine the validity of the NIDCAP® by studying the motor reactions of preterm infants in response to a continuum of stressor intensities which range from no stimulus to a painful stimulus. First, along with other valid biobehavioural measures of pain and in infants at 32 weeks corrected gestational age (GA), I examine the frequency of NIDCAP® movements during blood collection. Then, in a within subjects cross-over study (random order), I compare preterm infant biobehavioural pain reactions to responses during a tactile procedure. The secondary aim is to increase the accuracy and specificity of preterm infant pain assessment by determining whether NIDCAP® behaviours are reliable pain indicators and whether these indicators distinguish between pain and stress responses.

Of the 26 NIDCAP® stress cues, 14 movements (flex arms and legs, extend arms and legs, hand on face, finger splay, fisting, salute, yawn, sit on air, frown, tongue extension, air plane, eye floating) are associated with intrusive and painful procedures. Finger splay, fisting and hand on face are particularly salient stress cues in infants born at earlier GA (< 30 weeks). Contrary to the NIDCAP®, twitches decrease during the stressor phases in both studies. In addition, in response to painful and tactile

procedures, preterm infant body movements are often exaggerated, whereas facial responses are dampened.

In conclusion, the dualistic classification of the NIDCAP® needs revision; it also should incorporate alternative explanations of preterm infant movements. The use of body movements as stress response indicators is promising; however, facial reactivity is the most specific behavioural pain indicator in preterm infants. Future research is needed to refine further the measurement of stress responses in this vulnerable population.

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Acknowledgments

First, I would like to thank Dr. Ruth Grunau, mentor and PhD supervisor, who provided me with a world class, highly supportive interdisciplinary environment in which to work. Also, I would like to thank the other members of my PhD committee Dr. John Gilbert, Dr. Joanne Weinberg and Dr. Janet Werker who, like Dr. Grunau, facilitated a fascinating and efficient learning experience for me. I am also grateful to Sandra Bressler, Director of the Therapy Departments at British Columbia Children's and Women's Hospital, who encouraged me to pursue this advanced education and who arranged for my leave from my clinical duties. I would next like to thank Dr. Jo Powell, Dr. Grosvenor Powell, Dr. Kal Holsti and Peter Holsti Kaye for their continuous support and encouragement during this work and Dr. Matthew Holsti for answering technical questions when I found myself in the forest of hormone pathways. Finally, I would like to thank Dr. Michael Whitfield for supporting both my research and clinical endeavours over the last 18 years.

I am grateful to the staff and families of the Special Care Nursery at B.C.'s Children's Hospital for their participation in the clinical studies, Colleen Fitzgerald, Study Co-ordinator, Gisella Gosse and Adi Amir for data collection, and Colleen Jantzen for carrying out reliability NFCS coding, all of whom are staff of the Biobehavioral Research Unit of Centre for Community Health and Health Evaluation Research, B. C. Research Institute for Children's & Women's Health. Also I would like to thank Linda Williams, Clinical Supervisor, Physiotherapy Department, B. C.'s Children's Hospital, for carrying out NIDCAP® reliability coding.

Support for this work was provided by operating grants from the National Institutes of Health grant HD39783, the Canadian Institutes of Health Research grant

MOP42469, a Human Early Learning Partnership and the British Columbia Ministry of Children and Family Development grant 02-2410, and a Canadian Institutes of Health Research/Canadian Occupational Therapy Foundation Post-Doctoral Fellowship.

CHAPTER 1

Introduction

Twenty years ago, the environment of neonatal intensive care units (NICU) and the role of developmental specialists were vastly different from today. In each room of the nursery were rows of incubators in which infants, wearing only a diaper, lay flat on their backs, their arms and legs splayed. Radios played much of day; and the staff, often shouting up and down the rows, worked hurriedly to provide highly technical care to very tiny infants (See Appendix 1). The environment was often overwhelming for them and for staff (Lucey, 1977). Bright lights and high levels of noise were routine (Wolke, 1987); continuous alarms sounded, phones rang and the overhead paging system could sound at any time. Babies were handled often and for long periods of time. Parents were allowed to visit only during specific times, and they were never encouraged to stay when their infants required invasive procedures. Siblings of the infants were not permitted to come into the nursery for fear of spreading infection.

At that time, developmental specialists provided neurodevelopmental assessments and intervention only when the babies were medically stable. These assessments which involved handling the infants, were based upon the medical model, and were largely evaluations of reflexes, muscle tone and basic sensory function. Intervention focused on providing developmentally appropriate stimulation such as looking at and touching toys, facilitating more flexed postures and ensuring age appropriate feeding skills (e.g. Semmler, 1990). The rationale was that, because these infants had been deprived of "normal" stimulation, stimulation was what they needed.

Then in the early 1980's, a developmental psychologist, Dr. Heideleise Als described a new theory of development and proposed a radically different approach to

providing neonatal intensive care and developmental intervention. Her theory, the synactive theory of development (Als, 1982), was appealing because the theory was holistic and interdisciplinary, and the intervention strategies made sense intuitively. She linked the disabilities observed in preterm infants with their exposure to a developmentally unexpected environment, that of the neonatal intensive care unit. At that time, Als had little supporting evidence to show that early exposure to stress altered development. She specifically hypothesized that applying a new model of care, the Newborn Individualized Developmental Care and Assessment Program (NIDCAP®), would improve developmental outcomes of these high risk infants.

The focus of the NIDCAP® was to make the neonatal nursery environment much more protective of the infant's brain; that is, the environment should provide a quiet and developmentally sensitive place for high-risk newborns to recover and to grow. The model of care was family and baby centred, and it required that all disciplines in the nursery make a fundamental shift in their care giving philosophy. Als encouraged nurseries to paint their walls soft welcoming colors, to allow parents to stay with their infants whenever they wanted and to allow other family members to be involved and to see the new baby. She suggested that infants, unless there was a pressing medical need to do otherwise, be handled only when they were awake; that they should be bundled and nested, their limbs supported in a flexed position; and that their incubators be covered with quilts to shield them further from light and noise (See Appendix II). Als proposed that radios be removed, that phones have indicator lights rather than bell indicators, and that overhead paging systems be replaced with video cameras to locate staff.

In addition to vast changes in the nursery environment, Als provided a different strategy for assessing the developmental status of high-risk infants. Rather than wait until the infant was medically stable, Als believed that developmental assessments should begin on the baby's admission, should be done weekly throughout the baby's stay in the nursery and should involve no direct physical contact with the infant until they were close to term. Applying the NIDCAP®, developmental specialists were to observe each infant at the bedside and record the frequency of eighty five infant behaviours. Moreover, these observations were to be completed across a range of procedures so as to capture the range of responses that each infant had to procedures of varying levels of intensity, including those which were painful. Als believed that these behaviours were indications of the ability of each infant to communicate actively and to interact with the environment, and that by applying the principle of dual antagonistic systems, each behaviour could be interpreted as signs of stress or stability. The goal of these assessments was to record the infant's individual developmental agenda and to provide suggestions for altering the baby's environment and care to support optimal development.

Since Als introduced her pioneering ideas, multiple lines of evidence show that the nursery environment was and is vastly over-stimulating (Fiedler & Robinson,1995; Lasky, 1995). Increasing evidence from animal and human studies shows that early exposure to stressors, such as those experienced in the NICU, may alter brain development (Garg, Narsinghani, Bhutta, Rovanaghi & Anand, 2003; Bhutta & Anand, 2002). Additionally, the changes in brain development are linked with long-term alterations in development and behaviour. Significantly for this dissertation, early pain and stress responses of preterm infants induce behavioural and physiological changes

which then may directly and indirectly contribute to their neurodevelopmental and behavioral impairments (Grunau, 2002, 2003). Such evidence supports Als' supposition that early exposure to stressors alters development.

When Als introduced the synactive theory, she did not have empirical data supporting her claims; in the ensuing years, a number of researchers have tested her primary hypothesis by evaluating preterm infant outcomes following the implementation of the NIDCAP®. A number of studies have found preterm infants cared for using the NIDCAP® model showed improvements in respiratory status, increased physiological stability, and lower incidence of intraventricular hemorrhage. These infants also tended to be fed earlier, were discharged earlier and had better scores on early developmental testing. Even with these positive findings, questions regarding the efficacy of the NIDCAP® have remained because many of the randomized trials have methodological flaws in their design, few studies have evaluated developmental outcomes beyond preschool ages and some studies showed no differences in medical outcomes. In fact, a theoretically more fundamental reason may explain the conflicting outcomes: the individual principles underlying the theory have had limited empirical validation.

The synactive theory applies the principle of dual antagonistic systems of motor development as an overriding principle of development and also extends the principle to the interpretation of preterm infant movements such that infant behaviours are dualistically classified as either stress or stability movements. However, clinicians are also directed to remain flexible in their interpretations of these movements. In one instance, a specific movement may be an indication of stability; while in another, an indication of stress.

Assessing the validity of this principle of interpretation of preterm infant movements is vital because the NIDCAP® is the only developmentally relevant, comprehensive tool for recording behavioural and physiological stress responses in preterm infants and because the results of NIDCAP® assessments directly affect the clinical care of these infants. For example, if NIDCAP® assessments lead clinicians to believe that infants are in "pain" or are too "stressed", they may be given sedatives or analgesics. Sedatives often do not act specifically as analgesics; using them when pain is present is inappropriate, since the detrimental physiological side effects of pain would not be controlled. In contrast, administering analgesics may act differently in the brain according to whether or not pain is present (Rahman, Fitzgerald, Aynsley-Green & Dickenson, 1997). Thus administering analgesics only when pain is present may be critical for preventing undesirable, long-term side effects of opioid use. Moreover, accurate identification of pain behaviours allows appropriate use of non-pharmacological interventions, such as non-nutritive sucking, sucrose and kangaroo care (Johnston et al., 2003; Stevens et al., 1999; Stevens & Ohlsson, 2001).

Three previous studies have examined the interpretation of NIDCAP® movements in biobehavioural studies of preterm infant pain (Grunau, Holsti, Whitfield & Ling, 2000; Grunau, Oberlander, Holsti & Whifield, 1998; Morison et al., 2003), and in agreement with Als, found a small number movements were associated with highly intrusive or painful events. However, these studies have limitations such as a small sample size, short observation periods or incomplete use of the NIDCAP® assessment tool.

Building on previous work, this dissertation has both a theoretical and a clinical focus. The primary aim is to examine the validity of the dual antagonistic systems as

applied to the classification of NIDCAP® behaviours by studying preterm infants' motor responses across a continuum of stressor intensities, including stimuli which are acutely painful. First, concurrently with valid biobehavioural measures of pain, we examine the presence of NIDCAP® movements during blood collection. Then, I compare preterm infant biobehavioural pain responses to their responses to an event which is not painful, but nevertheless stressful. In this way, I can identify behaviours which are associated with a known stressor and ensure that I assess the NIDCAP® during stimuli which vary in intensity.

The secondary aim is to aid clinicians in making preterm infant pain assessment more accurate and specific. Although not specifically designed to assess pain, NIDCAP® assessments are completed during painful procedures. Moreover, using the NIDCAP® to assess pain is appealing because it provides specific descriptions of movements which are developmentally appropriate for preterm infants. Indeed, accurate pain assessment is critical for directing appropriate pain management strategies. However, pain assessment in preterm infants is complex, and despite the growing number of infant pain assessments, many of these tools have been developed for term infants or lack appropriate tests of their psychometric properties. Therefore, I will determine whether NIDCAP® behaviours are reliable pain indicators and whether these indicators will help clinicians distinguish between painful and stressful procedures in this high risk population.

This dissertation is organized so that all the background regarding stress and pain (Chapters 2, 3 and 4) precedes the critical analysis of the synactive theory of development (Chapter 5). This sequencing is to make it easier for the reader to link the theory in Chapter 5 with the empirical work presented in Chapters 6 and 7. In addition,

although Chapters 6 and 7 have been integrated into the dissertation, they are also independent articles, one in press and one under review for publication. As such, some repetition of the introductory content and of the methods was necessary to provide a context for the subsequent study aims and methodology.

Accordingly, in Chapter 2, I define stress and present the multiple lines of evidence which describe the effects of stressors on the developing central nervous system. I include a description of the development of the stress system, the hypothalamic-pituitary-adrenal (HPA) axis, and I present the physiology of the stress response. In order to understand how the HPA axis functions in infants, I combine current knowledge of animal and human studies to show how early exposure to various forms of stimulation and stressors affect both the HPA axis and the developing brain. In addition, I compare the function of the HPA axis in term and in preterm infants.

As we show in Chapter 2, few researchers have studied HPA axis responses to care giving tasks in preterm infants. Thus, to understand further how early exposure to stress alters long-term development, in Chapter 3, I examine preterm infant responses to pain, an area of neonatology which has had increasing study in recent years. I summarize the anatomy of pain processing in adults. Then I describe how pain processing is unique in infants and review the effects of early exposure to pain on early and later development.

In Chapter 4, to make the logical link between preterm infant pain processing and the concurrent measures of pain used in the empirical work, I review and provide the rationale for the biobehavioural measures used for pain assessment against which I compare the NIDCAP® assessments. Next, in Chapter 5, citing examples from the studies of foetal movement, preterm infant pain research and other theoretical models

of motor development, I examine the merits of the central principle of dual antagonistic systems. This principle is fundamental to the synactive theory because it not only determines how NIDCAP® behaviours are currently interpreted, but it is generalized to the developmental process itself.

Chapters 6 and 7 present the empirical work. In Study 1 (Chapter 6), I assess preterm infant responses to blood collection by applying the NIDCAP®, along with other biobehavioural measures of pain, in a large number of preterm infants and under well-controlled conditions. From this set of responses, I describe a subset of behaviours which are associated with pain. In addition, I focus on differences between facial and body movement responses to pain in infants born at earlier gestational ages.

In Chapter 7, Study 2, I compare preterm infant responses during blood collection with those during a clustered nursing care procedure routinely used in the neonatal intensive care unit. Here a subset of NIDCAP® behaviours is found to be reliable indicators of stress responses across a range of stressors. However, facial reactivity remains the most specific behavioural pain indicator in preterm infants.

Finally, I summarize the empirical findings by concluding that the interpretation of preterm infant movements, as proposed by the synactive theory of development, requires revision. I propose that the way in which flexible interpretations of infant movements is used in the NIDCAP® is inappropriately timed and placed. I end by presenting two ways in which developmental specialists might improve the clinical application of this theory and might direct future research which would refine the measurement of stress responses in this high risk population.

CHAPTER 2

Stress and the Preterm Infant

With the advent of neonatal intensive care in the early 1970s came the capacity to "rescue" infants who were born before term, and who previously would have died. For example, recent advances in the care of extremely low gestational age infants enable more than 50% of these infants to survive (Chan et al., 2001; Effer et al., 2002). However, as the level of sophistication increased, the level of invasiveness of this technology also increased. Infants who, early in the application of neonatal intensive care, were subjected to simple care-giving tasks such as diapering and tube feeding, became exposed to more noxious procedures such as endotracheal intubation, ventilation, repeated blood tests, insertion of peripheral lines and surgery (Barker & Rutter, 1995; Johnston, Collinge, Henderson & Anand, 1997; Porter, Wolf & Miller, 1999; Simons et al., 2003; Stevens et al., in press; Whitfield & Grunau, 2000). In addition to noxious procedures, preterm infants are now exposed to other stressors such as acute and chronic illnesses, maternal separation, unpredictable handling patterns, multiple medications, continuous lighting and high levels of noise.

When neonatal intensive care was emerging as a new medical specialty, clinicians knew intuitively that the neonatal intensive care unit (NICU) environment was vastly different from the protective environment of the uterus. But understanding the full implications of the impact of being born prematurely into such an environment was compromised because little was known about biobehavioural and hormonal responses and the stress system (the hypothalamic-pituitary-adrenal [HPA] axis) in infants. Indeed, initially, clinicians believed that these tiny infants were too immature to feel the stress and pain associated with neonatal intensive care; therefore, their care tended to focus

primarily on the physiological needs of the infant almost to the exclusion of the infants' developmental needs.

However, recognizing the special nature of the developmental process of these infants, Als, a developmental psychologist, developed a theory and systematic method of assessing and treating the developmental needs of high-risk newborns, the synactive theory of development (Als, 1982). Even before basic biological, animal and human research demonstrated links between early stress exposure and later development, Als attributed the developmental differences between preterm infants and full term infants to their stay in a developmentally unexpected environment, the NICU.

As we shall see in Chapter 5, although Als does not define stress per se, she integrates the concept of stress into her theory by generalizing the principle of dual antagonistic systems of motor development (Als, 1982): all development is biphasic. When developmental specialists assess preterm infants' development, Als' synactive theory directs us to interpret all behaviours as either stress or stability indicators. However, we will find limited empirical validation of this dualistic behavioural classification system, and we argue that some of the movements which she interprets as indicators of stress may not, in fact, reflect stress. These judgements vitally affect medical and nursing management and the well-being of vulnerable neonates.

These reservations aside, the synactive theory of development is central because it extended the therapeutic focus of neonatal intensive care to one which addresses the infant's developmental needs, and it provided the basis for the only multidimensional, non-invasive, developmentally relevant assessment of preterm infant movements. Moreover, Als' pioneering ideas regarding the links between preterm infants' early stressful experiences and later alterations in development are now supported by recent animal and human evidence which shows that the

neurophysiologic components required for generalized stress responses, including those for pain responses, are functional by mid-gestation (Flowers, 1985; Kostovic & Rakic, 1990 and as reviewed in Coskun & Anand, 2000; Tsariki, Chrousos & Margioris 2002). Furthermore, recent studies indicate that early exposure to stressors not only may alter developmental processes such as mylenation and neurogenesis which ultimately result in reductions in the size of limbic and other regions of the brain(Teicher et al., 2003), but also may change future responses to stressors by re-setting the HPA axis (e.g. Anisman, Zharia, Meaney & Merali, 1998; reviewed by Ladd et al., 2000). Changes such as these have been linked with long-term alterations in development and behaviour in both animal and human studies (reviewed by Bhutta & Anand, 2002; Grunau, 2000, 2002, 2003; reviewed by Pryce & Feldon, 2003).

It is in the context of this new information regarding the function of the HPA axis, and of the effects of early exposure to stress on the developing neonate, that examination of the validity of the synactive theory should take place. Therefore, the purposes of this chapter are to define stress; to describe the normal development and function of the HPA axis; and combining both animal and human studies, to review the effects of chronic stress responses on the brain. Next, I will summarize how stress responses affect the developing brain and describe term infant cortisol responses to a variety of stressors. Finally, I will review the physiological differences in the HPA axis in preterm infants and present what is known about preterm infants' cortisol responses to procedures in the NICU.

2.1 The Concept and Definition of Stress

The idea of studying the effects made by forces on a system can be traced to early conceptions of the word "stress" (Chrousos, Loriaux & Gold, 1988). These forces were thought to alter or to threaten balance. The early philosopher Empedocles (450

B.C.) first referred to homeostasis as a harmonious balance between the essential elements. Hippocrates, who was also writing at this time, applied the concept of harmony to living beings, linking health with balance and disease with disturbed balance (Chrousos et al., 1988). In the early 19th century, Claude Bernard described the "milieu interieur" and the necessity of maintaining life through relatively stable internal functioning (Kopin, 1995). At the beginning of the 20th century, Walter Cannon described the ways in which physiological systems were maintained within certain ranges and applied the term "homeostasis" to these stable ranges (Kopin, 1995). He also determined that organismic systems responded to opposing forces differently from physical systems and that "secondary, irrelevant effects" could be damaging (Kopin, 1995). Hans Selye (1907-1982) elaborated on Cannon's ideas and was instrumental in popularizing the concept of stress. Selye operationally defined stress as a "...state manifested by a specific syndrome which consists of all the non-specifically-induced changes within a biological system " (Selye, 1976, p. 64). In more recent literature, stress is defined as a state of threatened balance (Chrousos et al., 1988). It includes "...an event or events which is interpreted as threatening to an individual which elicit physiological and/or behavioural responses." (McEwen, 2000b, p. 173). Stressors, those things which disrupt the physiological balance, may be either positive or negative events.

One of the limitations of the way in which the term "homeostasis" had come to be used is that it made no distinction between the physiological systems which are essential for maintaining life and those which maintain those systems (McEwen, 2000b). McEwen & Stellar (1993) borrowed a term from cardiology, "allostasis", to try to distinguish between these two types of systems. They apply the term "homeostasis" to those systems which are maintained over a very narrow range, such as oxygen

saturation and body temperature, systems which are critical for life. These physiological systems vary only slightly to adapt to changes within the environment (McEwen, 1998; McEwen, 2000b; McEwen & Wingfield, 2003). The term "allostasis" is applied to those systems which help maintain homeostatic systems, such as the HPA axis and autonomic nervous system. These systems are extremely dynamic, allowing constant and frequent variations in response to perceived and anticipated environmental demands (McEwen & Wingfield, 2003). Although variation in these latter systems does not directly cause death, in the long term, high demands on these systems can have adverse effects on the body. McEwen and others (McEwen & Stellar, 1993; McEwen, 2000b) define allostatic load as the wear and tear on the body as a result of repeatedly adapting to adverse physiological or psychological situations. Allostatic load is described as a causative factor in diseases, such as coronary artery disease (Steptoe et al., 2002); however, this concept might also be applied to preterm infants given the multiple stressors they experience early in postnatal life.

2.2 The Development of the HPA Axis

The primary stress response system which helps maintain homeostasis is the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis includes the hypothalamus, particularly the paraventricular nucleus (PVN), the anterior pituitary, and the adrenal cortex as well as the hippocampus and other higher cortical areas (Vander, Sherman & Luciano, 1998). During normal pregnancy, the development of the HPA axis begins very early in gestation. The sulcus of the hypothalamus can be observed by the 5th week of gestation. Although the mature anterior pituitary gland is not fully formed until the 11th week, it is able to produce ACTH which then regulates steroid production by the adrenal glands. The vascular link between the hypothalamus and anterior pituitary is mature by the 12th week and, though not functionally mature, the hypothalamic-pituitary complex is

anatomically mature by the 20th week of gestation. Similarly, the adrenal cortex can be identified early, by the 4th week of gestation. During the subsequent four weeks, the adrenal cortex develops the inner foetal zone, which forms 80-90% of the adrenal cortex, and the definitive zone comprising 10% of the cortex. Although researchers have traditionally described the foetal adrenal cortex as having only two compartments, recent ultrastructural studies have also shown a third zone, the transitional zone, between the foetal and definitive zones. Functional studies indicate that the transitional zone may be able to produce cortisol after mid gestation, and is, therefore, like the zona fasciculata of the adult adrenal gland (Mesiano & Jaffe, 1997). Further, a very small medulla can be visualized. At term, the permanent cortex accounts for 35%, foetal zone 50%, and the medulla 15% of the gland. Following parturition, the adrenal gland shows remodelling of foetal zone cells (a transformation to cells typical of the zona fasciculata), regresses in size by half over the first month of postnatal life, is smallest at the end of the first year, and becomes completely mature by the 15th year of age.

Like the hypothalamic, anterior pituitary and adrenal gland development, the foetus' cortisol secretion undergoes concomitant developmental changes. Human foetuses are capable of producing cortisol between 10 and 20 weeks gestation, but this production is likely accomplished by converting progesterone. At mid-gestation, all of the foetus' serum cortisol is supplied by the mother, even though by 16 weeks the foetus has the capacity to produce its own cortisol (Mesiano & Jaffe, 1997). By 30 weeks gestation, the foetus has the steroid metabolizing enzymes required to produce cortisol *de novo* from cholesterol. And as the pregnancy continues, the foetus gradually takes over cortisol production so that by term, it can produce more than 50% of its own cortisol. As in animals, cortisol secretion is very high just after birth, diminishes during the first few days of life, and then subsequently increases. The half-life of neonatal

cortisol is almost twice as long as in adults. Similar to cortisol production, corticosteroid binding globulin (CBG) shows specific developmental patterns. Although foetuses have increasing concentrations of cortisol with increasing gestational age, at birth, their levels are half those of adults. [1]

2.3 Neuroendocrinology of the Stress Response

Specialized cells within the tissues of the hypothalamus, the anterior pituitary and the adrenal cortex make and secrete the primary secretory products of the HPA axis: corticotropin releasing hormone (CRH), adrenocorticotropic (ACTH) and the glucocorticoid hormone. In humans this hormone is cortisol; in rats it is corticosterone (McEwen et al., 1997).

The hypothalamus receives neural input directly or indirectly from almost every part of the brain and is an integrative centre for many important body functions (See Figure 1). The paraventricular nucleus (PVN) in the hypothalamus produces CRH which is stored in secretory granules. When a stimulus reaches the hypothalamus, the signal stimulates release of CRH. CRH is secreted in a pulsatile manner and has an ultradian rhythm. CRH is transported down axons from the PVN to terminals where it is released into the primary capillary plexus in the median eminence of the hypothalamus. The primary plexus of the hypothalamic-hypophyseal portal system consists of a rich capillary bed. These capillaries are characterized by their fenestrations ("windows") which allow large molecules (3-40 amino acids) to pass through them. The closed

[1] Unless specifically referenced, the above information taken from Winter, 1998 & Tsakiri, et al., 2002.

Stress Pathways in the Brain

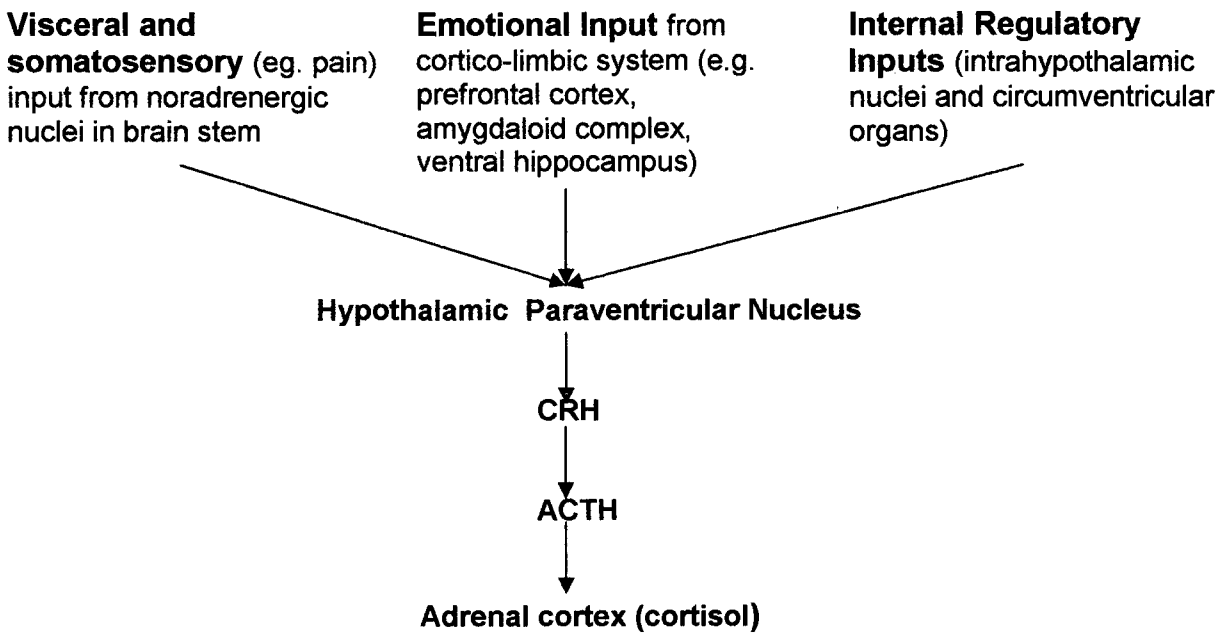
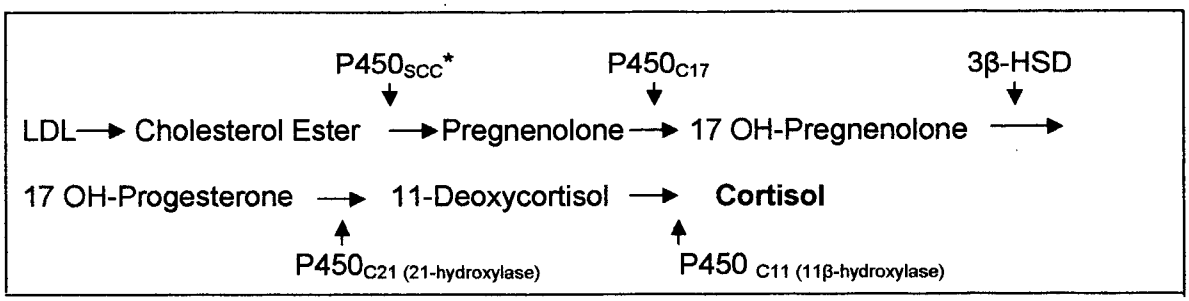


Figure 1. Stress Pathways in the Brain

hypothalamic-hypophyseal portal system ensures that high concentrations of CRH reach the anterior pituitary cells.

Once CRH reaches the anterior pituitary cells, it binds to specific G-protein receptors. The G proteins then use the cAMP intracellular signalling pathway to stimulate increased protein production. CRH stimulates an increase in expression of the gene coding for proopiomelanocortin (POMC), a precursor of adrenocorticotropic hormone (ACTH), and other hormones such as β endorphin. Thus, increased gene expression for POMC increases the synthesis and secretion of ACTH. Moreover, the G protein-cAMP pathway allows differing rates of ACTH production, a rapid pathway activated through membrane receptors, and a slower pathway activated through nuclear receptors which stimulate transcription. ACTH is secreted directly into the secondary capillary plexus of the anterior pituitary. Again, fenestrations in the secondary capillary

plexus vessels allow ACTH to pass into the peripheral circulation and to move to the zona fasciculata of the adrenal cortex. ACTH is important for maintenance of cells in the adrenal cortex and acts on these cells to increase the production of cortisol from cholesterol (See Figure 2).



*$P450_{scc}$, $P450_{c17}$, 3 β- HSD, $P450_{c21}$ and $P450_{c11}$ are all converting enzymes.

Figure 2. Production of Cortisol from Low Density Lipoproteins (LDL)

A second releasing factor, arginine-vasopressin (AVP), also produced in the PVN, may influence the production rate of ACTH, but this influence is secondary to that of CRH. Even though AVP alone does not strongly influence the production of ACTH, it is likely to be the hormone which activates the HPA axis to respond to novel stressors if repeated exposure to stressors has dampened the ACTH response. The glucocorticoids (GCs) are the final end product of the activation of the HPA axis and provide negative feedback through both short and long feedback loops. In the short feed back loop, cortisol feeds back to the anterior pituitary and reduces the production of ACTH. In the long feedback loop, cortisol feeds back to the hypothalamus, reducing the release of CRH. ACTH also feeds back directly to the hypothalamus to reduce production of CRH.

a. Types of corticosteroid receptors

There are two types of corticosteroid receptors: mineralocorticoid (MR), also known as Type I (Lupien & McEwen, 1997), and glucocorticoid (GR), or Type II

receptors (Lupien & McEwen, 1997). These receptors have differing, but complementary functions. MRs have a high affinity for cortisol (corticosterone) and aldosterone, while GRs have a high affinity for dexamethasone, a synthetic steroid. MRs are relatively insensitive to dynamic changes in cortisol levels (80%-90% of the receptors are occupied at basal cortisol levels); in contrast, GRs are highly responsive to changes such as those of stress (10-15% are occupied at baseline, whereas 75% are occupied during stress).

The patterns of anatomical distribution of these two receptors also differ. MRs are distributed predominantly in the limbic system and in brainstem motor nuclei; GRs are more widely distributed throughout the cerebral cortex, limbic system, PVN, and hypothalamic nuclei. MRs are involved in regulating on-going behaviours; they synchronize and co-ordinate circadian activities such as feeding, sleeping, and are involved in the subtle adjustments of basal HPA activity (De Kloet, Rosenfeld, Van Eekelen, Sutanto & Levine, 1988). In addition, MRs participate in the processes of interpretation of environmental stimuli and expression of appropriate behavioural and neuroendocrine responses.

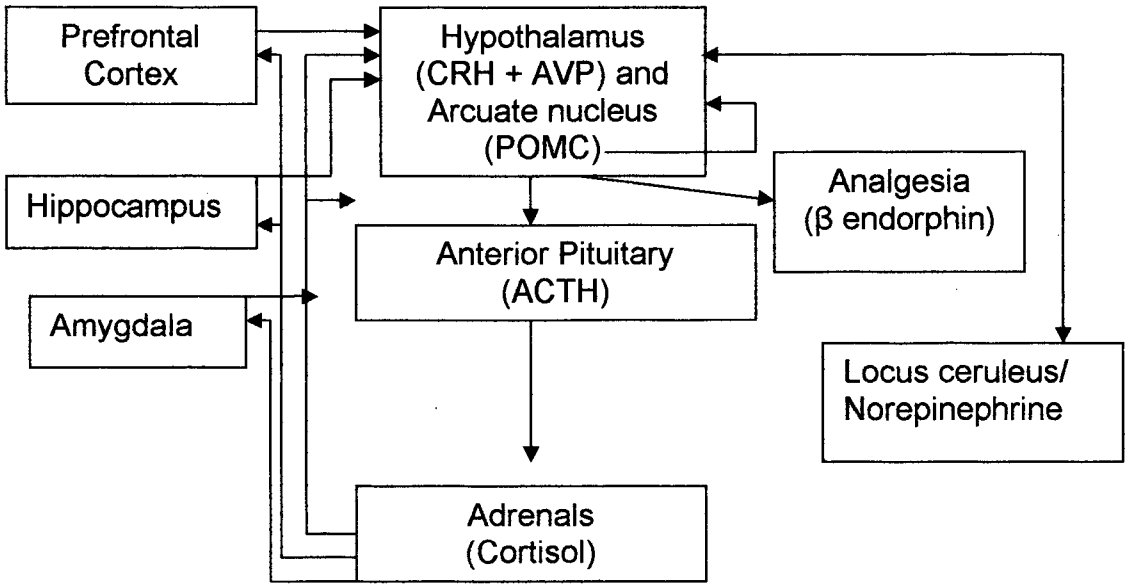
GRs are more widely expressed and are involved in the regulation of the ANS, as well as behavioural and neuroendocrine responses to stress (De Kloet et al., 1988). They are also thought to be involved in learning and memory (Raber, 1998). Studies indicate that low basal levels of corticosterone predominantly occupy MRs, and that GRs are activated when corticosterone levels rise (eg. circadian peak and stress) (De Kloet, Vreugdenhil, Oitzl & Joëls, 1998). The MR feedback to the HPA axis is probably a proactive mechanism; whereas, the GR feedback mechanism is probably reactive. The balance of the MR and GR-mediated effects on the stress system is important to the set point of HPA activity (De Kloet et al., 1998).

b. How cortisol binds to a steroid receptor.

Steroid hormone receptors are members of the cytosolic receptor family. When activated by hormone binding, the receptors may act directly as transcription factors by binding to specific DNA sequences. In the absence of cortisol, the steroid receptor is inactive by way of chaperone proteins (heat shock protein 90; HSP90) which maintain the receptor that is folded in the appropriate conformation to respond rapidly to signals. When cortisol binds with receptor, it induces a conformational change. The receptor dissociates from HSP90, and hormone-receptor complexes associate to form homodimers. The homodimers, actively transported to the nucleus, bind to the glucocorticoid response element of the target genes. Thus when stress hormones, such as cortisol, bind to a receptor, alterations in gene expression occur because cortisol triggers messenger RNA coding for specific proteins that can ultimately influence neuronal structure and function (Cicchetti & Walker, 2001).

2.4 HPA Axis and Other Stress-Response Networks

The HPA axis does not function in isolation, but is part of a larger network of central and peripheral systems. It is beyond the scope of this dissertation to describe in detail all the interconnecting networks; however, a general description of these systems is important for understanding the effects of stress responses on the developing brain. In addition to the hypothalamus, catecholaminergic neurons of the locus ceruleus (LC) and other central cell groups act as central coordinators of the stress-response system. Moreover, negative feedback to the HPA axis comes not only from the adrenal cortex, but also from the prefrontal cortex, the hippocampus and the amygdala (See Figure 3). In recognition of the central role of the limbic regions in regulating the HPA axis, recent stress response literature refers to the L-HPA system.



*The hypothalamus and locus ceruleus/norepinephrine systems also interact during stress to affect other systems such as the reproductive, immune, growth, thyroid and gastrointestinal systems.

Figure 3. Stress-Activated Networks*

The effects of chronic stressor exposure are systemic, affecting the brain and many other systems. Growth and reproduction, for example, are intimately linked to the HPA axis. When animals or humans are subjected to stressors, it is initially adaptive to redirect energy away from maintenance activities, such as growth and reproduction. CRH suppresses gonadal function in both males and females, and prolonged exposure to stressors suppresses growth hormone. Chronically stressful conditions can produce both stunted growth and infertility. Preterm infants may be particularly vulnerable to suppression of growth due to the multiple stressors they are exposed to in the NICU.

Thyroid function is also suppressed by chronic HPA activation. Decreased secretion of TSH, and conversion of T3 and T4 is adaptive in the short term because energy is conserved, but in the long-term, suppression of thyroid function may lead to depression. In addition to effects on growth, reproduction and thyroid function, chronic exposure to stressors alters metabolism. Under chronically stressful conditions, animals and humans exhibit increased fat deposits around the abdomen and insulin resistance. Chronic exposure to stressors also effects gastrointestinal function by delaying gastric emptying and by stimulating colonic transit and fecal excretion. Finally, chronic exposure to stressors negatively affects immune function. For example, when infected with a bacteria or virus, an animal produces inflammatory cytokines. Under normal conditions, GCs suppress the inflammatory effects of these cytokines, thereby keeping the system in balance. [2]

2.5 The Effects of Chronic Stressor Exposure on the Brain (Adult Animal Models)

Before describing the specific effects of stressors on the developing neonate, we will expand our understanding of the effects of chronic GC exposure in the adult central nervous system (CNS) because recent neuroimaging studies of former preterm infants show changes in the CNS which are similar to those seen in adults who are exposed chronically to stressors (Issacs et al., 2000; Nosarti et al., 2002; Peterson et al., 2000, 2003).

One of the most important areas of research which has evolved over the last 40 years involves the effects of exposure to acute and chronic stressors on the brain. Much of this work came about from the surprising finding from studies on rats that the hippocampus contains large numbers of glucocorticoid receptors (McEwen, Weiss & Schwarz, 1968; McEwen, Weiss & Schwarz, 1969). This finding crucially indicated that

[2] This section is summarized from Stratakis & Chrousos, 1995.

brain centres other than just the hypothalamus might be involved in the stress response.

The differing effects of GCs on the brain are largely due to differing functions of the two GC receptors and the type of stress experienced. In rats, activation of MRs by GCs is associated with the ability to form memories through sensory integration, whereas activation of GRs is related to the ability to acquire and consolidate memory (Lupien & McEwen, 1997). In humans, acute increases in GCs enhance the formation of memories which are associated with a highly emotive event (McEwen, 2000a). However, in general, research with both animals and humans shows associations between prolonged chronic stressor exposure and cognitive impairments (McEwen & Sapolsky, 1995). While rats exposed to chronic stressors show impaired spacial memory, humans show impaired performance in declarative memory (the ability to voluntarily remember previous information) (Lupien & McEwen, 1997; McEwen & Sapolsky, 1995).

The effects of chronic stressor exposure on the brain can be described in terms of the ways in which GC exposure affects hippocampal plasticity. Adrenal steroids, along with other mediators, play four roles in the plasticity of the hippocampus in response to stressors. First they participate in the regulation of branching and length of apical dendrites of pyramidal cells in Ammon's horn as was demonstrated in adult male rats after 21 days of repeated restraint stress (Magarinos, Garcia, & McEwen, 1997). Second, along with excitatory amino acids (EAAs) such as glutamate, they regulate the replacement of nerve cells in the dentate gyrus; stress responses (increased GCs) induce the release of glutamate, serotonin and gamma-amino butyric acid (GABA) which all appear to be involved in dendritic remodelling (McEwen, 2000a). Dendritic remodelling may, however, be protective in that it may be an attempt by the brain to

limit the increased excitatory input that stressors cause to the somewhat unstable (high vulnerability to stress) CA3 region of the hippocampus (McEwen, 2000a). It is important to note that all the rat experiments describing the effects of chronic stressor exposure on hippocampal plasticity used male animals. Female rats do not appear to show dendritic remodelling in the CA3 pyramidal neurons following repeated restraint stress (Galea et al., 1997).

Third, adrenal steroids modulate the excitability of hippocampal neurons; they influence the magnitude of long-term potentiation and produce long-term depression (De Kloet et al., 1998; Lupien & McEwen, 1997; McEwen, 1999, 2000a, 2000b). Increased adrenal steroid secretion, as a result of repeated restraint stress, inhibits long-term potentiation in CA3 and the dentate gyrus (McEwen, 1999).

Finally, adrenal steroids play a role in neurogenesis in the dentate gyrus as is demonstrated by the effects of adrenalectomy which, in rats, is found to increase apoptosis. This cell death results in increased neurogenesis. In adult rats, low levels of adrenal steroids (quantities which occupy MRs predominantly) block dentate gyrus apoptosis, but in the newborn rat, occupation of GRs is additionally required to prevent neuronal loss (Gould, Tanapat & McEwen, 1997; Woolley, Gould, Sakai, Spencer & McEwen, 1991). Many of these effects are reversible after a period of time (7-10 days in rats [Conrad, Margarinos, LeDoux & McEwen, 1999]). Some effects of long-term exposure to increases in adrenal steroids may be irreversible: the atrophy of the human hippocampus noted in Cushing's syndrome, recurrent depression, post-traumatic stress disorder (McEwen, 2000a) and the smaller brain volumes seen in ex-preterm children (e.g. Isaacs et al., 2000; Peterson et al., 2003).

2.6 Effects of Early Exposure to Stressors on the Developing Neonate

Just as animal models have increased our understanding of the effects of chronic stress responses on the brain, so neonatal animal studies and foetal studies of HPA development and function are vital to our understanding of preterm infant responses to stressors. There is growing evidence that early environmental manipulations or exposure to stressors may influence future responses to stressors by re-setting of the HPA axis, and may have major consequences on behaviour and on long-term health (Matthews, 2000). Some of the types of manipulations studied in neonatal animal models are highly relevant for the preterm infant because they include the effects of early handling, prolonged maternal separation and neonatal endotoxin exposure, conditions to which preterm infants are repeatedly exposed in the NICU. Researchers have reported that a developing brain which is exposed to high levels of glucocorticoid (GC) will exhibit catabolic and neuroanatomical changes such as reduced myelination, inhibition of cell proliferation, reduced axonal growth, and inappropriate formation of dendritic spines and synaptogenesis (De Kloet et al., 1988). Moreover, exposure to greater than physiological amounts of GCs produces delayed development of HPA circadian rhythms and responses to stressors, and delays in the acquisition of adaptive behaviour (De Kloet et al., 1988; Sapolsky & Meaney, 1986). However, when reviewing the effects of various stressors on the developing organism, one must first take into account developmental differences which may exist between neonatal and adult stress-reactivity models, particularly in rat models.

Initial studies which examined the effects of stressors on rat pups found, in the early postnatal period, that the adrenocortical response to stressors was significantly reduced (De Kloet et al., 1988; Levine, 1994; Sapolsky & Meaney, 1986). Although the pups had an initial HPA response to stressors on post-natal day 1 (P1), their response

subsequently dropped and remained low from P2-P14. It was hypothesized that this response was protective in keeping the developing brain from exposure to the damaging effects of high levels of GCs.

Initially, the stress non-response period was so named because it appeared that the HPA system was impervious to stressors during the first two weeks of life in rat pups. However, when the sensitivity of the assays improved, changes in responses to a variety of stressors, such as cold, ether, maternal separation, endotoxin, and sleep deprivation could be detected (Anisman et al., 1998; Hairston et al., 2001; Walker, Scribner, Cascio & Dallman, 1991). Furthermore, researchers demonstrated that neonatal rats could respond to a succession of stressors (Walker & Dallman, 1993). As such, this period was renamed the stress hypo-response period (SHRP). In attempting to better understand the nature of the SHPR, Viau, Sharma & Meaney (1996) reported that, in the neonatal rat, the functional GC signal itself is not diminished; rather it is the number of GC receptors (density) in the target tissues which is the major rate-limiting step in the GC signal.

Thus, neonatal rats respond to a number, and to a succession, of stressors, these responses being of smaller magnitude than those seen in the adult rat. Not only does the HPA axis in the neonatal rat respond to a variety of stressors, scientists have explained that the type, timing and length of exposure to stressors early in life alters the HPA axis set point on into adult life.

Research shows that early handling produces changes in HPA function in rats and boars (Weaver, Aherne, Meaney, Schaefer & Dixon, 2000). However, the nature of the changes varies depending upon the animal model used. The following sections will focus primarily on the effects of early handling on neonatal rats.

Early handling is a model of intervention whereby rat pups are repeatedly removed from their litter for brief periods (5-15 minutes) of time. In general, early handling alters HPA function later in life in rats. Multiple studies have shown that early handling may reduce the neuroendocrine response to subsequent stressors experienced during adulthood. In addition, the HPA response to stressors becomes more efficient in that it returns to baseline more quickly in handled rats than in non-handled rats (Anisman et al., 1996; Ladd et al., 2000; Meaney et al., 1991,1993; reviewed by Meaney et al., 1996). The nature of these altered responses appears to be developmental in that there are critical periods when handling is effective (first 21 days of life), and in that the changes last throughout the rat's life. Moreover, the alterations in HPA function appear to be protective; older handled rats do not show the same neurodegenerative processes as non-handled rats, probably because they have reduced exposure to high levels of GCs throughout life (e.g. Lehmann et al., 2002).

Although the mechanisms behind this alteration in HPA activity are very complex and probably involve interactions of many neurohormonal systems, the effects on the brain are very specific. For example, after early handling, there is an increase in GR receptor density and therefore, binding capacity, in the hippocampus and in the frontal cortex. The effects of other central neurotransmitter systems may influence handling effects on GR receptor density. For example, as adults, handled rats have increased dopamine responses. Moreover, serotonin affects GR receptor expression. Handling, via increased thyroid hormones, raises serotonin levels which in turn increase GR receptor density specifically in the cells of the hippocampus (reviewed by Meaney et al., 1996).

The alterations in the hypothalamus are different from those in the hippocampus in response to early handling. First there is no increase in GR receptor density.

However, other specific changes in the hypothalamus include lower levels of CRH and AVP in the paraventricular nucleus of handled rat pups because of the enhanced negative feedback from the increased GR receptor density in the hippocampus and prefrontal cortex. Moreover, early handling does not alter basal ACTH or corticosterone levels.

Researchers report that interactions between mother and pup appear to play a role in mediating this alteration. After the pups have been returned to the nest, maternal behaviour changes (increased licking, feeding) towards the pup. It is these changes in maternal behaviour, and therefore increased stimulation of the pup, which are thought to play a role in the resetting of the HPA axis (Liu, et al., 1997; Anisman, et al., 1998). Finally, behavioural alterations, such as improved spatial learning (Lehmann et al., 2002), less anxiety-related behaviour (Chapillon, Patin, Roy, Vincent & Caston, 2002), and altered responsivity to subsequent stressors such as pain (Sternberg & Ridgeway, 2003) occur as a result of exposure to early handling.

Unlike brief periods of handling, extended separation (3-24 hours) of rat pups from the mother and litter have deleterious effects on HPA function. Prolonged maternal separation induces heightened HPA activity (elevated basal plasma ACTH levels, elevated ATCH responses to stress, CRH hyper-secretion, and reduced CRH pituitary binding sites), and increased density of CRH binding sites in the frontal cortex, amygdala, hypopthalamus, hippocampus and cerebellum (as reviewed in Anisman et al., 1998; and Pryce & Feldon, 2003). Rat pups that spend extended periods away from their dam show heightened responses even to mild psychological stressors, such as novelty (Plotsky & Meaney, 1993). Scientists hypothesize that the mechanisms involved in these alterations of HPA function are caused by localized insufficiency in inhibitory mechanisms (GC and GABA) which leads to changes in excitatory

neurocircuits (e.g. CRH, serotonin, noradrenalin). These effects are so strong that they can alter HPA function during the relatively protective period of the SHRP (e.g. Smotherman, 1983; Walker et al., 1991).

Similar to the effects of prolonged maternal separation, early exposure of rat pups to endotoxin also produces alterations in later HPA axis function. These effects include increased plasma ACTH and corticosterone responses to stress, decreased GR receptor density, increased levels of median eminence CRH and hypothalamic CRH mRNA expression, and reduced GC negative feedback sensitivity (Shanks, Larocque & Meaney, 1995; Shanks & Meaney, 1994). Although scientists speculate that the increased pup's body temperature induced by endotoxin reduces maternal care-giving and this change in maternal behaviour mediates the changes in the HPA axis (Francis et al., 1996), the endotoxin model may have particular relevance to the preterm infant who may experience repeated infections while in the NICU.

In addition to studies on the effects of early handling, maternal separation and exposure to endotoxin, prenatal stress exposure complements the literature on the effects of GCs on the developing brain. In foetal studies, communication of stressful responses between the foetus and mother occurs through the placenta. The placenta, in effect, acts like a "central nervous system" in that it receives, processes and responds to different stimuli (Sandman et al., 1994). Normally the foetus is partly protected against maternal stress hormones because the placenta contains 11β-hydroxysteroid dehydrogenase Type 2 (11β-HSD2) which quickly converts cortisol to inactive cortisone. But excessive exposure to either endogenous or exogenous ACTH causes inhibition of this enzyme, leaving the foetus vulnerable to the effects of maternal stress (Clark, 1998; Lou et al., 1994). Additionally, scientists hypothesize that CRH

mediates the effects of the maternal environment on foetal development (Wadhwa, Sandman & Garite, 2001).

In primate models as in rodent models, foetal stress responses reduce the numbers of GR receptors. Primate mothers subjected to stressors deliver infants who go on to have lower neuromotor scores (Coe, Lubach & Schneider, 1999; Schneider, Roughton, Koehler & Lubach, 1999; reviewed by Weinstock, 2001) and enhanced responsivity to stressors (Clarke & Schneider, 1993; Clarke, Wittwer, Abbot & Schneider, 1994) compared to infants born to non-stressed mothers. This association has also been reported in the human infant literature (de Weerth, van Hees & Buitelaar, in press; Huizink, Robels de Medina, Mulder, Visser & Buitelaar, 2003).

In humans, one finds associations between mothers who were exposed to prenatal stressors and reduced birth weight and preterm delivery (e.g. Wadhwa, Sandman, Porto, Dunkel-Schetter & Garite, 1993; reviewed by Weinstock, 2001). Studies also report associations between maternal stressor exposure and later emotional disturbances such as hyperactivity-attention deficits (Schneider et al., 1999; reviewed by Weinstock, 2001). Finally, there is some evidence that human foetuses exposed to antenatal steroids have smaller head circumferences at birth (Lawson, 2001), although this evidence is not conclusive. It remains unclear in the human literature whether antenatal exposure to stressors permanently alters HPA activity in the offspring.

2.7 Human Neonatal Response to Stressors

As we have seen, the HPA axis is fully present anatomically by mid-gestation. Moreover, human studies report increases in foetal cortisol in response to painful stimuli, these being separate from maternal HPA responses (Gitau, Fisk, Teixeria, Cameron & Glover, 2001; Fisk et al., 2001). By the end of gestation, neonates exhibit

clear responses to stressors, show graded responses depending on the intensity of the stimulus, and habituate to repeated non-painful stimuli.

For example, Gunnar and colleagues (1981) found a 3-4-fold increase in cortisol levels in term infants 30 minutes after circumcision. Other painful stimuli, such as heel lance, also elicit an increase in cortisol (Gunnar, 1989). Moreover, term infants have increased cortisol levels to non-painful events such as physical restraint (Malone et al., 1985), physical examination (Gunnar, 1989) and developmental assessment (Gunnar, Isensee & Fust, 1987). They can even modulate their responses depending on the intensity and frequency of the stressor exposure. Thus, they show a lesser cortisol response to physical exam than to a painful stimulus (reviewed by Gunnar, 1992), and they habituate to repeated stressors (Gunnar, Connors & Isensee, 1989).

As in rats, human infants have a homologous dampening of their cortisol responses to stressors (Ramsey & Lewis, 1994; reviewed by Gunnar & Donzella, 2002). The hyporesponsive period seems to appear gradually over the first year of life. Although scientists have not yet definitively determined when this period ends (Gunnar, Brodersen, Krueger & Rigatuso, 1996; Gunnar & Donzella, 2002; Larson, White, Cochran, Donzella, & Gunnar, 1998), it seems likely that the hyporesponsive period extends into the preschool years (Gunnar & Donzella, 2002).

Whereas healthy term infants show predictable and graded HPA responses to varying stimuli, the cortisol responses of other groups of children (toddlers up until 12 years of age) who are raised in extreme deprivation are below basal levels (e.g. Gunnar, Morison, Chisholm & Schuder, 2001). Some infants, such as those with colic, have flattened morning cortisol levels, with little change in their cortisol levels when they are exposed to stressors (e.g. White, Gunnar, Larson, Donzella & Barr, 2000). Such findings, for the most part, have been unexpected. Investigators are now concerned

that flattening of daytime patterns of cortisol and drops in cortisol levels to stressors (hypocortisolism) may be indicators of developmental risk, but this conclusion has not been definitively established (Gunnar & Vazques, 2001; Heim, Ehlert & Helhammer, 2000).

2.8 HPA Function in the Preterm Infant

Preterm infant HPA function, cortisol secretion and responses to specific stressors are more complex than that in term infants. Unlike term newborns, preterm infants are delivered before their HPA system is fully mature, and they can no longer rely on maternal support for their production of cortisol. As a result, the HPA axis of preterm infants appears to have some functional differences from those of full term infants.

Although the HPA axis is anatomically present by 20 weeks of gestation, in general, preterm infants have functionally mature adrenal and pituitary activity by 26-27 weeks gestational age. But unlike neonatal rats, preterm infants show no sex differences in HPA function (Hanna, et al., 1993; Heckman, Wudy, Haack & Pohlandt, 1999; Midgley et al., 1998; Ng et al., 1997a). It appears that even at 28 weeks gestational age, preterm infants' plasma ACTH concentrations are similar to those in adults (Wittekind, Arnold, Leslie, Luttrell & Jones, 1993). ACTH levels show a developmental pattern; they increase immediately after birth, decline shortly after birth, and then gradually increase with increasing postnatal age (Wittekind et al., 1993; Midgley et al., 1998). Unlike their well counterparts, sick preterm neonates show increased ACTH levels during the first week of life (Ng et al., 2002). But even with increased physiological doses of ACTH, many preterm infants have inadequate cortisol production (Scott & Watterberg, 1994).

The data on the availability of cortisol precursor molecules is less consistent. Some researchers have suggested that preterm infants have increased concentrations of steroid precursors such as 17-OH-pregnenolone, 11-deoxycortisol (Lee et al., 1989) and 17-OH-progesterone (al Saedi, Dean, Dent & Cronin, 1995; Doerr, Sippell, Versmold, Bidlingmaier & Knorr, 1988; Hughes et al., 1987); however, Fujitaka et al. (1997) counters that the studies determining concentrations of these steroid precursors used radioimmunoassay which made determining true concentrations difficult because the antibodies of cortisol precursors, cortisone, and cortisol were cross-reactive.

More recently, Bolt et al. (2002b) found that 17-OH progesterone (17-OHP) levels varied depending on the gestational age of the infants tested. In those infants < 30 weeks gestation, ratios between cortisol and 17-OHP were lower than for those of more mature infants. Finally, for preterm infants who do show reduced cortisol levels, some scientists attribute these findings to diminished steroidogenic enzymes (Hingre et al., 1994; Lee et al., 1989) although this hypothesis is also debated. For example, Fujitaka et al. (1997) reported that preterm infant levels of 21 hydroxylase (P450c21) and 11β hydroxlyase (P450c11) were not decreased, but gestational age may influence the concentrations of these enzymes; Bolt et al. (2002b) suggested that the activity of 11β-HSD may change with increasing gestational age, a developmental pattern which may have affected the findings of earlier studies.

Not only do preterm infants at older gestational ages produce cortisol in similar ways to term infants, they can transport it equally well. Indeed, preterm infants' concentrations of corticosteroid binding globulin are similar to those levels found in full term infants (Kari et al., 1996; Hanna et al., 1997; Metzger et al., 1993).

Although it appears that preterm infants at older gestational ages can produce cortisol comparable to that of term infants, among healthy preterm infants, levels of

cortisol precursor molecules, CBG and basal cortisol are highly variable, although within-subject measures are more stable (Jett et al., 1997). During the first week of postnatal life, some investigators report increased basal cortisol in preterm infants compared to full term infants (Ng et al., 1997b, Ng, 2000, Scott & Watterberg, 1994). Others report cortisol levels below acceptable ranges, particularly in extremely low birth weight preterm infants (<1000grams) (Hingre, Gross, Hingre, Mayes & Richman, 1994; Kari, Raivio, Stenman & Voutilainen, 1996; Lee et al., 1989). These lowered levels may be protective since many preterm infants with low basal cortisol do not show clinical signs of hypocortisolism (Ng, 2000). An alternative explanation by Hanna, et al. (1993), Fujitaka et al. (1997) and Ng (2000) is that sick preterm infants may have lower levels of cortisol because they may fail to identify their illness as a stressor, or their hypothalami may be unable to secrete CRH under stressful conditions.

Despite these varied reports, the most recent literature supports total serum cortisol concentrations in preterm infants > 30 weeks gestational age as being equivalent to those of full term infants (Alkalay, Klein, Nagel & Pomerance, 1996; Fujitaka et al., 1997). Fujitaka et al. (1997) attribute the previous findings of lowered cortisol to the lack of measurement of cortisone concomitantly with cortisol. Moreover, reports of elevated cortisol levels in some studies may be explained by either inconsistencies in the timing of sampling which do not control for cortisol surges (Bettendorf et al., 1998), or by the presence of chorioamniotitis (Watterberg, Scott & Naeye, 1997). Even if basal cortisol levels are similar to term infants, preterm infants' patterns of cortisol secretion may be different from full term infants; they appear to secrete cortisol in bursts of longer duration, but of lower amplitude (Metzger et al., 1993).

In summary, preterm neonates > 30 weeks gestational age have plasma ACTH concentrations which are similar to those of term newborns. In contrast, they may have reduced steriodogenic enzymes which predispose them to increased levels of steroid precursor molecules although this hypothesis has not been definitively confirmed. Nevertheless, preterm infants have similar levels of CBG to term newborns, and appear to be able to secrete cortisol as do their term born peers, but they may show longer bursts with lower amplitudes. Unlike more mature preterm infants, younger (<30 weeks gestational age) preterm infants may have either lower basal levels of cortisol than they need to respond appropriately to the stressors they experience in the NICU or they may have response levels of cortisol which do not reflect the levels of stress they are exposed to (Bolt, Weissenbruch, Lafeber & Delemarre-van de Waal, 2002a).

2.9 Preterm Infants' Responses to Specific Stressors

Compared to the numbers of studies investigating term infant HPA responses to specific stressors such as circumcision, discharge or developmental examination, very few studies describe similar responses in preterm infants. Instead, much of the preterm infant literature takes up the effects which specific conditions or medications have on the HPA axis. For example, exposure to antenatal and postnatal steroids, hormones given to mature lung function, have suppressive effects on the HPA axis (e.g. Banks et al., 2001; Yeung & Smith, 2002). Varying severity of illness has differing effects on the HPA axis in preterm. In some studies, cortisol levels were higher in sicker infants (e.g. Banks et al., 2001; Barker & Rutter, 1996), but in other studies, cortisol levels are lower(Scott & Watterburg, 1994; Huysman et al., 2000); and finally, in one study, cortisol levels started lower and became higher with increasing postnatal life (Ng et al., 2002). But because of variation in determining illness severity, it remains difficult to interpret results on the effects of illness severity on cortisol.

Even though the focus of research in the preterm infant has been primarily to describe the maturity of, and the association of neonatal illness to the function of the HPA axis rather than to study specific preterm infant HPA reactivity to NICU procedures, Anand, Sippell, and Aynsley-Green (1987) have published a seminal article on preterm infants' adrenocortical stress response to surgery at 28 weeks gestation. These infants exhibited increased levels of 11-deoxycorticosterone, but no change in cortisol. The authors attribute this lack of cortisol response to the immaturity of the steroid biosynthesis pathway (Anand et al.,1987). A few others have used serum cortisol levels to evaluate interventions such as sedation (Barker & Ruttner, 1996; Guinsburg et al.,1998; Orsini, Leef, Cosarino, Detorre & Stefano, 1996; Pokela, 1993; Pokela, 1994), massage (Acolet, et al., 1993), or intrauterine-like sound stimulation (Giannakoulopulos, Muthy, Modi & Glover, 1995). Typically preterm infants have shown appropriate HPA responses with decreases in cortisol levels to such interventions. In addition to the paucity of research examining preterm infants' HPA responses to care-giving tasks or procedural stressors in the NICU, there are also no long-term studies examining HPA function in children born prematurely.

2.10 Summary

Preterm infants in the NICU face multiple stressful procedures and events. The synactive theory of development, and its clinical application the NIDCAP®, integrates the concept of stress in that all behaviours observed using this theory should be dichotomously interpreted as either stress or stability behaviours. However, the interpretation of these behaviours has had little empirical evaluation. Nevertheless, before addressing validation of the stress and stability behaviours in synactive theory, we must first understand the most current definition of stress, how a variety of stressors have been shown to affect brain morphology in developing animals and humans, how

the human neonatal stress response system functions, and how preterm infants may differ from full term babies.

Stress can be defined as a state of threatened balance which includes events that elicit physiological and/or behavioural responses. Stressors are both positive and negative stimuli which disrupt the physiological balance. Physiological responses to stress are activated through the hypothalamic pituitary adrenal axis (HPA) and other systems (e.g. limbic system and autonomic nervous system). The end product of this activation is the production of cortisol which feeds back to shut off the response. When this system is chronically activated, it induces alterations in brain structure and function which ultimately change behaviour.

Animal studies have shown that exposure to early adverse experience permanently alters GC receptor gene expression in the hippocampus, amygdala and frontal cortex. Moreover, the resting state activity in hypothalamic CRH/AVP neurons is also altered. The result of these changes ultimately alters the signal to the pituitary. Although rat pups exposed to early handling (stimulation) show improved negative feedback efficiency, protracted maternal separation or endotoxin exposure leads to reduced GC inhibition of ACTH release and thereby produces a heightened HPA response to stressors as the pups develop into adults. Growing evidence also demonstrates that human foetal brain development is altered in response to prenatal stressors and exposure to prenatal glucocorticoids. Such evidence supports the importance of our understanding the effects of exposure to stressors early in life on the developing brain.

Like their term peers, preterm infants have a functional HPA system. They secrete ACTH adequately by 28 weeks gestational age. Preterm infants also show similar levels of CBG to term infants. Although healthy preterm neonates > 30 weeks

gestational age appear to secrete cortisol like that of their term born peers, they may have reduced steriodogenic enzymes which predispose them to increased levels of steroid precursor molecules. Alternatively, very immature preterm infants may have lower basal and response levels of cortisol than they should, a condition which may impair their ability to respond appropriately to the stresses they experience while in the NICU. Finally, compared to the numbers of studies investigating preterm infant cortisol levels associated with various clinical conditions, few studies report cortisol responses to specific procedural stressors. While some scientists have used serum cortisol levels to evaluate specific NICU interventions, no investigators have examined the long-term effects of the NICU experience on HPA function.

Although currently the direct effects of early exposure to stressors on later preterm infant HPA function are not known, researchers have studied the effects of a specific stressor, early exposure to pain in preterm infants. In the next chapter I will begin by describing neonatal pain processing and then describe the current understanding of the short and long-term neurodevelopmental effects of early pain exposure.

CHAPTER 3

Neonatal Pain Processing and the Effects of Early Pain Exposure

Research which examines preterm infants' HPA axis and cortisol responses to care giving in the NICU is limited; however, the effects of early exposure to pain, as a specific stressor, have had relatively more empirical study. In Chapter 2, I indicated that, early in the history of neonatal intensive care, clinicians believed that preterm infants were too immature to respond to a variety of stressors. At that time, the state of understanding regarding infant pain was that newborn infants were relatively insensitive to pain. This belief was held because pain was defined as a subjective phenomenon, neonatal responses to pain being "reflexive", neither perceived nor remembered. Further, it was believed that, in theory if not in fact, having a high threshold to pain would be adaptive in protecting the infant from pain experienced during birth (McGraw, 1941). Moreover, this belief was maintained because there was almost a complete absence of research examining pain physiology and reactivity in infants. In addition, clinicians were concerned that, even if these infants did "feel pain", treatment of pain with analgesics would produce respiratory depression. Since nursing ratios were much lower than those used today and availability of ventilators limited, clinicians were reluctant to provide opioid analgesics (Whitfield & Grunau, 2000).

Then, in the mid -1980's, pivotal animal and human research showed that neonatal peripheral and central pain processing was different from those in adults, rendering infants more vulnerable and sensitive to pain (Fitzgerald, 1985; Fitzgerald & Koltzenburg, 1986). Shortly thereafter, while studying preterm infants during surgery, Anand and colleagues published a series of articles which described neonatal stress response and poor surgical outcomes which resulted from inadequate pain control (Anand & Aynsely-Green, 1988; Anand at al., 1987). At about the same time,

Fitzgerald, Millard and McIntosh (1988, 1989) provided further evidence that preterm infants felt pain; that preterm infants showed hypersensitivity in the flexor withdrawal reflex following heel lance, as in adults; and that the hypersensitivity could be diminished if the infants were treated with local anesthesia. Concurrently, pain measurement in infants was addressed with the development of a psychometrically reliable and validated pain scale (Grunau & Craig, 1987). These multiple lines of evidence, as well as improved ability to measure pain, influenced a gradual philosophical shift away from the belief that neonates did not experience pain, and towards the belief that pain was deleterious and that appropriate management of neonatal pain improved clinical outcomes. Consequently, clinicians and basic scientists undertook more rigorous studies of pain processing in human neonates and in neonatal rat pups.

I will begin this chapter by summarizing the anatomy of pain. Then I will identify the neurodevelopmental differences in neonatal pain processing and describe the role of early pain exposure in altering peripheral and central sensory development. Finally, I will examine links between early pain exposure and long-term alterations in preterm infant development.

3.1 The Anatomy of Pain

Pain signaling and modulation involves a number of parallel and overlapping somatosensory pathways. Making strict anatomical distinctions among these pathways is difficult because specific neurons often send collateral projects to several different nuclei (Willis, 1994). The neuroanatomy and physiology of the developing pain system

is complex; the purpose of this section is to summarize our general understanding of the neuroanatomical systems involved in pain processing in humans. [1]

Injury to the skin stimulates three types of primary sensory afferent fibres to send pain signals to specific laminae in the dorsal horn of the spinal cord. Aδ fibres rapidly transmit the pain to lamina I and V; the C fibres, slower signaling, unmylenated fibres, transmit the signal to lamina I, II and V; and Aβ fibres carry information regarding vibration and position to lamina III, IV, and V. Excitatory neurotransmitters such as glutamate and Substance P activate n-methyl d-asparate (NMDA) receptors which transfer the signal from the primary sensory afferents across the synapse in the dorsal horn laminae. These neurotransmitters also appear to mediate the long-term effects of pain because they have a more generalized role in the developing brain (reviewed by Coskun & Anand, 2000). For example, NMDA receptors allow calcium to enter into cells; this, in turn, leads to alterations in gene regulation (reviewed by Bhutta & Anand, 2002).

Once in the dorsal horn, the pain signal is transmitted primarily by four ascending somatosensory tracts: the spinothalamic tract (STT), the spinomesecephalic tract (SMT) and the spinoreticular tract (SRT) (the latter two also together known as the spinobulbar tract) and the spino-limbic tract. The spinothalamic tract is regarded as the primary pain signaling pathway to the brain. Within the spinal cord, pain signals cross over the midline in the ventral grey commisure at the spinal level near the entry level of the primary afferent fibers. In addition, the pain signals may be transmitted through interneurons, where the signal is sent to other spinal cord neurons such as flexor

[1] Unless otherwise indicated, this section is summarized from reviews by Craig & Dostrovsky, 1999; Willis, 1994; Willis & Westlund, 1997

motorneurons. As the pain signal passes up the spinal cord, it is transmitted along the parabrachial region of the pons and then ascends to a number of thalamic nuclei including the ventral posterior lateral, the ventral medial, the ventral lateral, the central lateral, the parafascicular and the medial dorsal nuclei. It is important to note that in adults, STT cells have restricted receptive fields. This is in contrast to the wider receptive fields found in infants. The functional relevance of this difference will be discussed later in this chapter.

Pain entering the spinal cord is also transmitted via the spinobulbar tract (SBT) and terminates in four main areas of the brainstem. The first termination area is brainstem catecholamine cell groups such as the ventrolateral medulla and the locus coeruleus. Projections to the ventrolateral medulla go on to terminate in the hypothalamus and stimulate the release of ACTH in response to noxious simulation. Additionally, pain signals may be sent to the parabrachial nucleus; the periaqueductal gray, the major integration site for homeostasic control and limbic motor output; and to the reticular formation. Considered part of the spinobulbar tract, the SRT also transmits pain signals. Unlike signals in the STT, pain is transmitted through the SRT on both sides of the spinal cord to both the right and left thalami. These signals then appear to terminate in the pontomedullary portion of the reticular formation and contribute to increased arousal, to the motivational-affective, the somatic and autonomic motor reflexes associated with pain. Although the STT, SMT and SRT have been defined, researchers also propose a multisystem pathway, the spino-limbic tract, which includes signals from the spinoreticular tracts, and which ascend from the reticular formation and terminate in the medial thalamus, hypothalamus and limbic structures. Furthermore, direct spinohypothalamic and spinoamygdalar pathways are involved in pain processing.

In addition to subcortical regions involved in pain processing, cortical regions involved in the sensory-discriminative and motivational-affective aspects of pain include the primary and secondary sensory cortex (SI and SII), the anterior insula and the anterior cingulate gyrus (See Figure 4).

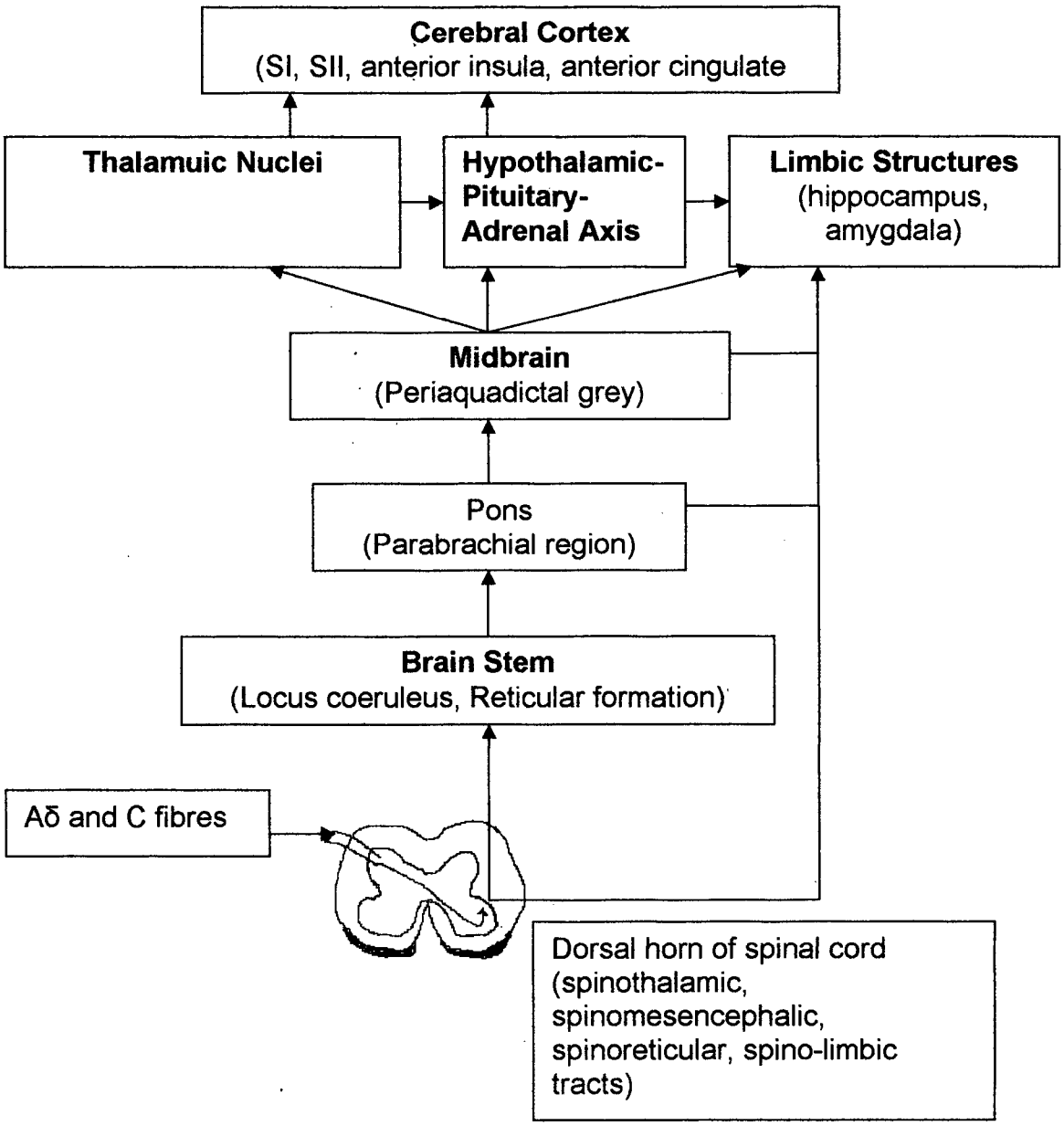


Figure 4. Pathway of Ascending Pain Signals

Descending pain modulatory pathways originate from multiple brain structures. Some thalamic nuclei, such as the ventral posterior lateral and vental posterior medial nuclei, are involved in reduction of pain. Limbic structures, including the hypothalamic-pituitary-adrenal axis, provide analgesia through production of β endorphin. When stimulated, the periaquaductal grey region inhibits nociceptive dorsal horn neurons and appears to be involved in complex behavioural responses to painful or stressful events. The dorsolateral pons, including the locus ceruleus, subceruleus and parabrachial area, also contribute to analgesia by simulating secretion of serotonin and noradrenalin. In addition, the raphe nuclei, reticular formation and the anterior pretectal nucleus are involved in antinociception.

Of the several pain inhibitory neurotransmitters, gamma amino butyric acid (GABA) is the most important. Serotonin and noradrenalin are also neurotransmitters involved in the descending modulation of pain. Moreover, opiates, such as enkephalins, endorphins and dynorphins, work to release adrenergic and serotonergic pathways from the inhibiting effects of GABA. This, in turn, increases the activity in the descending pathways and suppresses pain.[2]

3.2 Neonatal Pain Processing

Pain processing in neonates differs substantially from that of the adult. Indeed, since the seminal work of Fitzgerald's group (as summarized in the review Fitzgerald, 2000), we know that preterm infants are at risk for *enhanced* pain sensitivity due to differences in the developing nervous system. At birth, the skin is innervated by both Aδ fibres and C fibres, but the process of full innervation of the sensory nerves to the

[2] Although many more neurotransmitters are involved in pain processing, I have chosen to mention the primary ones.

skin continues to take place for some weeks after birth. Using animal models, researchers have shown that tissue damage in the early postnatal period causes a profound and lasting sprouting response of local myelinated A fibre and unmyelinated C fibre sensory nerve terminals, a response which remains long after the injury (Reynolds & Fitzgerald, 1995; Reynolds, Alvarez, Middleton & Fitzgerald, 1997). This alteration in sensory innervation is greatest when wounds are performed at birth; the sprouting response decreases progressively with increasing age at wounding (Reynolds & Fitzgerald, 1995; Reynolds, et al., 1997). Moreover, the sprouting response produces a long lasting hypersensitivity and lowered mechanical threshold in the injured region (Alvarez, Torsney, Beland, Reynolds & Fitzgerald, 2000; De Lima, Alvarez, Hatch & Fitzgerald, 1999; Reynolds & Fitzgerald, 1995). This response may be mediated by the local production of nerve growth factor that follows tissue damage (Constantinou, Reynolds, Woolf, Safieh-Garabedian & Fitzgerald, 1994); and importantly, this response cannot be altered by application of local sensory nerve blocking anaesthetics (De Lima et al., 1999).

Not only do neonatal rats show significant alterations in peripheral sensory nerve terminals following skin wounding, but also functional differences in the spinal cord make them more likely to be hypersensitive and to have exaggerated pain responses. First, in preterm infants, thresholds to tactile and nociceptive stimulation are very low and gradually increase in post-conceptional age (Fitzgerald, Shaw & MacIntosh, 1988). In addition, studies using animal models demonstrate that the receptive fields of the connections between the afferents and spinal cord dorsal horn cells are much larger in the newborn and gradually diminish over the first 2 postnatal weeks; natural stimulation of these expanded fields produces long lasting excitation which may lower their threshold to additional stimuli (Fitzgerald, 1985). This excitation is known as the "wind

up" phenomenon (Woolf, 1996). Moreover, repeated low threshold stimulation of the A fibres can lead to sensitization of dorsal horn cell responses beyond the period of stimulation (Jennings & Fitzgerald, 1998). These animal studies are highly relevant for preterm infants who are exposed to repeatedly painful procedures and, as a result, may have expansion of the dorsal horn receptive fields, making them more likely to be activated (Fitzgerald & Walker, 2003). Such changes can permanently alter neuronal circuits that process pain in the spinal cord (Ruda, Ling, Hohmann, Peng & Tachibana, 2000; Torsney & Fitzgerald, 2001). For example, death of the dorsal root ganglion cells leads to the central dorsal root terminals of nearby intact nerves sprouting in the spinal cord to occupy areas normally exclusive to the sectional nerve (Fitzgerald, 1985; Shortland & Fitzgerald, 1994). These sprouts then form inappropriate functional connections with the dorsal horn cells in new regions far from their normal areas (Fitzgerald, 2000), responses which are developmental in nature (Fitzgerald, 2000; Fitzgerald & Jennings, 1999).

Second, in adults, connections between A- and C-fibres and spinal cord neurons are precise; however, in neonatal rats, terminals may extend beyond their final resting place (Fitzgerald & Jennings, 1999). Thus, pain stimuli can evoke transmission of pain signals to many levels above and below the level of the stimulus. Finally, substance P and glutamate NMDA receptors are denser and more widely spread, thereby increasing the excitability of the neonatal spinal cord (Andrews & Fitzgerald, 1997).

Not only is pain transmission in the neonate developmentally different peripherally and in the spinal cord, but also the ascending control of pain signals is developed long before the descending inhibitory controls (Fitzgerald & Koltzenburg, 1986). As a consequence, neonates have less ability to utilize the endogenous analgesic system to modulate painful stimulation as it enters the CNS; and thus, the

effects of repeated pain exposure may be greater in infants than in adults (Fitzgerald, 2000). Moreover, this lack of inhibition may result in body and extremity responses to sensory inputs that may be exaggerated, generalized, unpredictable and disorganized (Fitzgerald, 2000).

As a result of developmental differences in pain processing, enhanced pain sensitivity in neonates can be observed functionally in both animal models (e.g. Teng & Abbott, 1998) and in preterm infants who are exposed to repeated painful events early in life. For example, infants below 35 weeks postconceptual age show altered peripheral nociceptive sensitization as demonstrated by lowered thresholds to tactile stimulation (sensitization); their thresholds decrease further (primary hyperalgesia) following repeated pain exposure (Andrews & Fitzgerald, 1994). Secondary hyperalgesia (pain arising from the tissue surrounding a wound) is exhibited in the preterm infant even when the injury is to the contralateral extremity (Andrews & Fitzgerald, 1999).

In addition to secondary hyperalgesia, preterm infants also develop allodynia (pain arising from previously innocuous stimulation) as a result of central sensitization (Fitzgerald et al.,1988, 1989; Fitzgerald & de Lima, 1999). This finding is significant for the preterm infant who, as a result of central sensitization, may perceive non-painful events, such as diapering (Grunau, Holsti, Whitfield & Ling, 2000), as being painful. Indeed, increases in c-fos, an immediate early gene, are seen in adult spinal cords only following the application of a painful stimulus, whereas neonatal rats demonstrate significant fos responses after both noxious and innocuous peripheral stimulation (Jennings & Fitzgerald, 1996). As previously described, repeated low threshold stimulation can also lead to sensitization of dorsal horn cell responses beyond the

period of stimulation (i.e. wind-up). Thus, for the preterm infant, even an initial response to a single painful event may become exaggerated with subsequent handling.

3.3 Effects of Early Pain on CNS Development

In addition to changes in peripheral sensory systems and the spinal cord, early pain exposure may affect the structural and functional connections within the brain. During early brain development, activity dependent synapses form precise connections with their appropriate target cells, and redundant or unused synapses die (apoptosis) (Penn & Shatz, 1999; Rabinowicz, de Courten-Myers, Petetot, Xi & de los Reyes, 1996). By 20 weeks gestation, thalamocortical connections are established, and by 24-26 weeks gestation, nociceptive fibers from the thalamus fully penetrate the primary somatosensory cortex (reviewed by Coskun & Anand, 2000). Animal research suggests that the normal development of the thalamic nuclei is dependent upon appropriate peripheral sensory stimulation (Feldman & Knudsen,1998). Moreover, immature neurons appear to be more vulnerable to excitotoxic damage (McDonald, Silverstein & Johnston, 1988); thus inappropriate sensory stimulation, such as repeated exposure to pain, may alter the synaptic connections in the preterm infant (reviews by Coskun & Anand, 2000; Fitzerald, 2000; Bhutta & Anand, 2002). Scientists hypothesize that alterations in sensory development occur through the induction of NMDA-dependent long-term potentiation and depression (Fox, 2002; Fitzgerald & Walker, 2003). Alterations in superspinal pain processing may be observed functionally in preterm infants who show dampened facial responses to heel lance following repeated exposure to painful events (Johnston & Stevens, 1996; Grunau, Oberlander, Whitfield, Fitzgerald & Lee, 2001). This interpretation must be made cautiously, however, because little is known about the maturation of higher order pain processing (Fitzgerald & Beggs, 2001).

Further alterations in preterm infant CNS development may occur as a result of pain exposure because neonatal physiological responses to painful stimuli include acute increases in heart rate and in blood pressure, variability in heart rate, increased venous and intracranial pressure, and decreases in arterial oxygen saturation and skin blood flow, all of which may cause or extend early intraventricular haemorrhage and produce ischaemia/reperfusion injury associated with periventricular leukomalacia.[3] Complications such as these are associated with increased risk for neurodevelopmental abnormalities (reviewed by Whitfield & Grunau, 2000).

3.4 Long-Term Effects of Early Pain Exposure

Although a causal relationship between neonatal pain and long- term developmental outcomes has yet to be established, there is evidence that early pain exposure changes subsequent biobehavioural pain expression (Grunau, 2002). Term infants who had undergone circumcision showed greater behavioural pain scores and cried longer at subsequent routine vaccinations than did non-circumcised infants (Taddio, Goldback, Ipp, Stevens, & Koren, 1995; Taddio, Katz, Ilersich, & Koren, 1997). Moreover, Taddio, Shah, Gilbert-MacLeod and Katz (2002) showed that term infants who were exposed to repeated heel lances learned to anticipate pain in the first 36 hours of life. Similarly, Goubet, Clifton & Shaw (2001) reported that preterm infants demonstrated anticipatory changes in heart rate following repeated heel lances.

In addition, alterations in reactivity and dampening to procedural pain in preterm infants having extremely low birth weight (ELBW: ≤800 grams) following discharge from the NICU and later, in infancy and childhood, may be related to early pain exposure.

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[3] Apoptotic cell death and necrotic cell death are distinct in morphology and cause; however, both lead to alterations in the CNS (Giffard, & Fiskum, 2003).

For example, former extremely low birth weight infants (ELBW; < 800 grams) assessed at 4 months corrected chronological age (CCA) exhibited subtle differences in their ability to recover from acute pain applied to a pain-naïve site when compared to term controls (Oberlander, Grunau, Whitfield, Fitzgerald, Pitfield & Saul, 2000). Specifically, ELBW infants showed sustained sympathetic response which remained during recovery. Moreover, ELBW infants whose facial activity returned to baseline more quickly (Early Recovery) had been exposed to greater numbers of prior pain procedures and surgeries than infants whose facial activity recovered later in time, although limited numbers in the two groups did not allow statistical analysis. At 8 months CCA, ELBW infants exposed to greater numbers of painful procedures since birth showed significantly dampened facial and heart rate responses during recovery following finger lance (Grunau, Oberlander, Whitfield, Fitzgerald, Morison & Saul, 2001). Changes from 4 to 8 months suggested that differences may become more marked over time.

Further indirect evidence supports the notion that early pain exposure alters later development in infants born extremely low birth weight. Former ELBW preterm toddlers, as described through parent report, had lower pain sensitivity than term born controls (Grunau, Whitfield & Petrie, 1994). At school entry, former ELBW children have increased somatization (non-organically based pain) (Grunau, Whitfield, Petrie & Fryer, 1994; Sommerfelt, Troland, Ellertsen & Markestad, 1996). Interestingly, this difference in somatization does not persist into middle school years (Whitfield et al., 1997). Finally, at school age, former ELBW children rated pictures of children in pain during recreation as higher in pain intensity than their term born peers and attributed greater affect to medical pain (Grunau, Whitfield & Petrie, 1998).

More direct evidence between early pain exposure and changes in development has been shown in animal models. For example, neonatal rats exposed to tissue injury

show altered pain thresholds as adults (Anand, Coskun, Thrivikraman, Nemeroff & Plotsky, 1999; de Lima et al., 1999). Using inflammatory pain models, others have shown that noxious stimulation early in development produces long-lasting changes in pain pathways (Bhutta et al., 2001; de Lima et al., 1999; Lidow, Song & Ren, 2001; Liu, Rovnaghi & Anand, in press; Ruda et al., 2000). However, the mechanisms for such alterations remain poorly understood.

In conclusion, over the last 20 years, tremendous advances in our knowledge of the neurobiology of pain in neonates have been made. Pain processing involves a complex network of peripheral and central systems which is not yet fully understood even in adults. However, we do know that the developmental differences in peripheral and central pain systems make infants, particularly preterm infants, much more vulnerable to short and long-term alterations in CNS development as a result of repeated early pain exposure.

In Chapters 2 and 3, I have presented multiple lines of evidence which support Als' contention that early exposure to a developmentally unexpected environment alters preterm infant development. This evidence highlights the vital contribution which the synactive theory makes to neonatal and developmental medicine because, when Als proposed the synactive theory of development, much of this evidence was not available. While understanding this background knowledge is critical for those wishing to examine the validity of the synactive theory of development, this literature does not directly answer the fundamental question of this dissertation: should preterm infant movements be interpreted as signs of stress or stability as the synactive theory of development directs us?

One of the ways to examine whether preterm infant movements represent stress responses is to study their movements across a range of procedures which vary in

intensity, acutely painful procedures being toward one end of the range. However, the assessment which is used in the clinical application of the synactive theory, the Newborn Individualized Developmental Care and Assessment Program (NIDCAP®), was not specifically designed to assess pain. Accordingly, to ensure that pain its self is being measured, along with the NIDCAP®, valid and reliable measures of pain for preterm infants must also be used. In Chapter 4, preterm infant pain measurement will be examined.

CHAPTER 4

Pain Assessment in Preterm Infants

The focus of this chapter will be on the assessment of pain in preterm infants; however, given that pain cues from term infants were used to develop pain measures for preterm infants, the chapter will begin with a discussion of these indicators. Descriptions of physiological and behavioural responses of term infants to acute pain began to appear with greater frequency in the 1970s and early 1980's. For example, behavioural reactivity including crying, grimacing, body movements and changes in behavioural states were described as indicators of pain in infants (e.g. Gunnar et al., 1981; Rich, Marshall & Volpe, 1974; Owens & Todt, 1984). Furthermore, changes in plasma cortisol levels, heart rate, respiratory rate and oxygen saturations were some of the first physiological indicators associated with acute pain in infants (e.g. Gunnar et al., 1981; Rawlings, Miller, & Engel, 1980; Talbert, Kraybill & Potter, 1976; Williamson & Williamson, 1983). In addition, Izard was instrumental in demonstrating that infants display discrete facial expressions indicative of acute pain (e.g. Izard, Hembree, Dougherty & Coss, 1983). The development of the first reliable and validated behavioural measure specifically for assessing pain in infants was based on such work (Grunau & Craig, 1987).

Since that time, extensive research has focused on biobehavioural reactivity of infants to acute pain and on the development of pain assessment tools (for reviews see Abu-Saad, Bours, Stevens & Hamers, 1998; Franck & Miaskowski, 1997; Stevens, Johnston & Gibbins, 2000). To date, the most specific behavioural indicator of acute pain in term neonates is facial reactivity (Franck et al., 2000; Stevens et al., 2000). Sleep/wake states and body movements have also been examined as indicators of acute pain in infants (e.g. Dale, 1986; Fuller & Neu, 2000; Franck, 1986; Grunau & Craig, 1987); while these

indicators are associated with stressful stimuli, they have not been shown to be pain specific (Craig, Korol & Tillai, 2002; Stevens et al., 2000).[1]

Researchers examining physiological indicators of acute pain in infants, such as changes in heart rate and oxygen saturation, have determined that these indicators are also important to include in multidimensional assessment of pain because they provide different information from behavioural indicators (Franck & Miaskowski, 1997). However, they also caution that physiological changes during acute pain are less specific than facial reactivity, and as such, should not be used as sole measures of pain (Stevens et al., 2000). I will review the most commonly used physiological pain indicators later in this chapter.

While extrapolating observations of pain reactivity in term infants has been the most commonly employed strategy for developing pain assessment tools for preterm infants, this approach is limited because assessing pain in preterm infants is more complex than for term infants for a number of reasons. First, preterm infants respond with facial, motor and physiological changes to acute pain, but they differ from term born infants in that preterm infant responses are of smaller magnitude particularly at younger gestational ages (Craig, Whitfield, Grunau, Linton & Hadjistavropoulos, 1993; Johnston & Stevens, 1996; Johnston, Stevens, Yang & Horton, 1996; Johnston et al., 1999b). Second, due to neurological immaturity, preterm infants at earlier gestational ages may display different pain behaviours from infants at later gestational ages; these behaviours, therefore, may not be captured by pain scales based on pain cues observed in term infants. Third, no physiologic or behavioural threshold specifically marks the presence of pain. Finally,

[1] I will describe this literature in further detail in relation to pain assessment in preterm infants.

although using a single pain index makes assessment easy for clinicians, the physiologic and behavioural responses of preterm infants to painful stimuli are often dissociated (Morison, Grunau, Oberlander & Whitfield, 2001); therefore, reliance on a single pain index may not capture the range of responses in this population. Complexities such as these have led to the decision that the most promising indices of pain in preterm infants employ multidimensional measures and incorporate developmentally relevant pain indicators (Grunau, 2000; Morison et al., 2003; Stevens, Johnston & Grunau, 1995).

In this chapter, I will review the most commonly used physiological and behavioural indicators of acute pain in preterm infants, and I will describe the unidimensional and multidimensional pain assessment tools currently available. The purpose of this review is not to present the entire pain assessment literature, but rather to provide a focused description of pain indicators and tools available for preterm infants. Through this focus, I will provide a rationale for the specific biobehavioural measures I will use to examine the validity of the dualistic classification system of the synactive theory of development.

4.1 Physiological Indicators of Pain in Preterm Infants

Over the past fifteen to twenty years, many studies have described physiological indicators which change when preterm and term infants are exposed to invasive procedures; however, these indicators are relatively non-specific to pain. Nevertheless, physiological indicators should meet three criteria before they can be said to be true measures of pain: they should show graded responses with variation in intensity of the stimulus, they should show changes when pain relief is provided, and they should relate to other measures of pain (Sweat & McGrath, 1998). A fourth consideration is also important for clinicians in the NICU: the physiological indicators should be readily available and feasible in the clinical setting so as to facilitate rapid pain assessment and implementation of pain management strategies.

Multiple physiological parameters such as heart rate, oxygen saturation (SaO2), heart rate variability, vagal tone, blood pressure, respiratory rate, transcutaneous oxygen levels (tcPO2), transcutaneous carbon dioxide levels (tcCPO2), intracranial pressure (ICP), palmar sweating, and skin blood flow have been studied during infant pain. Since some of these indicators, such as vagal tone and heart rate variability, require sophisticated computer transformation and analyses after acquiring the clinical data, they are impractical for the clinician to use at the bedside. Moreover, other physiological parameters such as tcPO2, tcCPO2 and blood pressure show varying responses to acute pain (Craig et al., 1993; MacIntosh, Van Veen & Brameryer, 1993; Schwarz & Jeffries,1990). Although measuring respiratory rate as a physiological pain indicator is easily achieved using bedside monitors, it may show little variation in response to painful events (Bozzette, 1993; Mudge & Younger, 1989; Porter, Miller, Cole & Marshall, 1991; Weatherstone et al., 1993; Williamson & Williamson, 1993; Van Cleve et al.,1995). Respiratory rate is also influenced by severity of illness (Field & Goldson, 1984), by sleep/wake states (Prechtl, 1974) and by medications such as respiratory stimulants commonly used in neonatal intensive care nurseries (NICUs). ICP and skin blood flow are measures not used regularly in NICUs, and although palmar sweating appears to be pain sensitive, it is a measure of emotion in general and so can not be considered pain specific.

Heart rate is the most commonly used physiological indicator of pain. Heart rate is usually monitored in the NICU as part of standard clinical practice, and monitoring equipment is readily adapted for research purposes. Heart rate is measured as the number of beats over a specific period of time. In infants, heart rate increases during painful procedures such as circumcision (Benini, Johnston, Faucher, & Aranda, 1993; Marchette, Main, Redick, Bagg, & Leatherland, 1991; Rowlings, Miller, & Engel, 1980;

Weatherstone et al., 1993; Williamson & Williamson, 1983), during heel stick (Beaudoin, James & McAllister, 1991; Bozzette, 1993; Craig et al., 1993; Dinwiddie, Patel, Kumar, & Fox, 1979; Gonsalves & Mercer, 1993; Grunau et al., 1998; Grunau et al., 2001; Stevens, Johnston & Horton, 1993; Johnston & Stevens, 1996; Johnston, Stevens, Yang, & Horton, 1995, 1996; Kachoyeanos, Bollig, & Eggener, 1991; McIntosh, Van Veen, & Brameyer, 1994; Morison et al., 2003; Owens & Todt, 1984; Schwartz, & Jeffries, 1990; Stevens, & Johnston, 1994), and during other invasive procedures such as suctioning (Durand, Sangha, Cabal, Hoppenbrouwers, & Hodgman, 1989; Ninan, O'Donnell, Hamilton, Tan, & Sankaran, 1986), percutaneous silastic catheter insertion (Moustogiansi, Roohey, McColloch, & Raju, 1994), lumbar puncture (Porter et al., 1991) and venipuncture (Van Cleve et al., 1995). Moreover, heart rate drops when interventions are given to treat pain (Arnett, Jones, & Horger, 1990; Corff, Seideman, Vankaaraman, Lutes & Yates, 2001; Field & Goldson, 1984; Guinsberg, et al., 1998; Holve et al., 1983; Johnston et al., 2003; Maxwell, Yaster, Wetzel, & Niebyl, 1987; Mudge & Younger, 1989; Stevens & Ohlsson, 2001) and shows differential increases depending on the level of intensity of the stimulus (Craig et al., 1993; Johnston et al., 1995; Marchette et al., 1991; Owens & Todt, 1984; Porter et al., 1991, 1999; Johnston et al.,1994). Finally, heart rate has been positively associated with other pain measures (Johnston et al.,1995; Owens & Todt,1984).

Despite this large quantity of evidence supporting heart rate as a pain indicator in infants, a few studies document contradictory responses. Marshall, Deeder, Sharada, Berkowitz and Austin (1984) found a drop in heart rate during endotracheal intubation of preterm infants. This result may have been caused by the vagal response created by the insertion of the tube. Furthermore, when measuring heart rate over a very short period of time, Johnston et al. (1996) noted an initial drop in heart rate prior to rising in

infants undergoing routine immunization. Finally, Zahr and Balian (1995) clustered 19 nursing procedures, compared the influence of these with noise levels in three neonatal nurseries and found no specific changes in heart rate to either noise or interventions. In this study, however, invasive procedures were combined with tactile procedures.

Although heart rate is the most commonly studied physiological pain indicator in preterm infants, interpretation of the changes in heart rate during acute pain must take into account factors such as the law of initial values (Lacey, 1956), gestational age of the infants (Field & Goldson, 1984; Craig et al., 1993; Johnston et al., 1996; Owens & Todt, 1984), sleep/wake states (Johnston et al., 1999b; Stevens et al., 1994), illness severity and when the infants had last been exposed to a painful procedure (Johnston et al., 1999b), as these factors have been shown to influence heart rate.

Like heart rate, oxygen saturation (O2 sat) is a physiological indicator which is commonly used at the bedside in the NICU and is readily adapted for research use. Oxygen saturation is a measure of the percentage of hemoglobin that is carrying oxygen at a given time (Sweet & McGrath, 1998). Measures of mean O2 sat have been shown to drop during acutely painful procedures (Benini et al., 1993; Bozzette, 1993; Maxwell et al., 1987; Schwartz & Jeffries, 1990; Stevens et al., 1993; Johnston et al., 1995; Van Cleve et al., 1995) and to increase when analgesics are given to treat pain (Arnett et al., 1990; Benini et al., 1993; Maxwell et al., 1987). Furthermore, reports indicate that O2 sat changes were greater during painful stimulus than non-painful stimulus (Craig et al., 1993; Johnston et al., 1995; Johnston et al, 1995).

In summary, heart rate and oxygen saturation are the two most commonly used physiological indicators of pain in preterm infants. These indicators satisfy the criteria as true indices of pain; that is, they show graded responses with variation in intensity of the stimulus, they show predictable changes when pain relief is provided, and they can be

related to other measures of pain. Finally, they are readily available in the clinical setting and easily adapted for research purposes.

While physiological measures of pain are indispensable, they should not be used in isolation because no specific change in heart rate and oxygen saturation indicates the threshold of pain. For the most accurate results, pain assessment must combine physiological with behavioural indicators.

4.2 Behavioural Indicators of Pain in Preterm Infants

A number of behavioural indicators have been studied during infant pain. The indicator usually associated with pain is crying. From an evolutionary point of view, however, cry did not develop as a specific pain signal, but rather as a graded sign of distress (McGrath, 1998). Nonetheless, using cry as a pain indicator is appealing because a relatively consistent pattern in the onset of crying follows a painful stimulus across postnatal age, and the acoustic and temporal characteristics of pain cry may help us distinguish between painful and non-painful events (Franck & Miaskowski, 1997). However, using cry as a behavioural indicator of pain in preterm infants is problematic for two reasons. First, many preterm infants require mechanical ventilation, a procedure which precludes using cry as an indicator. Second, as many as 50 % of premature infants may not cry after a painful event (Johnson, Stevens, Craig & Grunau, 1993 Johnston et al., 1999a). Third, no acoustic or temporal features are specific to pain particularly in individual infants (as reviewed by Barr, 1998; Johnston et al., 1999a; McGrath, 1998).

In addition to crying, generalized body movements are commonly observed in infants responding to a painful stimulus (Franck, 1986; Craig et al., 1993). In a small sample of full term infants, Franck (1986) concluded that reaction time, by either direct observation or photogrammetric methods, provides a precise quantification of body

movements. However, Craig and colleagues (1993), who described general arm, leg and torso movements in response to heel lance in both preterm and term infants, found that body movements are less specific to pain than facial response. Moreover, gross body movements, as a single measure of response to acute pain, are not useful because it is difficult to control for stimulus intensity and for sleep/wake state; because thus far, they have been shown to be less specific than facial reactivity; and because few objective measures are available.

Alternatively, some researchers have described specific body movements which are associated with pain. The flexion withdrawal reflex (FWR) observed in the neonate is a clear stereotypical response to tactile and nociceptive stimulation whereby the leg of the infant flexes away from a stimulus applied to the plantar aspect of the foot. The threshold for the response can be measured using von Frey filaments or hairs (calibrated in size for graded mechanical stimulation) applied to the plantar aspect of the foot and measuring the force required to reproduce the response (Fitzgerald, Shaw & McIntosh, 1988). During the FWR, the infant may also extend the contralateral leg; noxious stimulation can elicit this reflex in preterm infants as early as 26 weeks gestational age (Andrews & Fitzgerald, 1994).

In the adult, this nociceptive reflex response is directly related to the perception of pain (Willer, 1977). However, in neonates, the reflex, although associated with the anatomical structures associated with pain perception, is not an indicator of pain specifically because eliciting the reflex does not always require a noxious stimulus (Fitzgerald, 2001). Nevertheless, this response may be useful clinically as an indicator of lowered pain thresholds (hyperalgesia) and of pain caused by non-noxious stimuli (allodynia) (Stevens et al., 2000). For this reason, investigators have included knee or

leg flexion in their pain indices (Evans, Voglepohl, Bourguignon & Morcott, 1997; Franck, 1986; Lawrence et al., 1993).

Others have identified specific body movements such as rigidity, clenching of the fists, withdrawal and "flinching" as potential body reactions to heel lance (Bozette, 1993). Splaying of fingers and toes, arching, and limb and neck extension have also been used in some assessments; but the presence or absence of these movements is usually combined with other behavioural and physiological pain indicators rather than studied in isolation (Halimaa, Vehviläinen-Julkunen & Heinonen, 2001; Slevin, Daly & Murphy, 1998; Sparshott, 1996). Furthermore, many of these movements have not been studied in preterm infants.[2]

Changes in sleep/wake states appear to be useful pain indicators when used in combination with other indicators. Sleep/wake states, which begin to develop prenatally (Nijhuis, Prechtl, Martin & Bots,1982), have been used to describe clusters of behaviours and to explain brain mechanisms which modify the responses of infants (Parmelee, Wenner, Akiyama, Schultz & Stern,1967; Prechtl, 1974). Sleep/wake states can be measured by direct observation and through polygraph recordings of respiratory patterns, by eye movements together with gross motor movements (Franck & Miaskowski, 1997). In full-term infants, painful stimuli evoke measurable short and long term changes in sleep/wake cycles, including increases in wakefulness during and shortly after circumcision and increases of quiet sleep with corresponding decreases in active sleep the night following circumcision (Anders & Chalemain, 1974; Emde, Harmon, Metcalf, Keonig, & Wagonfeld, 1971). Preterm infants also show significant

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[2] The use of specific body movements as pain indicators will also be discussed in more detail in Chapter 6.

changes in sleep/waking states during acutely painful events (e.g. Grunau, et al., 2001; Morison, et al., 2003). Indeed, sleep/wake states must be considered when measuring preterm infant pain responses because Grunau and Craig (1987) demonstrated that term infants in quiet sleep had the least facial activity during heel lance, whereas those in quiet-alert showed the greatest facial activity. Stevens et al. (1994) confirmed these findings in preterm infants with those infants during quiet sleep showing fewer facial actions. In addition, infants in active sleep or quiet awake showed the greatest proportion of facial action. While most clinicians are aware of sleep/waking states, extensive training is required to detect subtle changes in state (Stevens et al., 2000; Franck & Miaskowski, 1997).

Although the assessment of sleep/wake states should be included during preterm infant pain assessment, assessment of facial activity is comparatively the most specific behavioural indicator of pain. Infants have a stereotypical group of facial movements in response to an acutely painful event including eye squeeze, brow bulge, deepened nasolabial furrows, taut tongue and open mouth. These features have been observed in infants as young as 23 weeks gestational age (Grunau et al., 2001); however, the intensity and duration of particular facial actions varies with obstetric history, neonatal history (including history of pain exposure), gestational age at birth and at the time of the pain assessment, with gender, and as we noted in the previous section, with the sleep/wake state of the infant (Craig et al., 1993; Grunau et al., 2001; Grunau & Craig, 1987; Stevens et al., 1994). For example, Grunau, Craig and Drummond (1989) found that term infants who had had more stressful deliveries showed increased facial responses to acute pain. Although the relationship between obstetrical management and acute pain reactivity has not been examined in preterm infants, increased facial reactivity can be seen in preterm infants who are handled just

prior to an acutely painful procedure (Porter, Wolf and Miller 1998). Confirming these findings, Lindh, Grunau, Holsti, Oberlander, Fitzgerald & Solimano (in preparation) reported that preterm infants who had experienced diaper changing prior to heel lance showed increased facial reactivity when compared to infants who had not been handled before blood collection. Moreover, preterm infants who experienced blood collection prior to tactile procedures also demonstrated increased facial reactivity (Holsti, Grunau, Whitfield & Oberlander, under review).

The gestational age of the infant at the time of the pain assessment also influences facial responses to pain. Provided that the assessments are undertaken close to birth, facial reactivity increases with increasing gestational age (Craig et al., 1993; Johnston et al., 1995; Porter et al.,1999). On the other hand, dampening of facial responses is observed in infants who were born earlier and who had been in the NICU longer, had greater numbers of painful procedures and had been exposed to post-natal steroids (Grunau et al., 2001; Johnston & Stevens, 1996). Dampened facial reactivity may also be seen in preterm infants who have experienced an acutely painful event just prior to the one being assessed (Johnston et al., 1999b). Prior morphine exposure, however, has been associated with "normalized" rather than with decreased responses (Grunau et al., 2001). Finally, female preterm infants assessed within the first week of life express more facial reactivity to pain than male infants (Guinsberg et al., 2000).

Despite the variability in intensity and duration of facial activity during acute pain, studies show that facial activity contributes more to adult judgments of severity of infant pain than does cry (Craig, Grunau, & Aquan-Assee, 1988; Hadjistavropoulos, Craig, Grunau, & Johnston, 1994). Moreover, facial activity is actually less variable and more consistent in infants than cry or generalized body movements (Stevens et al., 2000). Therefore, as previously mentioned, facial activity is the most reliable and consistent

behavioural indicator of pain in infants (Franck & Miaskowski, 1997; Steven,s et al., 2000); as such, it is considered the "gold standard" as an indicator of acute pain in infants (Franck et al., 2000; Stevens et al., 2000).

In summary, some researchers use cry as a behavioural measure of pain in neonates, but this response is precluded for preterm infants who are intubated. Others have included body movements as indictors of pain, but except for the flexor withdrawal response, these have had little empirical study. Currently, the most reliable and well-studied behavioural indicators of pain in preterm infants are facial actions and changes in sleep/wake states.

4.3 Pain Measurement Tools

Identifying the presence or absence of pain requires the use of reliable and valid pain measures which can be used for both research and clinical assessment. The tools which are currently available can be divided into two categories: unidimensional measures and multidimensional measures. Unidimensional measures of pain use either a single type of variable, such as facial activity, or single dimensions of pain such as behavioural measures (Stevens et al., 2000). Multidimensional measures combine types of pain indicators and may include contextual factors.

4.3.1.Unidimensional Measures

While a number of unidimensional tools are available for use in research and in clinical settings, many of these lack tests of their psychometric properties and/or are used only in research settings. Table 1 summarizes the psychometric evaluation of some of the most frequently used infant pain measures.

Currently, the most well-researched unidimensional pain scale for use with preterm infants is the Neonatal Facial Coding System (NFCS; Grunau & Craig, 1987).

Table I. Psychometric Properties of Unidimensional Neonatal Pain Tools

TOOLS*	VALIDITY					RELIABILITY			FEASIBILITY/ CLINICAL UTILITY
	Face and Content	Criterion-related	Construct	Discriminant	Con-vergent	Inter-rater	Intra-rater	Consistency	
IBCS	Yes	-	-	-	-	Yes r=0.83	-	-	-
BPS	-	-	Preliminary	-	-	-	-	-	-
MAX	Yes	-	Yes	-	Yes r=0.87	Yes r=0.83	-	-	Requires extensive coding and interpretation
Baby FACS	Yes		Yes	-					Labour intensive
NFCS	Yes	Yes	Yes	-	Yes r=0.89	Yes >0.85	Yes >0.85	-	Established at bedside
LIDS	Yes	-	Yes	-	-	Yes r=0.74-0.88	Yes R=0.81-0.96	-	-
RIPS	-	-	-	Yes, p<0.001 Sensitivity=0.31-0.23 Specificity=0.68-0.90	-	Yes ICC=0.53-0.83	-	-	-
PRBS	-	-	-	Yes, p< 0.0001	-	Yes, r=0.65-0.84	-	-	-
CSS	-	-	Preliminary	Yes, p<0.001	-	Yes	-	α=0.79-0.88	-
CHIPPS	Yes	Yes	Yes	Yes, sensitivity=0.92-0.96, Specificity=0.74-0.95	-	Yes, r=0.93		α=0.96 infants α=0.92 toddlers	Not used with premature infant.

*Infant Body Coding System(ICBS; Craig, McMahon, Morison & Saskow,1984); The Behavioural Pain Score (BPS; Pokela, 1994; Maximally Discriminant Facial Coding System (MAX; Izard, 1979); Baby Facial Action Coding System (Baby FACS; Oster, 1978); Neonatal Facial Coding System (NFCS; Grunau & Craig, 1987); Liverpool Infant Distress Score (LIDS; Horgan, & Choonara, 1996); The Riley Infant Pain Scale (RIPS; Schade, Joyce, Gerkensmeyer & Keck, 1996); The Pain Rating Scale (PRBS; Joyce et al., 1994); The Clinical Scoring System (CSS; Barrier, Attia, Mayer, Amiel-Tison & Shnider, 1989); Children's and Infant's Postoperative Pain Scale (Buttner & Fink, 2000).

This scale is based on the Baby Facial Action Coding System (Baby FACS; Oster, 1978) approach to coding specific facial actions, and was adapted from the Facial Action Coding System (FACS) for adults which comprise every possible facial muscle movement (Ekman & Friesen, 1978). Much shorter than the Baby FACS, however, the NFCS consists of 10 facial actions that are most closely associated with pain. These comprise brow bulge, eye squeeze, naso-labial furrow, open lips, vertical and horizontal stretch mouth, lip purse, taut tongue, chin quiver and tongue protrusion.[3] Crucially, even though these behaviours were derived from observations of full term infants, the actions are reliably observed in preterm infants as young as 23 weeks gestational age (Grunau et al., 2001).

Tests of the psychometric properties of the NFCS indicate that the NFCS has face and content validity (Abu-Saad et al., 1998). Further, the NFCS has construct validity because it discriminates relative differences between tissue damaging and non-tissue damaging situations (Grunau, Johnston & Craig, 1990). Convergent validity has been demonstrated by comparing the NFCS with the Baby FACS (Craig, Hadjistavropoulos, Grunau & Whitfield, 1994). Not only is the NFCS a valid measure of pain, it is reliable; inter-rater and intra-rater reliability >0.85 have been established on both term and preterm infants (e.g. Craig et al., 1993; Grunau et al., 1990; Grunau et al., 1998; Grunau et al., 2001; Morison et al., 2003; Peters et al., 2003). Moreover, the NFCS has shown greater sensitivity and specificity in preterm infants when compared to

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[3] Since the NFCS was originally designed, researchers have reduced the number of facial actions to 8 movements in some cases (Guinsburg et al., 1997), 5 (Peters et al, 2003), 4 (Rushforth & Levene, 1994) and 3 movements in others (Stevens et al.,1996), while maintaining the specificity of measuring acute and postoperative pain.

another multivariate neonatal pain measure, the Neonatal Infant Pain Scale (NIPS; Lawrence et al., 1993) (Guinsburg, Berenguel, Xavier, Almeida, & Kopelman, 1997). Finally, even though most researchers using the NFCS have coded infants from videotape, four recent reports have used the NFCS at bedside using real time and demonstrate high inter-observer reliability, construct validity and clinical feasibility (Grunau et al., 1998; Grunau et al., 2001; Guinsburg et al., 2000; Rushforth & Levene, 1994).

4.3.2. Multidimensional Measures

We have seen that behavioural indicators, such as facial activity and sleep/wake states, are promising pain indicators. Yet given the complex nature of pain, and the challenges associated with accurate interpretation of behavioural responses to painful stimuli, researchers and clinicians have developed tools which combine physiological indicators with contextual factors and behavioural expressions of pain. Only a few of the many multidimensional measures of pain have been designed specifically for assessing acute pain in preterm infants. However, irrespective of how well multidimensional scales for preterm infants are designed, a major conceptual problem arises when such scales are used clinically. Bauer and colleagues (2004) found that oral glucose reduced facial activity and crying during venipuncture, but did not attenuate physiological responses. Thus, reductions in pain scores on multidimensional scales following anti-nociceptive interventions may not accurately reflect whether or not the effects of pain have been controlled. In addition, the arbitrary weightings applied to facial activity, sleep/wake states, and physiological variables make interpretations of composite scales complex (e.g. Premature Infant Pain Profile (PIPP); Stevens, Johnston, Petryshen & Taddio, 1996). The NIPS (Lawrence et al., 1993); the COMFORT scale (Ambuel, Hamlett, Marx & Blumer,1992); the CRIES (Krechl &

Bildner, 1995); the Scale for Use in Newborns (SUN), based upon the COMFORT Scale (Blauer & Gerstmann, 1998); the Distress Scale for Ventilated Newborn Infants (Sparshott, 1996); the Pain Assessment in Neonates (PAIN Scale; Hudson-Barr et al., 2002), a scale which combines indicators from the NIPS and the CRIES scales, either have been validated mainly with preterm infants > 28 weeks gestational age or have limited evaluation of their psychometric properties. A summary of the psychometric properties of multidimensional pain scales for use in preterm infants is presented in Table 2.

In conclusion, pain assessment in preterm infants is more complex than for term infants. Researchers and clinicians have developed a number of unidimensional and multidimensional pain measures for use with preterm infants. Unfortunately, most of these tools are limited in their evaluation of psychometric properties and/or clinical utility. Nevertheless, multidimensional assessment of pain using both physiological and behavioural indicators is essential since different parameters provide unique information. (e.g. Frank & Miaskowski,1997; Stevens et al., 2000). For these reasons, rather than use a single multidimensional measure of pain, I will combine separate biobehavioural indicators of pain for the empirical work presented in Chapters 6 and 7. The most promising of these indicators in infants > 28 weeks gestational age are changes in facial activity, shifts in infant sleep/waking state, and physiologic indices of heart rate and oxygen saturation.

Table 2. Psychometric Properties of Neonatal Multidimensional Pain Tools

TOOLS*	VALIDITY					RELIABILITY			FEASIBILITY/ CLINICAL UTILITY
	Face and Con-tent	Criterion -related	Construct	Discriminant	Concurrent	Inter-rater	Intra-rater	Con-sistency	
COM-FORT	Yes	r=.75 with VAS	-	-	-	r=0.84	-	α=0.90	Assesses level of sedation. Quickly administered, but more complex than SUN, not primarily for neonates
NIPS	Yes	Yes	p<0.001	-	r=0.53-0.84	r=0.92-0.97	-	α=0.88-0.95	Primarily for preterm infants, only one physiological indicator, easy to use, but variable scoring
CRIES	Yes	Yes	Yes	Yes, p<0.0001	Yes, r=0.49-0.73	r = 0.72	-	-	Easy to administer, inexpensive, easy to learn
PIPP	Yes	-	Preterms p=0.0001-0.02, terms, p<0.02, clinical setting, p<0.0001	-	-	ICC= 0.93-0.96	ICC= 0.94-0.98	α=0.59-0.76	Beginning established utility for post-operative preterm-term infants
DSVNI	Yes	Yes	--	-	-	-	-	-	-
SUN	Yes	-	Yes	-	-	-	-	-	Beginning utility compared to COMFORT/NIPS
PAIN	Yes	Yes, with NIPS (r=0.84-0.98)	Yes, preliminary	-	-	-	-	-	-

*Neonatal Infant Pain Scale (NIPS; Lawence et al., 1993); CRIES (crying, requires oxygen, increased vital signs, expression and sleeplessness (Krechl & Bildner, 1995); Premature Infant Pain Profile (PIPP; Stevens et al., 1996); Distress Scale for Ventilated Newborn Infants (DSNVI; Sparshott, 1996); Scale for Use in Newborns (SUN; Blauer & Gerstmann, 1998); Pain Assessment in Neonates (PAIN; Hudson-Barr et al., 2002).

CHAPTER 5.

The Synactive Theory of Development

Before much of the previously cited literature that documents the deleterious effects of stress and pain on the developing CNS was available, and when medical concerns focused primarily on stabilizing the physiological needs of preterm infants, Als, a developmental psychologist, developed a theory and systematic method of assessing and treating the developmental needs of high-risk newborns: the synactive theory of development (Als, 1982). The purpose of Als' synactive theory of development was to conceptualize high-risk infants' behavioural competence in relation to their environment and to explain how multidisciplinary teams, including parents, could optimally support their individual developmental needs (Als, 1982, 1986, 1995, 1999). Als attributed the developmental differences between preterm infants and full term infants that have been documented in long-term follow-up studies (e.g. Grunau, Whitfield & Davis, 2002; Vohr et al., 2003; Holsti, Grunau & Whitfield, 2002; Saigal, Pinelli, Hoult, Kim & Boyle, 2003; Whitfield, Grunau & Holsti, 1997; Wood, Marlow, Costeloe, Gibson, & Wilkinson, 2000) to the mismatch between the preterm infant's brain and the developmentally unexpected environment of the neonatal intensive care unit (Als, 1999). Developmental specialists use the synactive theory to guide their assessments and interventions of these infants by applying the model of care based upon the synactive theory of development, the Newborn Individualized Developmental Care and Assessment Program (NIDCAP®) (Als, 1986).

In the following chapter, I first describe the four sub principles of the theory which comprise Als' principle of synaction and the theory's model of care, the NIDCAP®. Next I will discuss a number of clinical studies that have evaluated whether the use of the NIDCAP® alters developmental outcome in high-risk infants. One study points the way

to my approach; it tests a principle of the theory itself. I will examine the merits of a central principle, the dual antagonistic systems. This principle is not only the most widely applied throughout the theory, it also determines the fundamental way in which preterm infant behaviours are interpreted.

5.1 Principles of the Synactive Theory of Development

The synactive theory of development is a synthetic theory in that a number of areas of study including ethology, neuroembryology, organismic psychology, and motor neurophysiology contribute to the overriding tenet of this theory, the principle of synaction (Als, Duffy, McAnulty, & Badian, 1989). The principle of synaction comprises four sub principles. First, the synactive theory draws on the principle of species adaptedness, an ethological principle, whereby infants work toward finding the highest level of adaptation and one which is most parsimonious for their particular environment. According to this theory, the premature infant is not an immature term infant, but an organism which is perfectly adapted to its intended environment, the uterus (Als, 1986). Moreover, the central nervous system (CNS) drives the infant to find the best adaptive behaviours, and although the CNS "drives" development, plasticity allows flexibility and complexity of responses (Als, 1999). Thus, the infant is viewed as a biologically programmed entity which interacts with its environment and actively participates in obtaining information from the environment to advance its own development.

The study of neuroembryology contributes a second principle to the application of the synactive theory. The principle of continuous organism-environment interaction states that the primary role of the CNS is to differentiate and to develop by interacting with the environment. The way in which the infant uses the environment to refine its development is through internalized feedback loops.

Als takes a third principle from organismic psychology, the principle of orthogensis in which global function moves towards differentiated function, and of syncresis in which subsystem functions synchronize through these means. An infant develops by moving from global functioning towards increasingly distinct and differentiated function whose outcome is also hierarchically integrated into subsystems. Developmental psychologists who accept this principle of organization are therefore led to identify subsystems and their interrelationships and to suggest that the task of the newborn is to synchronize the subsystems. The synactive theory's subsystems which function simultaneously include the autonomic, motor, state organizational, attention/interaction and self-regulatory/balancing subsystems. Als states that when function at one level is stable and integrated, the emergence of another subsystem is free to develop a new level of differentiation. Timing and intensity of stimulation change synchrony of the subsystems (Schneirla, 1965). Poor timing or intensity of stimulation alters cortico-cortical connections which, in turn, alter the CNS and subsequent development (Als, 1999).

The principle of continuous dual antagonistic integration, a principle taken from the neurophysiological studies of motor systems, is the final principle on which the synactive theory draws. This principle states that the motor system strives for smoothness between two types of responses, antagonists (withdrawing forces) and agonists (approaching forces) (Denny-Brown, 1966), and that approach and avoidance behaviors are the fundamental actions of the motor system. (Als, 1999) The principle of dual antagonistic systems is applied broadly throughout the theory not only to the motor systems, but also to all the other subsystems.

The principle of synaction itself is an integration of four others: the principle of adaptedness, of continuous organism–environment interaction, of orthogenesis and

syncresis, and of dual antagonist integration. Als states that '...development proceeds thorough the continuous balancing of approach and avoidance, yielding a spiral potentiation of continuous intraorganism system interaction and differentiation and organism-environment interaction aimed at bringing about the realization of hierarchically ordered species-unique developmental agenda.' (Als et al., 1989, p. 6).

Using this principle of synaction, Als proposes a biphasic model of development that specifies the degree of differentiation of behaviour and the ability of the infant at a given time to modulate and organize its own behaviour (Als, 1982). The focus is on the way in which an infant responds to the world rather than on the specific skills of an infant. The development is synactive in that the infant functions within five subsystems of simultaneously existing and interactive behaviours. These subsystems are the autonomic, motor, state organizational, attention and interacting, and self-regulatory/balancing systems. According to the synactive theory (Als, 1986), an organism will defend itself against stimulation if it is intense, is too complex or is inappropriately timed. Further if stimulation is properly timed and is appropriate in complexity and in intensity, the organism, while maintaining itself in balance, will search and move toward it. (Als, 1986)

5.2 Clinical Application of the Synactive Theory: The Newborn Individualized Developmental Care and Assessment Program (NIDCAP®).

The model of care that is based on Als synactive theory of development is the Newborn Developmental Care and Assessment Program, the NIDCAP®. Unlike many other infant developmental assessments, the NIDCAP® utilizes naturalistic observation techniques to observe an infant's development; that is, the examiner does not interact with the infant or care-giver during the assessment. The examiner observes the infant during a procedure that is part of the normal routine NICU care. While a NICU staff

member or parent provides the care, the examiner documents the frequency of 85 specific NIDCAP® behaviours on a continuous time sampling sheet every two minutes. The behaviours are grouped on the time sampling sheet under the five subsystems of functioning. For example, the autonomic system is assessed by recording the infant's breathing patterns, color changes and visceral stability; the motor system is assessed by observing the infant's muscle tone and movement patterns; and sleep/wake state organization is assessed by observing the available range of states and how the infant makes transitions between the states. The infant should be observed during a variety of procedures ranging in intensity from intubation and blood collection to feeding, bathing and being held by its parent. Each observation usually takes 60-90 minutes and includes a 10 minute baseline, the time it takes to perform the care-giving procedure, and a 10 minute recovery period.

Following each observation, a detailed descriptive narrative is written which includes an evaluation of the infant's environment and an evaluation of the how the infant has responded before during and following the care-giving task. More specifically, the observer documents the infant's avoidance behaviours (those thought to reflect stress, [Als, 1999]), the capabilities which the infant had to self-regulate and which, if any, strategies the care-giver used to provide developmental support and stability. These detailed assessments are repeated weekly; and in this way, a comprehensive understanding of each individual infant's development is achieved. Thus, the purpose of the assessment is to evaluate the extent to which the infant is able to differentiate and to modulate the various subsystems given the varying demands of the environment. Developmental specialists then suggest modifications to handling and to the environment that might reduce the stress behaviours and help the infant maintain a balanced and integrated state. The aim is to promote optimal development. The

constituents of the synactive theory of development and of its model of care, the NIDCAP® are summarized in Figure 5.

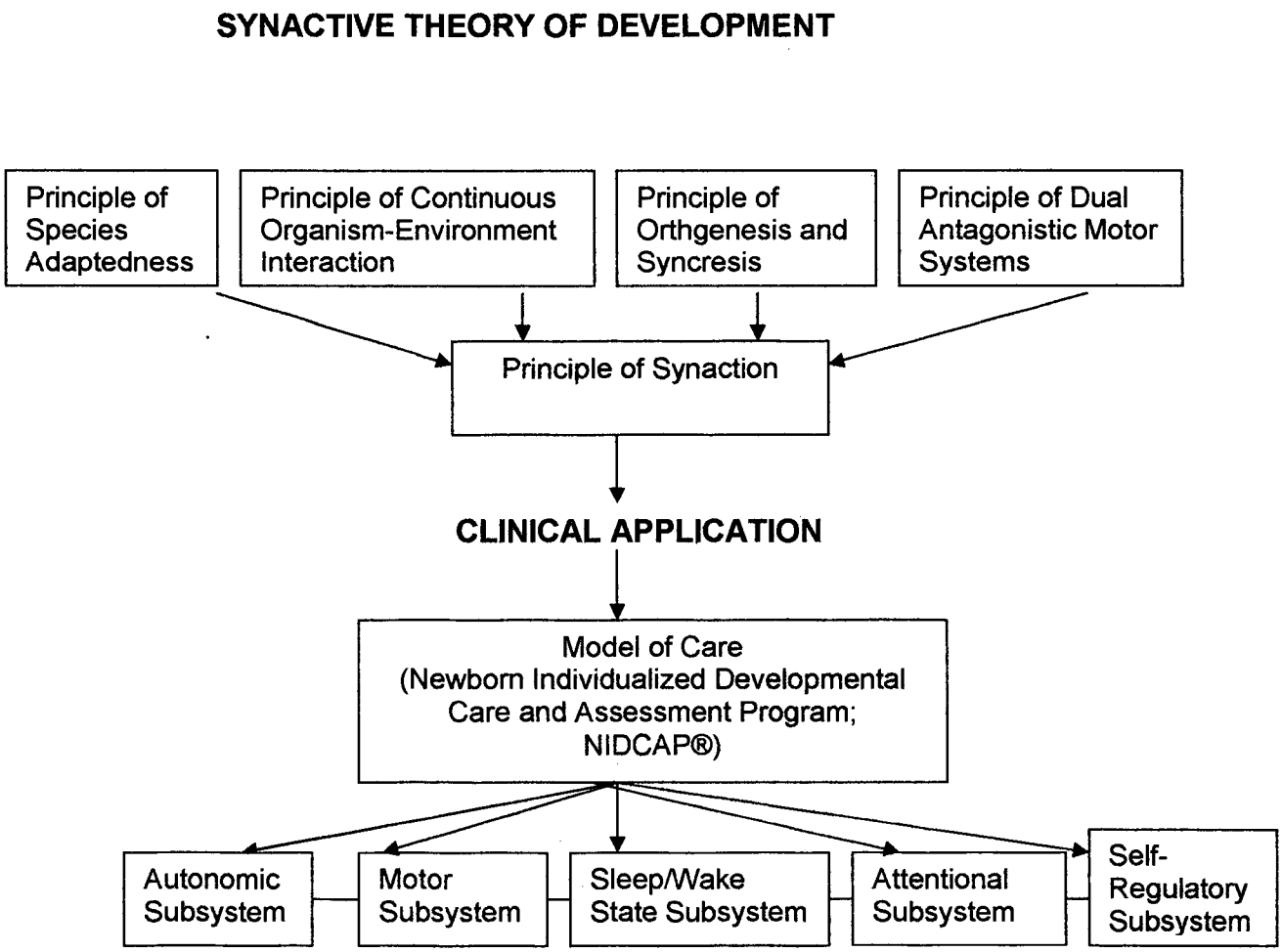

Figure 5. The Synactive Theory of Development

5.3 Synactive theory of development: Evaluating the NIDCAP®.

One of the measures of an effective theory of development is whether the hypotheses generated from the theory are testable and valid. Thus far, the major hypothesis tested from the synactive theory of development is one which measures clinical outcomes: that those infants who are cared for using the NIDCAP® model will show improvements in early and later development compared to infants cared for using the traditional medical model. Utilizing randomized, phase-lag, retrospective study

designs, or providing secondary analyses, a substantial number of studies have been published in which preterm infants, varying in gestational ages and illness severity, are cared for using the NIDCAP® model of care (Als, Brown, Gibes, Duffy, McAnulty & Blickman, 1986; Als et al., 2003; Als, Lawhon, Duffy, McAnulty, Gibes-Grossman, & Blickman, 1994; Becker, Grunwald, Moorman & Schtuhr, 1991; Becker, Grunwald, Moorman & Schtuhr, 1993; Brown & Heermann, 1997; Buehler, Als, Duffy McAnulty & Leiderman, 1995; Fleisher et al., 1995; Heller, Constantinou, Landenberg, Benito & Fleisher, 1997; Kleberg, Westrup & Stjernqvist, 2000; Mouradian & Als, 1994; Stevens, Petryshen, Hawkins, Smith &Taylor, 1996). Although these studies used different medical outcome measures, in general, they showed that preterm infants who had been cared for using the NIDCAP® model required less mechanical ventilation, had increased physiological stability, needed less sedation, had lower incidence of intraventricular hemorrhage, fed earlier and were discharged earlier. The most recent of these studies (Als et al., 2003) also reported reduced family stress and enhanced parental competence in the experimental group.

Some of the studies also reported that the preterm infants in the intervention groups had improved early brain scans, improved behavioural developmental (Buehler et al, 1995) and improved motor outcomes (Mouradian & Als, 1994). Moreover, a recent meta-analysis suggested that the NIDCAP® model of care may be of some benefit to preterm infants; however, the authors caution that this conclusion is based on only two randomized trials (Symington & Pinelli, 2003).

Even with these encouraging findings, questions remain as to the efficacy of the NIDCAP® for at least three reasons. First, critical evaluations of these studies raise serious methodological objections, pointing to their small numbers of patients, to patient selection bias, to inappropriate staff knowledge of treatment groups, to

overgeneralization of results, and to use of large numbers of dependent variables with inappropriate analysis (Garland, 1993; Lacy & Ohlsson, 1993; Lacy, 1995; Ohlsson, 1995; Saigal & Streiner, 1995; Symington & Pinelli, 2003; Tyebkhan, Peters, McPherson, Coté & Roberston, 1999).

However, in addition to methodological flaws which make many of the results difficult to interpret, very few studies report long term improvements in development (Kleberg et al., 2000; Kleberg et al., 2002), and some researchers state that even these few studies do not show that the NIDCAP® significantly influences neurodevelopment at 2-3 years of age (Aucott, Donohue, Atkins & Allen, 2002). Third, and most important, some studies evaluating the NIDCAP® model as a whole, or other studies in which specific NIDCAP® intervention strategies are employed, report no differences in medical outcomes (Ariagno et al., 1997; Constantinou, Thomas, Korner, & Fleisher, 2000; Kennedy, Fielder, Hardy, Tung, Gordon & Reynolds, 2001; Ohlsson, 2002). These negative findings are further supported by a second recent meta-analysis which concluded that there remains insufficient evidence of beneficial effects of the NIDCAP® model of care (Jacobs et al., 2002).

A more theoretically fundamental reason may explain the conflicting outcomes in the application of NIDCAP ® -- the underlying individual principles of the theory may require revision. Peters (2001) began to evaluate the validity of the concept of synchrony between the subsystems which is part of the principle of synaction. According to this principle, the subsystems (the autonomic, motor, state, attentional and self-regulatory subsystems) should function in synchrony. The purpose of Peter's study was to examine whether there was clinical evidence of synchronous associations between the motor and autonomic subsystems in preterm infants. Using a small pre-selected sample of NIDCAP® behaviours, Peters did find positive relationships between

movements which are proposed signs of stability, such as flexor movements, and positive changes in heart rate and oxygen levels. She also found associations between proposed signs of stress and negative clinical events, such as drops in heart rate and oxygen saturation.

While this is an important study because it is the first attempt to examine the principle of synaction, Peters' results must be interpreted cautiously. First, she did not include all NIDCAP® behaviours and second, evidence showing high concordance between behavioural and physiological measures in preterm infants remains controversial. For example, some researchers report that the correlations between behavioural and physiological indicators across infant pain studies appear only to be about 0.30 (Barr, 1998). More recently, however, Morison, Grunau, Oberlander & Whitfield (2001), in a study examining the association between autonomic and behavioural responses to acute pain in preterm infants, found that some infants who showed strong behavioural responses simultaneously showed low physiological responses; other infants show the reverse, having high physiological and low behavioural responses. They found that the correlation between facial reactivity and changes in heart rate to pain was 0.55.

I will now draw on research from foetal movement studies and from studies of preterm infant pain responses, and bring to bear claims from dynamic systems theory, to raise doubts with respect to the principle of dual antagonistic systems which is applied to the interpretation of preterm infant movement. The question under discussion is whether, as the synactive theory maintains, all behaviours observed in these infants may be categorized into stress behaviours or stability behaviours.

5.4 Is the Principle of Dual Anagonistic Systems Valid?

It is critical for developmental specialists using the synactive theory of development to understand that the dual antagonistic model of motor development is not only an individual principle, but is an overriding theme which describes development itself (Als & Duffy, 1982). That is, according to the synactive theory, the developmental process itself is biphasic (Als & Duffy, 1982). Thus, the fundamental way in which every infant movement and behaviour within each of the five subsystems is assessed and interpreted is determined by this principle.

When the synactive theory was first proposed in 1982 (Als, 1982), the principle of dualistic antagonism was applied to the interpretation of infant movement such that, in a given context, each movement could be either a stress or stability indicator. However, in clinical practice, it is unclear how strictly this principle is applied. Some publications provide tables or lists in which the NIDCAP® movements are divided into stress or stability indicators (Als, 1982; VandenBerg, 1995; Cheng & Chapman, 1997). Others describe how various behaviours are "conceptualized as stress....or regulatory behaviors..." (Buehler, et al., 1995). For example, "...extension behaviours are thought to reflect stress, and flexion behaviours are thought to reflect self-regulatory competence..."(Als, 1984, p. 15). However, Als also states that "... approach and self-regulation behaviours may shift into stress behaviours..."(Als, 1984, p. 15). Thus, whereas the theory provides general rules, in practice the interpretation of these movements is much more fluid. All the same, interpretation is not so fluid as to predict that movements which could be identified as indicators of a stress response may not, in fact, reflect stress responses or self-regulation in any context, but may be innate movements which are state-related, are reflexive or are required for normal neurological development. The examples which follow point to the need for further evaluation of the principle of dual antagonistic systems as applied to the interpretation of preterm infant movements.

One of the ways of determining whether the synactive theory provides an accurate interpretation of infant movement is to review studies which examine the movements under controlled conditions such as those observed in utero. Observing movements which occur in the developmentally "expected" environment, one can identify movements which are present when the foetus is optimally supported. MacLeod and Sparling (1993) did just that; they applied the synactive theory system (NIDCAP®) to observe foetal movement. They showed that 85% of the NIDCAP® movements could be observed on foetal ultrasound at 14-16 weeks. If one disregards physiological and autonomic behaviours (such as gag, spit-up) which would not be observed by foetal ultrasound, 48 behaviours remain. Currently, in some contexts, over half of these movements could be classified as stress indicators. Thus it would seem counterintuitive to conclude that all of these movements represent stress responses. Moreover, under some conditions, the NIDCAP® interprets twitches and startles as stress indicators; however, many other foetal ultrasound studies show specifically that twitches and startles are behaviours associated with sleep states in normal foetuses (De Vries, Visser & Prechtl, 1982, 1985, 1988; Dipietro, Hodgson, Costigan, Hilton & Johnson, 1996; Kisilevsky, 1998; Roodenburg, Wladimiroff, van Es, Prechtl, 1991, Visser, 1992). Thus, one must question whether the movements classified according to the synactive theory as indicators of stress are correctly interpreted when so many of them are observed in foetuses under optimal conditions.

In addition to studying the movements under optimal conditions, a second way of determining whether the NIDCAP® movements are correctly interpreted is to study the movements during procedures toward the other end of the stressor intensity continuum – during acute procedural pain. In an exploratory study of the relationship between the NIDCAP® movements and intrusive procedures, Grunau, Holsti, Whitfield & Ling (2000) found that, in a convenience sample of 64 infants <1001 grams exposed to increasingly

invasive stimuli (nasogastric feeds, diaper change, chest physiotherapy and endotracheal tube suctioning), 25 % of the infants displayed twitches during the baseline period before the procedures. No statistically significant change in the frequency of twitches occurred during procedures. Walden and colleagues also found no significant changes in the occurrence of extremity or body twitches during acute pain compared to a baseline period (Walden, et al. 2001). More recently, Morison et al. (2003) found that, following a painful procedure (heel lance) a majority of preterm infants had *lower* incidence of twitches and startles during post-lance than during baseline. These three studies appear to indicate that twitches, in particular, may not be signs of stress. Rather, they may, in fact, be necessary movements for *normal* infant development by influencing neuron cell death, synapse elimination, muscle fibre differentiation and formation of topographic maps (Blumberg & Lucas, 1995). Thus classifying these movements as indicators of stress may be incorrect.

Furthermore, according to the synactive theory, leg flexion may be an indicator of stability and leg extension an indicator of stress. However, other studies examining preterm infant pain responses show that preterm infant hip and leg movements, such as flexion and extension, are normal reflexive responses. For example, Maria Fitzgerald and colleagues have repeatedly studied the flexor withdrawal response in preterm infants, a response which occurs to both tactile and painful stimuli applied to the plantar aspect of the foot (e.g. Fitzgerald et al., 1988, 1989; Andrews & Fitzgerald, 1994; Andrews & Fitzgerald, 1999). Similar lower extremity responses can be elicited by both painful and tactile simulation to the abdomen (Kugelberg & Hagbarth, 1958; Andrews & Fitzgerald, 2002). Thus, studies such as these might direct one to interpret lower extremity movements as normal spinally mediated reflex responses rather than as stress or stability cues. Alternatively, and in support of AIs' interpretations, finger splay appears to be a reliable indicator of stress responses related to procedural pain in this

population (Grunau et al.,1998; Grunau et al., 2000; Grunau et al., 2001; Morison et al., 2000; Morison et al., 2003; Oberlander, Grunau, Fitzgerald & Whitfield, 2002; Slevin, Daly & Murphy, 1998; Walden et al., 2001).

In addition to studies of foetal development, studies examining the relationship of specific movements to known neonatal intensive care unit (NICU) stressors, and infant pain processing studies, other theoretical models may direct our interpretations of preterm infant movements. Clinical studies which evaluate the dynamics of infant movement and which are based on the theory of dynamic systems (Thelen & Smith, 1994) have begun to show that assumptions regarding the dual antagonistic model of motor control may need revision. For example, according to Thelen (1986), when full term infants kick when in the supine position, the extensor motion of the kick occurs because of forces of gravity acting on the leg; there is no *active* extensor muscle activity in that phase of the action. In some contexts, the synactive theory classifies leg extension movements, particularly those co-occurring with arching, as indicators of avoidance or "stress". However, if one finds no active extension in this position, the movement cannot be said to communicate stress. An alternative explanation outside the NIDCAP® interprets these movements as physical activities attributable to the biomechanics of movement.

Finally, it is intriguing that one of the earliest students of stress, Dr. Hans Selye, suggested that although muscle actions may only be bidirectional (flexion/extension), there are actually three possible reactions to a stimulus: flexion, extension or steadiness (Selye, 1976). It is the coordination of these *three* movements in response to a stressor which ultimately allows survival. Thus, binary options may not be the most accurate interpretation of preterm infant movement. Whether the theory is applied strictly or loosely, it is a system in need of empirical validation.

5.5 Conclusion

Currently, the synactive theory of development, a theory of preterm infant development, is widely used by developmental specialists in NICUs throughout the world and has radically altered care for vulnerable high-risk infants. Preliminary evidence indicates that some of the NIDCAP® movements which are purported to be indicators of stress do not occur in response to highly invasive stressors such as endotracheal suctioning, and others may be spinal reflexes. Moreover, many of the behaviours are observed when the foetus remains in utero under optimal conditions. Other movements, such as finger splay, appear to be reliable indicators of stress response behaviours. If some preterm infant movements cannot be related directly to varying intensities of procedures, other biomechanisms may explain them. Although initial studies have begun to examine the validity of the dualistic classification of preterm infant movement as proposed in the synactive theory of development, further empirical work employing concurrent measures of pain and stress in larger samples is required. It is to this empirical work which I now turn.

CHAPTER 6.

Study 1. Does the NIDCAP® Measure Stress Responses? Preterm Infants' Responses during Acute Pain in the Neonatal Intensive Care Unit. (A revised version of this chapter is currently in press. Holsti, L., Grunau, R.E., Oberlander, T.F., & Whitfield, M.F. (In press). Specific NIDCAP® movements are associated with acute pain in preterm infants in the NICU. Pediatrics).

Major advances in neonatal care now enable a high proportion of preterm infants to survive. During a time when medical concerns focused primarily on stabilizing the physiological needs of preterm infants, Als, a developmental psychologist, developed a theory and systematic method of assessing the developmental needs of preterm newborns, the Newborn Individualized Developmental Care and Assessment Program (NIDCAP®) (Als, 1982). Als hypothesized that early exposure to stress, or the mismatch of the preterm infant brain with the environment, could be linked to the long-term developmental impairments reported in these children at school age (Als & Gilkerson, 1997). Since the early 1980's, the NIDCAP® observation system has become widely used in neonatal intensive care units (NICU) around the world (Jacobs et al., 2002; Symington & Pinelli, 2003). With this system, infant responses, which include motor behaviours, state organizational behaviours and autonomically-related indicators (e.g. respiratory pattern), are recorded continuously in two-minute time blocks before, during and following a procedure (Als, 1984). Using the NIDCAP® model, infants can be assessed during any NICU procedure, including those which are painful. Based on the NIDCAP® theory, it is proposed that these infant responses indicate thresholds of stress or stability.

In the preceding chapter, I questioned the validity of a dualistic interpretation such as this and suggested that further empirical evaluation was needed (MacLeod & Sparling, 1993; Grunau, et al., 2000). Not only does this evaluation need to be done for theoretical

reasons, accurate interpretation of preterm infant movements is crucial for clinical reasons. For example, if NIDCAP® assessments lead clinicians to believe that infants are in "pain" or are too "stressed", they may be given sedatives or analgesics. Analgesics appear to act differently in the brain according to whether or not pain is present (Rahman et al., 1997). Therefore, appropriate administration of analgesics only when pain is present may be critical for preventing unwanted long-term side effects of opioid use (Ng, Taddio & Ohlsson, 2000; Mao, 2003).

One of the ways to validate the NIDCAP® system is to study the movements during acute pain. Acute pain responses in preterm infants have been well researched, and unlike other routine NICU handling such as bathing, procedural pain is undisputedly a stressor. Further, by studying the NIDCAP® movements during acute pain, not only can we validate the classification, but we could improve pain management by providing more accurate recognition of valid cues which may be useful for clinical pain assessment. Identifying and treating pain in preterm infants is a high priority for caregivers in the NICU, since long-term outcomes indicate that early pain exposure may alter nociceptive pathways (Anand et al.,1999; Anand, 2000), and may also contribute to changes in other areas of development (Grunau, 2002, 2003).

Thus, improved pain assessment and management is a clinical priority; however, accurate identification of pain responses is complex for a number of reasons. First preterm infants respond with facial, body and physiological changes to acute pain, but they differ from term born infants in that their responses are more variable and of smaller magnitude, particularly at younger gestational ages (Craig et al., 1993; Johnston & Stevens, 1996). Second, preterm infants at earlier gestational ages may display different pain behaviours than infants at later gestational ages due to neurological immaturity. Such behaviours may not be captured in the current pain scales because the behaviours

chosen have been based on those seen in term infants. Third, no physiologic or behavioural threshold specifically marks the presence of pain. Finally, although using a single pain index is easier for clinicians, the physiologic and behavioural responses of preterm infants to painful stimuli are often dissociated (Morison et al., 2001); therefore, reliance on current pain indices may not capture the range of responses in this population (Porter et al., 1999). Changes in facial activity, shifts in infant sleep/wake state, and physiological indices of heart rate and oxygen saturation are the most promising biobehavioural pain indicators in preterm infants (Craig et al., 1993; Franck et al., 2000; Stevens, Johnston & Grunau, 1995; Stevens, Johnston, Petryshen & Taddio, 1996).

In contrast to the research using facial activity, heart rate and oxygen saturation as pain cues, body movements have not been thoroughly evaluated. Some researchers include knee or leg flexion in their pain scales (Franck, 1986; Lawrence et al., 1993; Evans et al., 1997), and others use a measure of total body movements (Craig et al., 1993). These measures are problematic because, in the first case, leg flexion and extension is not pain specific; and in the second case, many movements are combined, thus making them more difficult to interpret. Alternatively, using behaviours from the NIDCAP® to assess pain is appealing because it provides specific descriptions of movements which are developmentally appropriate for preterm infants.

Several researchers have used the NIDCAP® to assess body movements of infants in the NICU, but these studies did not describe the procedures observed, did not include painful procedures or did not include the NIDCAP® specifically (Peters, 1998; Pressler, Helm, Hepworth & Wells, 2001; Slevin et al., 1998). Recently, one study examined NIDCAP® behaviours in preterm infants in response to events which varied in degree of intrusiveness (endotracheal suctioning, chest physical therapy, diaper change and nasogastric feed); however, this study used brief observation periods and did not include a

pain procedure (i.e. skin breaking) (Grunau et al., 2000). Only three studies have used the full NIDCAP® to study pain in preterm infants. The first of these investigations did not evaluate the system along with other reliable, valid behavioural and physiological pain measures, nor did it take into account gestational age at assessment, baseline behavioural state, or handling prior to the invasive procedure (Van Cleve et al., 1995). The second study measured pain responses over longer periods of time and compared the NIDCAP® with other reliable infant biobehavioural pain measures; however, it included only 11 infants, and the examiner completed the observations at bedside which may have allowed for observer bias. Moreover, it did not use continuous digitized monitoring methods for acquiring the physiological data (Walden et al., 2001). As in the second study, the third study used a small sample (10 infants) and it did not control for the time of each handling phase (Morison et al., 2003). The purpose of the present study was to evaluate the dualistic interpretation of the NIDCAP® movements by determining whether NIDCAP® movements were associated with validated pain cues in preterm infants.

6.1 Methods

6.1.1. Study Participants

The infants were recruited by a NICU-trained research nurse, and written informed consent was obtained from the mother or other legal guardian according to a protocol approved by the Clinical Research Ethics Board of the University of British Columbia. The study sample included 44 preterm infants in the level-III NICU in the Children's & Women's Health Centre of British Columbia, Vancouver, Canada. The infants were ≤ 32 completed weeks gestational age (GA) at birth, had no major congenital anomalies and had no reported illicit maternal drug use during pregnancy. Infants who had received analgesics or sedatives within 72 hours of the assessment, or had significant intraventricular hemorrhage and/or parenchymal brain injury (IVH Grade

III, IV, or PVL), were excluded. All infants were 32 weeks postconceptional age (PCA) (+/- 7 days) at time of testing. Infant characteristics are presented in Tables 3-5. Sample size estimates were calculated as though we were using a between groups design; this provides a conservative estimate given that we used a repeated measures design. GPOWER (Faul & Eldfelder, 1998) was utilized to calculate the estimate, and effect sizes entered into the program were based on changes in Neonatal Facial Coding System (NFCS) scores during blood collection at 31-33 weeks (Craig et al., 1993). Using this method, 15 infants were needed to detect differences between each Phase for a power of 0.90 with the statistical significance set at 0.05.

6.1.2 Measures

All infants were observed during blood collection that was required for clinical management. The three Phases of the procedure included in this study were a baseline period of 6 minutes of not handling immediately prior to the first contact by the lab technician (Baseline); a blood collection period of 6 minutes from insertion of the lancet into the heel, which included the heel lance and squeezing (Lance/squeeze); and an undisturbed recovery period of 6 minutes from the last contact of the lab technician (Recovery).

6.1.2.1. Infant State

Infant sleep/wake state was coded according to the NIDCAP® protocol (Als, 1984): 1 = deep sleep; 2 = light sleep; 3 = drowsy; 4 = quiet awake; 5 = active awake; 6 = highly aroused/crying. The predominant state over each 2 – minute period was coded for each Phase.

6.1.2.2. Facial Activity (Neonatal Facial Coding System: NFCS)

The Neonatal Facial Coding System (NFCS) is a reliable, well validated behavioural pain measure widely used in studies of term born (Grunau & Craig, 1987;

Grunau et al., 1990; Peters et al., 2003) and preterm infants (Craig et al., 1993; Grunau et al., 1998; Johnston et al., 1993; Stevens et al., 1994; Johnston & Stevens, 1996; Lindh, Wiklung, Sandman & Hakansson, 1997). Traditionally, the full NFCS has been applied to brief periods (e.g. 20 seconds per phase) to capture the acute pain response. However, for this study,

Table 3. Demographic Characteristics (n=44)

	Mean (SD)	**Range**	**N (%)**
Birth weight (g)	1289 (388)	590 - 2345	
Gestational age at birth (wk)	29.6 (2.0)	25 - 32	
Gender: Male			23 (52)
Small for gestational age			8 (18)
SNAP- II Day 1	12 (9)	0-34	
SNAP-II Day 3	3 (4)	0 -14	
Ventilation (days)	5.34 (9)	0-38	
Other respiratory support (days)	8.5(9)	1-32	
Dexamethazone (days)	0.05 (0.3)	0-2	
Pain Exposure*	60.36 (41)	2-157	
Morphine exposure†	0.29 (0.67)	0 - 3.99	
Ethnicity (Caucasian)			34 (77)
Maternal age (yrs)	32.1 (5.7)	19 - 47	

*Number of invasive (skin breaking) procedures from birth to the study day

†Morphine exposure = (daily average/kg per os dose/3 + daily average intravenous mg/kg) X days

Table 4. Infant Characteristics on the Study Day (n=44)

	Mean (SD)	Range	N (%)
Post-conceptional age on Study day (weeks)	32.3 (0.7)	31 - 33	
Postnatal age on Study day (days)	18.2 (13)	3-51	
Mechanical ventilation on Study day			4 (9)
Time since last feed (min)*	58.8 (4)	0-116	
Number of painful procedures in 24 h prior to Study day	1.5 (1)	0-11	
Time since last invasive procedure (min)	1242.2 (1187)	40-6690	

*Four infants were not on oral feeds

Table 5 Characteristics of Earlier and Later Born Infants

Characteristic	Early Born (<30 weeks) n = 25 Mean (SD)	Later Born (30-32 weeks) n = 19 Mean (SD)	P<
Postnatal age on Study day (days)	26.7 (11)	7.1 (3)*	0.0001
Pain Exposure*	83 (41)	30 (15)	0.001
Ventilation (days)	8.9 (0.6)	0.63 (1)	0.0001
Other respiratory support (days)	12.8 (9)	2.7 (3)	0.001
Number of invasive procedures during 24 hours prior to Study day	1.2 (1)	1.9 (3)	ns
Time since last handling	86.6 (55)	96.4 (47)	ns

*Number of invasive (skin breaking) procedures from birth to Study day

the frequency of NFCS brow bulge was coded continuously for 18 minutes using the Noldus Observer system (The Observer, 1995) (throughout 6 minutes of Baseline, 6 minutes of blood collection [Lance/squeeze], and 6 minutes after the last contact by the technician [Recovery]) to match the NIDCAP® coding. Brow bulge was selected as a proxy for upper facial actions since it has been shown to correlate highly with the other upper facial actions of the NFCS (Johnston et al., 1995). Lower facial actions were not used because they are sometimes obscured in preterm infants. Videotapes were edited for coding in random order of events, and coders were blind to all clinical information about the infants and to events. In order to establish reliability, both the primary NFCS coder (LH) and the reliability coder were trained on the entire tool to achieve a reliability coefficient of 0.87 (Grunau & Craig, 1987). In addition, reliability coding was carried out on 20% of the sample for a reliability coefficient of 0.88. For data analysis, the frequency of NFCS Brow Bulge was summed across all infants for each 6 minute Phase.

6.1.2.3. Full Body (NIDCAP®)

The NIDCAP® behaviours were coded continuously from video recordings of each infant for the 3 Phases (Baseline, Lance/squeeze and Recovery) and coding was carried out blind to all clinical information. While blinding to procedure was possible for facial coding, blinding to procedure is not possible for full body coding. Following published NIDCAP® procedures, the frequency of each infant's separate movements (e.g. each incidence of leg flexion or each incidence of fisting) was recorded systematically in 2- minute time blocks, (Als, 1984). The primary coder was an occupational therapist, and the reliability coder was a physiotherapist, both of whom were NIDCAP® certified. Reliability for the NIDCAP® was initially established during the certification process (Pressler & Hepworth, 2002). In addition, a randomly selected

sample of 5% of NIDCAP® video segments from the study (e.g. Baseline segment, Lance/squeeze segment or Recovery segment) was coded to evaluate reliability. NIDCAP® reliability was calculated by determining % agreement of occurrence (both coders indicating the presence or absence of a behaviour) within every 2 minute time segment during each 6 minute Phase for each infant. Inter-rater agreement was 87%. Physiological measures (heart rate and oxygen saturation) were recorded by custom computer software, and so were not scored using the NIDCAP® observation record.

6.1.2.4. Heart Rate

Continuous electrocardiographic (ECG) activity was recorded from a single lead of surface ECG (lead II), and was digitally sampled at 360 Hz off-line using a specially adapted computer acquisition system. Custom physiologic signal processing software (HR View Software, 1996) was used to acquire process and analyze heart rate. R waves were detected from the sampled ECG, and were used to form a smoothed instantaneous 4-Hz time series as described previously (Berger, Saul & Cohen, 1989). Mean heart rate (HR) was calculated for each 2 minute segment of each study period to correspond to the 2 minute NIDCAP® time blocks and averaged over 6 minutes of each of the 3 Phases (Baseline, Lance/squeeze, Recovery). Prior to statistical analysis, 25 (6%) of the two minute HR segments were dropped due to poor signal.

6.1.2.5. Oxygen Saturation

Continuous measures of oxygen saturation (O2 sat) were obtained from the bedside monitor using the same bedside computer apparatus as for HR above. Analogue signals were digitally converted to a 4 Hz digital signal. Mean and standard deviations were calculated for each infant during each 2-minute segment of each study period, as detailed above for HR. Physiologic recordings were scrutinized for accuracy prior to analyses and 12 (3%), 2 minute segments were dropped due to poor signal.

6.1.3. Background Data

A NICU-trained research nurse completed the prospective clinical chart review and obtained information from birth to day of testing including, but not limited to, the following: birth weight, gestational age at birth, Apgar score at 1 minute, illness severity using the Scale for Neonatal Acute Physiology (SNAP-II: Lee et al., 1999), amount of opioid and other analgesic and sedative exposure, numbers and types of invasive skin breaking procedures, respiratory support, type and time of last handling just prior to blood collection. Invasive procedures were defined as those involving skin breaking such as heel lance, venipuncture, insertion of arterial and venous lines, lumbar puncture and chest-tube insertion. In addition, number of endotracheal intubations was collected (see Table 3). Study day characteristics of the infants are presented in Table 4.

6.1.4. Procedures

Each infant was lying in the incubator undisturbed for a period of at least 30 minutes prior to recording. Heart rate data were collected by attaching the leads from the bedside monitor to a custom-designed computer data acquisition system. Two cameras (one positioned for close-up on the face, the other on the full body) were attached to a custom made recording set-up on a moveable cart, including two 9" video monitors. The signals were fed directly to two VCRs and a time code was imprinted automatically (See Appendix III). Each study phase was marked with an audible event cue signal recorded simultaneously on the videotape and physiologic acquisition systems. During recording, the incubator was partially covered with a blanket, and the infant's position was supported (nested) using a continuous roll around both sides and feet. At the time the infants were studied, 10 were supine, 27 were prone, and 7 were side lying with the face and full body in view for video coding. The infant's position was not altered before or during the procedure, since handling to alter the position may affect the infant pain

response. (Grunau, Linhares, Holsti, Oberlander & Whitfield, 2004) For the blood collection procedure, the research nurse applied a foot warming pack 5 minutes before the lab technician drew the blood. The research nurse determined which foot would be used for the blood collection according to which foot would be most easily accessed by the lab technician in order to minimize extreme stretching of the leg and foot during the procedure. Fifteen different lab technicians carried out the blood collection on the 44 infants. The lab technician's standard protocol involved checking the infant's identification band on the incubator, removing the warming pack from the foot, swabbing the heel with a small gauze pad with disinfectant, lancing the heel, and then gently squeezing the heel intermittently until the amount of blood was collected which was required for clinical care. A research technician set up the video camera and the VCR machines, operated the cardiac data acquisition computer and marked each Phase during the procedure.

6.1.5. Data Analysis

The frequency of the NIDCAP® behaviours was reviewed, and the 30 movements which occurred in less than 25 % of the infants were excluded from statistical analysis (See Figure 6) (Grunau et al., 2000). Total frequencies of each NIDCAP® movement were summed for each 6 minute Phase to reveal in a clinically meaningful way, the amount of infant movement exhibited throughout the procedure. Sleep-wake states were analyzed using nonparametric tests for related samples (Friedman and Wilcoxon Signed Ranks). Continuous measures (NIDCAP®, NFCS brow bulge, HR and O2 sat) were examined using repeated measures analysis of variance to compare biobehavioural responses across the 3 Phases of each procedure with sex as a between subjects factor. Bonferroni corrections were used to correct for overall error. Statistically significant ANOVA was followed by planned Student's t tests for paired

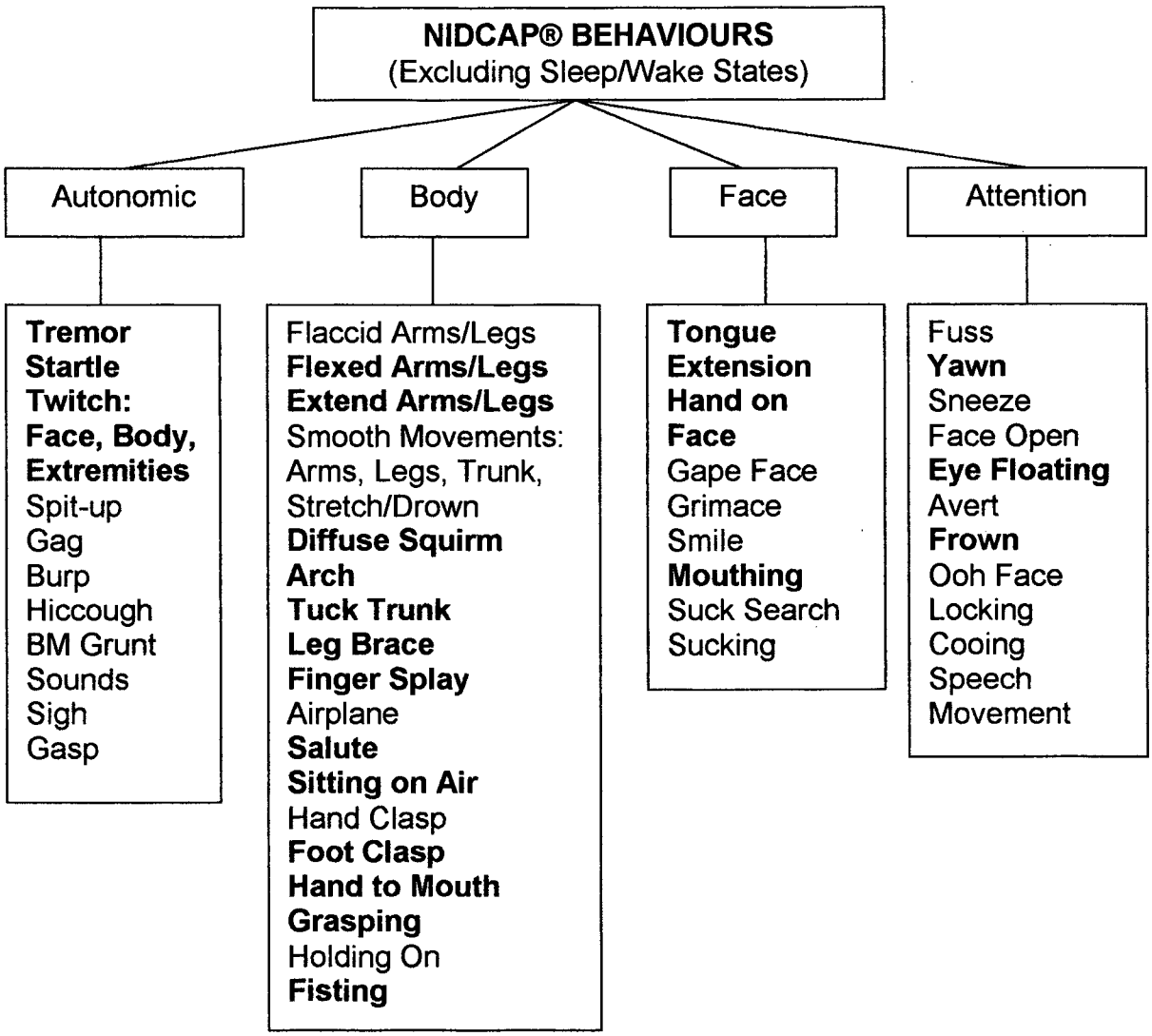

Figure 6. Newborn Individualized Developmental Care and Assessment Program (NIDCAP®) Behaviours (Bolded cues included in statistical analyses).

comparisons to identify differences between specific Phases. Pearson product-moment correlations were used to examine associations between perinatal variables, and to

describe relationships between the NIDCAP® and infant background characteristics during Lance/squeeze.

6.2 Results

6.2.1. Infant State

There was a main effect of Phase on Sleep/wake states (χ^2=47.0, p<0.0001). Most infants were in "sleep" (75%) or "drowsy" (25%) states during Baseline, and then shifted significantly from Baseline to Lance/squeeze (z = - 5.0, p < 0.0001). Seven percent of infants remained in "deep sleep" during Lance/squeeze, no infants were in "active sleep", 56% were "drowsy", and a further 36% were crying. Sleep/wake state also shifted significantly from Lance/squeeze to Recovery (z = -4.7, p < 0.0001); 70% of infants returned to sleep and only 2% remained highly aroused. There were no differences between Baseline and Recovery states.

6.2.2. NFCS

There were no statistically significant sex effects with any of the behavioural measures. The frequency of NFCS Brow Bulge changed significantly across the 3 Phases (F [1, 43] = 49.43; p < 0.0001). In addition, NFCS Brow Bulge remained elevated during the Recovery Phase compared to Baseline levels (t = -2.42, p < 0.02).

6.2.3. NIDCAP®

Of the 26 NIDCAP® behaviours included in the statistical analyses, the frequency of a subset of eight NIDCAP® behaviours, namely flex arms, flex legs, extend arms, extend legs, hand on face, finger splay, fisting and frown, increased significantly during Lance/squeeze. The frequency of a second subset of 5 NIDCAP® behaviours namely, twitch face, twitch body, twitch extremities, mouthing and foot clasping, decreased during Lance/squeezes. Additionally, the frequency of two NIDCAP® behaviours, diffuse squirms (F [1, 43] = 5.22; p < 0.008), and arching (F [1, 43] = 4.22; p < 0.03), decreased

significantly from Lance/squeeze to Recovery. Finally, during Recovery, all but one of the NIDCAP® behaviours returned to Baseline frequencies; the frequency of fisting (t=-2.5, p < 0. 02) remained increased. The total frequencies of the NIDCAP® behaviours across the three Phases of blood collection are shown in Table 6.[1]

Table 6. Changes in Frequency of NIDCAP® Behaviours Across The Phases

	Total Frequencies Across Phases			
NIDCAP® Behaviour	**Baseline**	**Lance/Squeeze**	**Recovery**	**P <**
Flex arms	38	88	49	0.01
Flex legs	118	310	117	0.0001
Extend arms	26	52	27	0.02
Extend legs	103	216	79	0.01
Hand on face	14	45	11	0.001
Finger splay	42	86	51	0.048
Fisting	0	12	7	0.004
Frown	12	51	16	0.0001
Twitch face	153	19	127	0.0001
Twitch body	74	12	60	0.001
Twitch extremities	371	39	287	0.0001
Mouthing	22	8	36	0.02
Foot clasping	18	1	16	0.01

[1] NIDCAP® results are also presented graphically in Appendix IV.

6.2.4. Heart Rate

Heart rate changed significantly across the 3 Phases ($F [1, 43] = 40.67$; $p < 0.0001$). Heart rate (mean ± SD) increased from Baseline 157.5 ± 12 to Lance/squeeze 174.0 ± 20 ($t = -6.74$, $p < 0.0001$), and decreased during recovery 156.3 ± 16 ($t = 9.48$, $p < 0.0001$). There were no statistically significant differences in heart rate between Baseline and Recovery.

6.2.5. Oxygen Saturation

Mean O2 sat changed significantly across the three Phases of Blood Collection ($F [1, 36] = 15.2$; $p < 0.0001$). Oxygen saturations dropped from Baseline 96.6 ± 3 to 92.9 ± 6 during the Lance/squeeze Phase ($t = 4.0$, $p < 0.0001$), and increased to 97.4 ± 2 during the Recovery Phase ($t = -4.2$, $p < 0.0001$). There were no statistically significant differences in oxygen saturations between Baseline and Recovery.

6.2.6. Infant Background Characteristics

Gestational age at birth and postnatal age (days) at testing were highly correlated ($r = -0.91$, $p<0.0001$); gestational age at birth was used to examine the relationships between infant characteristics and the NIDCAP® behaviours. Perinatal and Study day characteristics of the earlier born and later born infants are shown in Table 5.

During blood collection at 32 weeks, infants who were born at lower gestational ages (< 30 weeks) showed significantly higher frequencies of finger splay, fisting and mouthing during Lance/squeeze than infants born at later gestational ages. Infants who had been exposed to greater numbers of invasive procedures since birth showed significantly more finger splays and fisting. Moreover, those infants who had greater opioid exposure displayed significantly more fisting and hand on face. Infants who were sicker earlier in the neonatal course (SNAP II Day 1) displayed more facial twitches. Infants who continued to be physiologically unstable on day 3 (SNAP II Day 3) continued to show more facial

twitches, greater numbers of hand on face and fisting during Lance/squeeze than those infants who became medically more stable. Finally, infants in supine showed greater numbers of flex arms than infants in prone or sidelying (See Table 7).

Table 7. Correlations between Infant Background Characteristics, Position during Blood Collection and NIDCAP® Behaviours.

Infant Characteristics	**NIDCAP® Behaviour**	**r**	**P <**
Gestational age at birth	Finger splay	- 0.39	0.009
	Fisting	- 0.42	0.004
	Mouthing	- 0.42	0.03
Illness severity (SNAP-II Day 1)	Facial twitches	0.33	0.03
Illness severity (SNAP-II Day 3)	Facial twitches	0.40	0.01
	Hand on face	0.35	0.02
	Fisting	0.58	0.0001
Pain Exposure*	Finger splay	0.37	0.013
	Fisting	0.40	0.007
Morphine exposure†	Fisting	0.57	0.0001
	Hand on face	0.34	0.02
Infant Position	Flex Arms	-0.41	0.005

*Number of invasive (skin breaking) procedures from birth to Study day

† Morphine exposure = (daily average/kg per os dose/3 + daily average intravenous mg/kg) X days.

6.3 Discussion

Although the NIDCAP® has been widely used in NICUs throughout North America and Europe since the 1980s, this study is the first to examine the validity of the

interpretation of NIDCAP® movements under well-controlled conditions using a large number of infants. The infants in this study showed facial, behavioural state, heart rate and oxygen saturation responses similar to responses documented in other studies of responses of preterm infants to acute pain (Craig et al., 1993, Morison et al., 2001; Grunau et al., 2001a). However, we found that a significant proportion (53%) of the NIDCAP® movements either did not occur at all, or occurred in less than 25 % of the infants. These results are supported by our previous pilot study (Morison et al., 2003) and by those of Walden and colleagues (Walden et al., 2001), who discarded 50% of the NIDCAP® variables because they were not sensitive or specific for pain. Our findings are also consistent with other studies which recently reported that over one third of the movements described by the NIDCAP® are not observed in infants < 30 weeks gestational age in the NICU even when observed sequentially over a 7 week period; however, these investigators did not specify the procedures they observed (Pressler et al., 2001).

In contrast to our approach, others have described the NIDCAP® movements which occur in utero in healthy pregnancies. MacLeod and Sparling (1993) completed such a study using the NIDCAP® classification system to observe fetal movements. These investigators found that 85% of the NIDCAP® movements could be observed on fetal ultrasound as early as 14-16 weeks. If one disregards the physiological and autonomic behaviours (such as gag, spit-up) which would not be observed by fetal ultrasound, 48 NIDCAP® behaviours remain. Over half of these behaviours are classified in the NIDCAP® as stress indicators. Given that many of these behaviours are observed under optimal conditions in utero, it seems counterintuitive to consider any of these movements stress response behaviours.

Even though many NIDCAP® movements occurred infrequently or did not occur at all in our study, we did find a subset of eight NIDCAP® movements which appear to be reliable stress response behaviours in preterm infants. While some of the eight NIDCAP® movements we identified as stress-related cues are included in other pain measures, others have not been described as behavioural pain cues prior to this study. For example, the increased frequency of flexing and extending the extremities has been reported in other studies describing and assessing pain responses in both term born, (Rich et al., 1974; Evans et al., 1997) and preterm infants (Lawrence et al., 1993; Van Cleve et al., 1995; Sparshott, 1996). In particular, extending the legs appears to be a consistently observed stress cue (Grunau et al., 2000; Morison et al., 2003). In the NIDCAP® model, flexing of the extremities is usually considered self-regulatory unless the infant is assuming a fetal tucking position (Als, 1982, 1984). Thus, the increased flexor activity could be an indication of active self-regulation. Alternatively, the combined flexing and extending of the legs may be reflexive in nature (i.e. the flexor withdrawal response) (Fitzgerald, Shaw & McIntosh, 1988; Andrews & Fitzgerald, 1999); therefore, one might argue that these actions are neither indicators of stress or stability. In addition, since the flexor withdrawal response is not pain specific, the use of these movements for pain assessment is unlikely to improve the identification of pain if facial, sleep/wake state, heart rate and oxygen saturations are also used.

Unlike flexing and extending of the extremities, finger splay does appear to be a discrete indicator of a stress response. Moreover, finger splay may be a developmentally specific stress response behaviour since the infants who were born prior to 30 weeks gestational age had higher frequency finger splays to Lance than those born > 30 weeks. Not only did these earlier born infants show greater finger splays during Lance/squeeze, but also during the Baseline Phase. This finding is consistent with previous studies

examining pain responses in this population, (Grunau et al., 2000; Morison et al., 2003) and may be indicative of "sensitization" which results from greater early pain exposure (Fitzgerald et al., 1989; Andrews & Fitzgerald, 1994).

Perhaps an even more sensitive hand movement than finger splay is fisting. According to the NIDCAP®, repeated fisting is usually interpreted as an indication of stress (Als, 1984; Cheng & Chapman, 1997), and has also been described in one other studies examining preterm infants' responses to pain (Bozette, 1993). Furthermore, a recent study showed that fisting is considered by a majority of nurses to be a pain indicator. (Howard & Thurber, 1998) Like finger splay, fisting appears to be a sensitive stress response behaviour in infants born at earlier gestational ages (<30 weeks), and may be useful in identification of pain in those infants who are sicker early in postnatal life, and who require opioids during their care. This finding is important since facial responses to acute pain in sicker preterm infants are often diminished (Johnston & Stevens, 1996; Grunau et al., 2001a). In addition to fisting and finger splaying which have been previously associated with painful experiences in the NICU, the movement of hand on face (which involves a defensive-like action with the infant placing a hand on its face), is also hypothesized be a NIDCAP® stress cue in older infants.

The only NIDCAP® facial action which increased significantly during the Lance/squeeze was frowning (brow lowering). Frowning is a flexor motion which involves knitting of the eyebrows or darkening of the eyes and is usually considered an indication of state stability and attentional regulation (Als, 1982, 1984). In contrast, our findings show this movement associated with pain.

Whereas we found a subset of eight NIDCAP® behaviours associated with pain, we also found five behaviours which decreased significantly during the Lance/squeeze Phase. As in our previous study of stress responses during endotracheal suctioning,

twitches decreased, and startles did not change significantly across the Phases. Therefore, we conclude these are not specific stress response behaviours (Grunau et al., 2000; Morison et al., 2003). Many fetal ultrasound studies show that twitches and startles are behaviours associated with sleep states in the normal fetus (Visser, 1992; Dipietro, Hodgson, Costigan & Hilton, 1996; Kisilevsky & Low, 1998). Rather than being stress response behaviours, twitches may, in fact, be necessary movements for normal infant development which influence neuron cell death, synapse elimination, muscle fiber differentiation and formation of topographic maps (Blumberg & Lucas, 1996). Although we conclude that twitches may not be specific stress response behaviours, infants who were sicker on Day 1 and remained sicker on Day 3 had more facial twitches associated with the Lance/squeeze Phase at 32 weeks PCA, a finding that has not been reported in previous studies. We cannot attribute this finding to the infants being in active sleep during the lance, since none of our infants remained in this sleep state during the blood collection. Thus, in very specific situations, facial twitches may represent stress response behaviours.

Another NIDCAP® movement which decreased to Lance/squeeze was foot clasping. This finding was not unexpected since infants who are rapidly flexing and extending their legs are less likely to clasp their feet together. Similarly, the frequency of mouthing (more than one opening and closing of the mouth), a proposed NIDCAP® stability cue in younger infants, decreased during Lance/squeeze. Indeed, fetal ultrasound studies have shown that mouthing is a regularly observed movement in utero (i.e. under optimal, non-stressed conditions) (Roodenburg et al., 1991; D'Elia, Pighetti, Moccia & Santangelo, 2001). However, clinicians must be aware that repetitive mouthing may also be associated with seizure activity.

Like finger splay, the frequency of two behaviours, diffuse squirms and arching, both proposed NIDCAP® stress cues, occurred at high frequencies during Baseline. However, they differed from finger splay in that they did not increase to Lance/squeeze, but dropped significantly during Recovery. The high frequency of these movements during Baseline may indicate a higher basal arousal in preterm infants; however, even infants born > 30 weeks, and who had little prior pain exposure, showed these behaviours. Alternatively, these movements may not be specific stress response behaviours; rather their diminished frequency during Recovery may be an indication of fatigue caused by the length of the procedure.

Prior to this study, most of the literature describing the responses of these infants to acute pain used very short periods of observation. Our findings demonstrated that many preterm infants remain in a higher state of physiological and behavioural arousal not only during the tissue-damaging portion, but also during the entire blood collection. It may be that this higher and sustained level of stress contributes to altered reactivity and self-regulation observed later in these children (Oberlander, Grunau, Whitfield, Fitzgerald, Pitfield & Saul, 1999; Grunau et al., 2001b).

Although our study carefully controlled for age at assessment, length of assessment time and procedure order, there remain some limitations. First, it was not possible to be blinded to events when coding body movements. Instead, with the use of a second video camera, facial coding was carried out blinded to events, and was always completed prior to the coding of the NIDCAP® tapes. Second, the infants' position during the Lance was not controlled; more than half of the infants were positioned in prone during the assessment. It is standard practice in our nursery to promote prone positioning to support physiological stability, particularly for infants with respiratory difficulties (Hutchinson, Ross & Russell, 1979). We deliberately did not alter the positions in which the babies were

being nursed because change of position would likely alter biobehavioural reactions. Although a prone position would not have affected the facial responses during blood work, (Grunau et al, in press) it appeared to influence the ease of the infants to flex their arms for self-regulation and may have affected the frequency of one NIDCAP ® body movement, sitting on air, an action where the legs are flexed at the hips and extended at the knees. This movement would be very unlikely to occur in prone.

One of the most important findings of this study is that body movement responses to acute pain differ from facial responses. Contrary to the dampened facial activity associated with infants of lower gestational ages (Johnston et al., 1995), these infants responded with increased frequency of specific body movements during pain. Increased body movements during pain may be indicative of increased pain sensitivity due to sensitization, which is then followed by the "wind-up" phenomenon, both of which are spinal cord mediated effects (Fitzgerald et al., 1989; Andrews & Fitzgerald, 1994).

6.4 Conclusion

The NIDCAP® system categorizes preterm infant movements as stress and stability behaviours. However, our findings show that only a few NIDCAP® movements were associated with an event which is undisputedly stressful: blood collection. Therefore, many of the NIDCAP® movements may be misclassified, thereby leading to errors in interpretation of the state of the infant. Nonetheless, we found a subset of eight NIDCAP® movements which appear to be salient stress response movements in preterm infants at 32 weeks PCA. By adding a few discrete body movements to the assessment of pain in neonates, particularly hand movements such as finger splay, fisting and hand on face, we can use the NIDCAP® to provide additional behavioural cues which may make pain assessment more accurate, particularly for those infants born at early gestational ages.

While a subset of NIDCAP® movements appear correctly classified, recent studies report that other care giving tasks may be more stressful than blood collection. If this is the case, a study limited to an examination of preterm infants' responses to acute pain may not capture the full compliment of stress response movements. Additionally, recent animal research has shown that distinguishing between pain and stress may be vital for preterm infants because medications given for sedation and pain may act differently in the brain if pain is or is not present (Rahman et al., 1997). Therefore, further evaluation of the NIDCAP® using procedures of varying intensities will try to determine whether the full compliment of stress response movements has been identified. This evaluation will seek also to determine whether pain and stress reactions in preterm infants can be distinguished.

CHAPTER 7.

Study 2. Validation of the NIDCAP® on the Continuum of Stressors: Can we distinguish between pain and stress in preterm infants? (This chapter is currently under review. Holsti, L., Grunau, R.E., Weinberg, J., Whitfield, M.F., & Oberlander, T.F. [Under review]. Body movements, an additional important factor in discriminating pain from stress in preterm infants.)

Preterm infants, delivered into a developmentally unexpected environment, have vastly different early experiences from those of term infants. Preterm infants are exposed to multiple stressors such as acute and chronic illnesses, maternal separation, unpredictable handling patterns, multiple medications, repeated painful procedures, continuous lighting and high levels of noise. Initially, clinicians believed that these tiny infants were too immature to feel the stress and pain associated with this neonatal intensive care. However, recent evidence shows that the neurophysiologic components required for generalized stress responses, including those for pain responses, are functional by mid-gestation (Coskun & Anand, 2000;Tsakiri et al., 2002). Long-term alterations in pain responses have been reported following early repetitive pain exposure in both animals and in children born prematurely (Anand et al., 1999; Grunau et al., 2001b; Lidow et al., 2001) and a growing body of knowledge demonstrates that repeated exposure to other stressors also alters brain development. For example, studies using animal models have shown that the regulation of branching and length of apical dendrites of pyramidal cells in Ammon's horn (Magarinos et al. 1997), and hippocampal dendritic remodelling may be altered in response to chronic stressor exposure (McEwen, 2000). Chronic exposure to stressors may also influence the magnitude of long-term potentiation and produce long-term depression (McEwen, 2000). Although many of the effects of early exposure to stressors appear to be

reversible after a period of time (7-10 days in rats) (Conrad et al., 1999), these findings are highly relevant to the preterm infant because recent neuroradiological evidence reveals reduction in volume in the hippocampus (Isaacs et al., 2000; Nosarti et al., 2002; Peterson et al. 2003) and other brain regions (Peterson et al., 2003) in older children born prematurely. Indeed, these areas of neuroanatomical deficits correlate with the long-term functional developmental differences described in these children (Grunau et al., 2002; Holsti et al., 2002).

One of the difficulties faced when assessing pain in preterm infants in the NICU is that the infants' responses to pain and other stressors are non-specific and can be misinterpreted. Although both stress and pain activate the autonomic nervous system and the hypothalamic-pituitary-adrenal axis, the continuum of tactile and invasive stimuli has cumulative effects. Some have suggested that distinguishing between pain and stress is not clinically relevant (Andrews & Fitzgerald, 1997; McIntosh, 1997). However, appropriate management would differ depending on whether pain was present or not. While not all tactile events produce deleterious neuroendocrinological responses (Acolet et al., 1993), more recent evidence suggests that non-painful stimulation received during routine NICU care may be *more* stressful for preterm infants than painful interventions (Hellerud & Storm, 2002). Moreover, responses to tactile events may become heightened over time because preterm infants exhibit, not only primary and secondary hyperalgesia, but also allodynia (pain arising from previously innocuous stimulation) as a result of central sensitization (Fitzgerald et al., 1988, 1989). Further, pharmacological interventions used for pain management may act differently if pain is or is not present (Rahman et al., 1997). Conversely, sedatives often do not act specifically as analgesics; using them when pain is present is inappropriate, since the detrimental physiological side effects of pain would not be controlled. Administering analgesics only when pain is present may be critical for

preventing unwanted long-term side effects of opioid use.

In response to concerns with respect to difficulties in identifying and treating pain in preterm infants, considerable research in pain assessment has been carried out in recent years. Currently researchers argue that multidimensional assessment of pain using both behavioural and physiological indicators is essential since different parameters provide different information (Frank & Miaskowski, 1997). The most promising of these indicators in infants > 28 weeks gestational age are changes in facial activity, shifts in infant sleep/waking state, and physiologic indices of heart rate and oxygen saturation. In addition to changes in facial activity and shifts in sleep/waking states, recent studies have shown that some specific body movements, described in the Newborn Individualized Developmental Care and Assessment Program (NIDCAP®), are associated with acute pain responses in preterm infants. The NIDCAP® is an important tool because it is developmentally relevant for preterm infants, as well as being the only comprehensive tool available which measures behavioural and physiological thresholds to stress in preterm infants (Als, 1982). Using the NIDCAP® model, Morison and colleagues (2003) found that preterm infants assessed at 32 weeks post conceptional age responded to blood collection with increases in flexion and extension of the arms and legs, and finger splays. Holsti and colleagues confirmed these findings in a larger sample of preterm infants, and described additional NIDCAP® movements associated with acute pain such as fisting and hand on face, some of which were particularly salient cues for preterm infants born at earlier gestational ages (See Chapter 6; Holsti et al., in press; Holsti et al., 2003).

Compared to the numbers of studies evaluating preterm infants' responses to pain, few studies have provided detailed descriptions of preterm infants' behavioural and physiological responses to non-painful, but potentially stressful, care-giving tasks in the NICU (Peters, 1998; Peters, 2001; Sell, Hill-Mangan & Holberg, 1992; Slevin et al.,

1998). One study used diaper changing as a non-painful event with which to evaluate construct validity of the Premature Infant Pain Profile (PIPP) (Ballantyne, Stevens, McAllister, Dionne & Jack, 1999). These researchers reported lower pain scores during the non-painful handling than with heel lance. However, when Blauer and Gerstmann used diapering as a "non-painful" event with which to compare 3 infant pain scales, two of the scales rated diaper changing as more "painful" than endotracheal suctioning (Blauer & Gerstmann, 1998). These authors concluded that this finding represented a lack of specificity of the scales for measuring pain. More recently, Hellerud & Storm showed that diaper changing produced greater physiological changes than did heel lance in preterm infants (Hellerud & Storm, 2002). But the difficulty with using pain scales to measure infants' more generalized stress responses is that they may miss salient stress response cues not associated with pain.

Studies using the NIDCAP® to evaluate the effects of routine care giving in the NICU are not only few in number, but either pooled all handling to make general comments regarding the infants' responses (Sell et al., 1992), did not include the full range of NIDCAP® behaviours (Stevens & Glazer, 1992), did not specify the procedures observed in the study (Pressler et al., 2001), or did not include diaper changing as one of the procedures being evaluated (Peters, 1998, 2001). Thus, the aims of this study are to describe, in detail, biobehavioural responses of preterm infants to a routine cluster of care-giving tasks (including diapering, measuring abdominal girth by placing a tape measure around the abdomen, mouth care and taking an axillary temperature [Clustered Care]), and to determine whether there are specific behaviours which distinguish pain from tactile responses. Stressors function on a continuum, with painful stimuli being toward one end of the gradation. For this study, stress will be

defined as a reaction induced by non-invasive tactile stimulation; pain will be defined as a more severe form of stress that is associated with a tissue breaking event.

7.1 Methods

7.1.1 Study Participants

The study sample comprised 54 preterm neonates (24 female, 30 male) born ≤ 32 completed weeks gestational age, in a major regional level-III NICU at the Children's & Women's Health Centre of British Columbia, Vancouver, Canada. Infants with a major congenital anomaly, significant intraventricular hemorrhage (IVH Grade III), and/or parenchymal brain injury (IVH Grade IV and/or periventricular leukomalacia [PVL]), as well as infants who had received analgesics or sedatives within 72 hours of the targeted study session, were excluded. All infants were 32 weeks postconceptional age (+/- 7 days) at time of the study. Forty-four infants were appropriate for gestational age, eight were small for gestational age, and two were large for gestational age. Sample size estimates were calculated as though we were using a between groups design; this provides a conservative estimate given that we used a repeated measures design. GPOWER (Faul & Erdfleder, 1998) was utilized to calculate the estimate, and effect sizes entered into the program were based on differences in NFCS scores in term infants between a painful and non-painful event (Grunau et al., 1990). Using this method, 16 infants were needed to detect differences between each Phase for a power of 0.95 with the statistical significance set at 0.05.

7.1.2. Procedures

The infants were recruited by a NICU research nurse, and written informed consent was obtained from the mother according to a protocol approved by the Clinical Research Ethics Board of the University of British Columbia. Videotaping and physiologic recording were carried out continuously. Heart rate data were collected by attaching the leads from

the bedside monitor to a custom-designed computer data acquisition system. Two cameras (one positioned for close-up on the face, the other on the full body) were attached to a custom made recording set-up on a moveable cart, including two 9" video monitors. The signals were fed directly to two VCRs, and a time code was imprinted automatically. Each study phase was marked with an inaudible event cue signal recorded simultaneously on the videotape and physiologic acquisition systems. A research technician set up the video cameras, VCRs, and operated the computerized cardiac data acquisition system and marked each event. A single research nurse carried out clustered nursing procedures (Clustered Care) in a set order: changing the diaper, measuring girth, taking the axillary temperature, cleaning the mouth with gauze and sterile water. Blood collection (Pain) following heel warming was carried out by a lab technician who cleansed the heel, applied a lancet, and squeezed the heel to collect blood. Each infant was tested on two occasions always on different days that were no more than 13 days apart. Assignment to Pain versus Clustered Care as the first procedure was randomized when babies were entered into the study. For this study, three phases of Pain (Baseline, Lance, Recovery) and Clustered Care (Baseline, Clustered Care, Recovery) were analyzed.

7.1.3. Measures

7.1.3.1. Infant State

Infant sleep/wake state was coded every two minutes according to the NIDCAP® protocol (Als, 1984): 1 = deep sleep; 2 = light sleep; 3 = drowsy; 4 = quiet awake; 5 = active awake; 6 = highly aroused/crying. The predominant state over each 4 minute period was coded for each Phase.

7.1.3.2. Facial Activity (Neonatal Facial Coding System: NFCS).

The Neonatal Facial Coding System (NFCS) is a reliable, well validated behavioural pain measure widely used in studies of term born (Grunau & Craig, 1987; Grunau, et al. 1990; Peters, et al. 2003) and preterm infants (Craig et al.,1993: Grunau et al., 1998; Lindh et al., 1997), and has been shown to distinguish relative differences between tissue invasive events and non-tissue invasive tactile stimulation (Grunau et al., 1990). Traditionally, the full NFCS has been applied to brief periods (e.g. 20 seconds per phase) to capture the acute pain response. However, for this study, the frequency of NFCS brow bulge was coded continuously for 12 minutes using the Noldus Observer system (The Observer, 1995) (throughout 4 minutes of Baseline, 4 minutes of the blood collection [Lance/squeeze] and Clustered Care, and 4 minutes after the last contact by the technician [Recovery]) to match the NIDCAP® coding. Brow bulge was selected as a proxy for upper facial actions since it has been shown to correlate highly with the other upper facial actions of the NFCS (Johnston et al., 1995). Lower facial actions were not used because they are sometimes obscured by tape used to secure tubes to the face of preterm infants. Videotapes were edited for coding in random order of events, and coders were blind to all clinical information about the infants and to events. In order to establish reliability, both the primary NFCS coder (LH) and the reliability coder were trained on the entire tool with a reliability coefficient of 0.87 (Grunau & Craig, 1987). In addition, reliability coding was carried out on 20% of the sample with a reliability coefficient of 0.88. For data analysis, the frequency of NFCS brow bulge was summed across all infants for each 4 minute Phase.

7.1.3.3. NIDCAP®.

The NIDCAP® behaviours were coded continuously, from video recordings of each infant, for the 3 phases of blood collection (Baseline, Lance/squeeze and Recovery), and Clustered Care (Baseline, Clustered Care, Recovery), and coding was carried out blind to

all clinical information. While blinding to procedure was possible for facial coding, blinding to procedure is not possible for full body coding. Following published NIDCAP® procedures, the frequency of each infant's separate movements (e.g. each incidence of leg flexion or each incidence of fisting) was recorded systematically in 2 minute time blocks (Als, 1984). The primary coder (LH) was an occupational therapist, and the reliability coder was a physiotherapist, both of whom were NIDCAP® certified. Reliability for the NIDCAP® was initially established during the certification process (Pressler & Hepworth, 2002). In addition, a randomly selected sample of 5% of NIDCAP® video segments from the study (e.g. Baseline segment, Lance/squeeze segment, Clustered Care segment or Recovery segment) was coded to evaluate reliability. NIDCAP® reliability was calculated by determining % agreement of occurrence (both coders indicating the presence or absence of a behaviour) within every 2 minute time segment during each 4 minute Phase for each infant. Inter-rater agreement was 87%. Physiological measures were recorded by custom computer software, and so were not scored using the NIDCAP® observation record.

7.1.3.4. Heart Rate

Continuous electrocardiographic (ECG) activity was recorded from a single lead of surface ECG (lead II), and was digitally sampled at 360 Hz off-line using a specially adapted computer acquisition system. Custom physiologic signal processing software was used to acquire, process and analyze heart rate (HR View, 1996). R waves were detected from the sampled ECG, and were used to form a smoothed instantaneous 4-Hz time series as described previously (Berger, et al., 1989). Mean heart rate (HR) was calculated for each 2 minute segment of each study period to correspond to the 2 minute NIDCAP® time blocks and averaged over 4 minutes of each of the 3 Phases (Baseline, Lance/squeeze, Recovery or Baseline, Clustered Care, Recovery). Prior to

statistical analysis, 42 (5%) of the two minute HR segments were dropped due to poor signal for that phase.

7.1.3.5. Oxygen Saturation

Continuous measures of oxygen saturation (O2 sat) were obtained from the bedside monitor, using the same bedside computer apparatus as for HR above. Analog signals were digitally converted to a 4 Hz digital signal. Mean and standard deviations were calculated for each infant during each 2 minute segment of each study period, as detailed above for HR. Physiologic recordings were scrutinized for accuracy prior to analyses and 20 (3%), 2 minute segments were dropped due to poor signal.

7.1.4. Background Data

A NICU-trained research nurse completed the prospective clinical chart review and obtained information from birth to day of testing including, but not limited to, the following: birth weight, gestational age at birth, Apgar score at 1 minute, illness severity using the Scale for Neonatal Acute Physiology (SNAP-II: Lee, et al., 1999), amount of opioid and other analgesic and sedative exposure, total number and types of invasive skin breaking procedures, respiratory support, type and time of last handling just prior to blood collection. Invasive procedures were defined as those involving skin breaking such as heel lance, venipuncture, insertion of arterial and venous lines, lumbar puncture and chest-tube insertion. In addition, number of endotracheal intubations was collected (see Table 8). Study day characteristics of the infants are presented in Table 9 based upon data obtained up to the first observation.

7.1.5. Data Analysis

The frequencies of the NIDCAP® movements were reviewed, and 30 movements which occurred in less than 25 % of the infants were excluded from statistical analysis. Total frequencies of the remaining 26 NIDCAP® movements were summed for each 4

minute Phase. Sleep-wake states were analyzed using nonparametric tests for related samples (Wilcoxon Signed Ranks and Friedman).

Table 8. Demographic Characteristics to Study Day 1 (n=54)

	Mean (sd)	**Range**	**N (%)**
Birth weight (grams)	1257 (423)	500 – 2345	
Gestational age at birth (weeks)	29.3 (2.2)	24 – 32	
SNAP- II Day 1	12 (9)	0 – 38	
SNAP-II Day 3	3 (5)	0 -17	
Ventilation (days)	8.3 (12)	0 – 46	
Other respiratory support (days)	7.2 (7)	0 - 28	
Dexamethazone (days)	0.32 (1.4)	0 – 8	
Pain exposure if Pain first procedure*	67.17 (47)	7 -202	
Pain exposure if Clustered Care first procedure*	74.61 (56)	9 – 246	
Morphine exposure if Pain first Procedure†	0.87 (2.2)	0 – 9.6	
Morphine exposure if Clustered Care first procedure†	0.66 (1.6)	0 – 8.3	
Ethnicity (Caucasian)			39 (72)
Maternal age (years)	31.4 (5.7)	19 – 47	

*Number of invasive (skin breaking) procedures from birth to the first study day

†Morphine exposure = (daily average/kg per os dose/3 + daily average intravenous mg/kg) X days

Table 9. Infant Characteristics on the First Study Day (n=54)

	Mean (sd)	**Range**	**N (%)**
Post-conceptional age (weeks)	32 (0.7)	31 -33	
Postnatal age (days)	19	3 – 49	
Mechanical ventilation			9 (2)
Time since last feed (minutes)*	56.2 (32)	0 – 152	
Number of painful procedures in 24 hour prior to first study	2 (2)	0 – 11	

*Four infants were not on oral feeds

Continuous measures (NIDCAP®, NFCS brow bulge, HR and O2 sat) were examined using repeated measures analysis of variance (ANOVA) to compare biobehavioural responses across the 3 phases of each procedure with sex as a between subjects factor. Since infants were positioned in one of three positions during the Pain procedure (18 in supine, 32 in prone, 4 in side lying), but all were in supine for the Clustered care, position was entered as a covariate for the ANOVA for the Pain procedure only. Bonferroni corrections were used to correct for overall error. Statistically significant ANOVA was followed by planned Student's t tests for paired comparisons to identify differences between specific Phases within each observation. Student's t tests were also used to examine differences between frequencies of NIDCAP® movements occurring during Lance/squeeze with those occurring during Clustered Care. Pearson product-moment correlations were used to examine associations between perinatal variables, and to

describe relationships between the NIDCAP® and infant background characteristics during Lance/squeeze and Clustered Care.

7.2 Results

7.2.1. Infant State

The infants differed in their behavioural state during the Baseline Phases of the two procedures. Greater numbers of infants were in active sleep during the Baseline Phase of the Pain procedure, whereas more infants were in quiet sleep during the Baseline Phase of the Clustered Care procedure ($z = -2.2$, $p < 0.03$). State changed significantly across Phases (Baseline, Lance or Clustered Care, Recovery) during both the Pain ($\chi^2 = 62.2$, $p < .0001$) and Clustered Care ($\chi^2 = 69.2$, $p < 0.0001$) conditions. Additionally, infants showed greater arousal during the Lance/squeeze compared to the Clustered Care Phase ($z = -4.0$, $p < 0.0001$). There were no statistically significant differences between Pain and Clustered Care Recovery states.[1] See Figure 7.

7.2.2. Facial Activity (Neonatal Facial Coding System: NFCS)

There were no differences in the frequency of brow bulge during the Baseline Phases of Pain and Clustered Care. The frequency of brow bulge changed significantly across the 3 Phases of both the Pain ($F [1,52] = 52.0$, $p < 0.001$) and Clustered Care ($F [1,52] = 19.2$, $p < 0.0001$) procedures with infants showing greater frequencies of brow bulge during the Lance/squeeze and Clustered Care Phases. There were no sex differences. Finally, the infants showed greater numbers of brow bulge during the Lance/squeeze Phase compared to the Clustered Care Phase ($t = 3.8$, $p < 0.0001$).

[1] The total average time of handling did not differ between procedures (5 minutes).

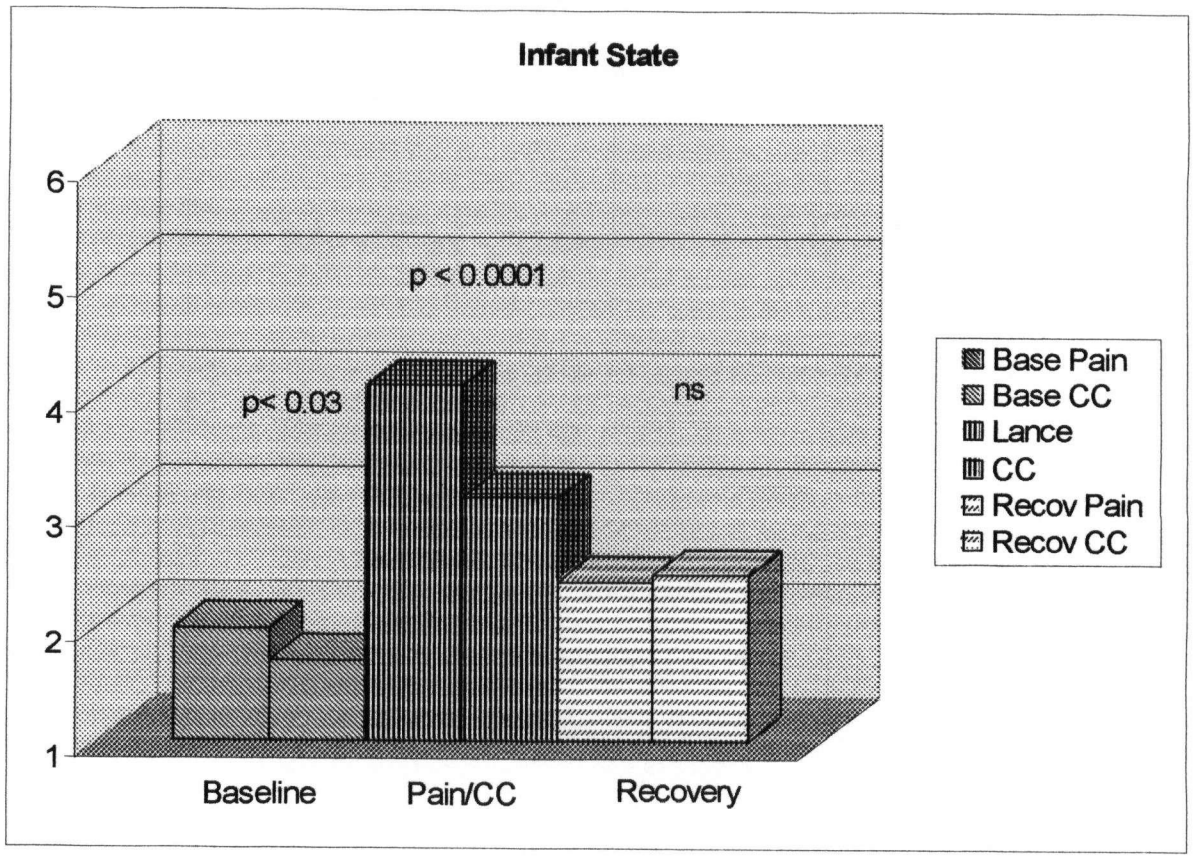

Figure 7. Infant Sleep/Wake State Across Three Phases Of Pain And Clustered Care (CC) Procedures.

7.2.3. NIDCAP®

7.2.3.1. Pain Procedure

Of the 26 NIDCAP® behaviours included in the statistical analysis (See Table 10), a set of 5 NIDCAP® movements (flex legs, hand on face, finger splay, salute, and frown) showed an overall main effect with increased frequencies of movements during the Lance/squeeze Phase. There were no sex effects, nor position interactions with these 5 behaviours (See Table 11). Moreover, there were no differences between the

Table 10. NIDCAP® Behaviours Included In Statistical Analyses.

NIDCAP® Behaviours	
Tremor	Tongue Extension
Twitch Face, Twitch Body, Twitch Extremities	Hand on Face
Flex arms, Flex legs	Mouthing
Extend Arms, Extend Legs	Finger Splay
Diffuse squirm	Airplane
Arch	Salute
Trunk Tuck	Sit on air
Hand to Mouth	Yawn
Grasping	Eye Float
Fisting	Frown
Leg Brace	Foot Clasp

Table 11. Frequencies of NIDCAP® Behaviours Which Increased Across Phases of Pain And Clustered Care

NIDCAP® Behaviour*	**Pain**			**Clustered Care**			**ANOVA**			
	Baseline Mean (sd)	Lance/ Squeeze Mean (sd)	Recovery Mean (sd)	Baseline Mean (sd)	Clustered Care Mean (sd)	Recovery Mean (sd)	Pain		Clustered Care	
							F	P<	F	P<
Flex Arms	0.8 (1.7)	1.4 (2.1)	0.7 (1.8)	0.3 (0.7)	1.1 (1.3)	0.7 (1.1)	8.1	0.001	9.4	0.0001
Flex Legs	1.8 (2.8)	4.6 (5.5)	1.8 (3.3)	1.0 (2.0)	2.6(2.5)	1.7 (3.5)	5.5	0.01	5.6	0.01
Extend Legs	1.6 (2.7)	3.1 (5.0)	1.1 (2.4)	1.0 (2.3)	3.5 (2.5)	1.3(3.0)	8.6	0.001	16.9	0.0001
Hand on Face	0.11 (0.4)	0.6 (1.1)	0.2 (0.4)	0.1 (0.5)	1.0 (1.2)	0.2 (0.7)	3.8	0.04	11.9	0.0001
Finger Splay	0.5 (1.4)	1.3 (1.9)	0.8 (1.3)	0.2 (0.7)	2.9 (2.4)	0.8 (1.4)	5.1	0.009	40.3	0.0001
Salute	0	0.1 (0.3)	0	0.1 (0.2)	0.7 (1.4)	0.1 (0.3)	3.5	0.05	13.5	0.0001
Frown	0.2 (0.6)	0.9 (1.2)	0.2 (0.6)	0.1 (0.3)	0.6 (1.0)	0.2 (0.5)	11.4	0.0001	8.2	0.002
Yawning	0.2 (0.4)	0.4 (1.0)	0.3 (0.7)	0.1 (0.3)	1.5 (1.6)	0.3 (0.6)	3.4	0.04	36.9	0.0001
Tongue Extension	0.4 (1.1)	0.3 (0.8)	0.4 (0.9)	0.1 (0.5)	1.2 (1.5)	0.3 (1.3)	0.9	ns	14.4	0.0001
Extend Arms	0.6 (1.5)	1.1 (1.6)	0.5 (1.1)	0.3(0.7)	2.0 (1.6)	0.5 (1.1)	1.1	ns	31.6	0.0001

NIDCAP® Behaviour	Pain			Clustered Care			ANOVA			
	Baseline Mean (sd)	Lance/ Squeeze Mean (sd)	Recovery Mean (sd)	Baseline Mean (sd)	Clustered Care Mean (sd)	Recovery Mean (sd)	Pain		Clustered Care	
							F	P<	F	P<
Airplane	0.1 (0.5)	0.1 (0.4)	0.1 (0.4)	0.1 (0.3)	0.4 (1.0)	0.1 (0.3)	0.4	ns	6.1	0.01
Sit on Air	0	0.16 (0.2)	0.1 (0.5)	0	0.3 (0.5)	0.1 (0.6)	3.2	0.05	6.0	0.005
Hand to Mouth	0. 4 (1.3)	0.6 (1.1)	0.4 (1.1)	0.2 (0.4)	0.1 (0.3)	0.5 (1.3)	1.2	ns	4.7	0.02
Fisting	0	0.2 (0.5)	0.1 (0.4)	0	0.3 (0.6)	0.1 (0.5)	1.6	ns	6.1	0.008

frequencies of these behaviours between Baseline and the Recovery Phases. An additional behaviour, flex arms ($F [1, 51] = 8.1$; $p < 0.001$) also increased significantly during the Lance/squeeze Phase with no sex effects, but those infants in prone had fewer instances of flex arms during the Lance/squeeze Phase ($p < 001$). An eighth movement, yawning also increased during the Lance/squeeze Phase ($F [1, 50] = 3.4$; $p < 0.04$) with those infants in prone yawning more than those in supine or side lying ($p < 0.01$). Further, a ninth movement, extend legs, increased during the Lance/squeeze Phase ($F [1, 51] = 8.6$; $p < 0.001$) with girls showing greater frequencies of this movement than boys ($p < 0.001$), and those infants in prone showing fewer instances of leg extensions ($p < 0.009$). Sitting-on-air (full extension of the legs into the air) increased significantly across the 3 Phases with highest frequencies during the Recovery Phase ($F [1, 51] = 3.6$; $p < 0.05$); however, there were no statistically significant differences in frequencies between each individual Phase. Finally, one movement, twitch extremities, decreased during Lance/squeeze ($F [1, 51] = 5.0$; $p < 0.01$). Appendix V presents NIDCAP® changes during Pain in graphical form.

7.2.3.2. Clustered Care Procedure

There were no sex effects with any NIDCAP® movements during the Clustered Care procedure. Of the 26 NIDCAP® movements included in the statistical analyses, 14 movements increased during the Clustered Care procedure, 9 of which were those observed during the Lance/squeeze (See Table 11). Grasping also increased between Baseline and Clustered Care and remained elevated during the Recovery Phase ($F [1, 52] = 4.3$; $p < 0.02$). Eye floating showed increased frequencies during the Recovery Phase ($F [1, 52] = 8.1$; $p < 0.003$). Furthermore, the infants continued to show increased finger splays ($t = -2.7$; $p < 0.01$), hand to mouth activity ($t = -2.1$, $p < 0.04$) and yawning ($t = -2.5$, $p < 0.01$) during Recovery when compared to Baseline frequencies.

Four NIDCAP® movements decreased significantly during the Clustered Care (twitch body {F [1, 52] = 12.3; p < 0.0001}, twitch face {F [1, 52] = 19.6; p < 0.0001}, twitch extremities {F [1, 52] = 19. 0; p < 0.0001} and mouthing {F [1, 52] = 9.9; p < 0.001}). Appendix VI presents NIDCAP® changes during Clustered Care in graphical form.

7.2.4. NIDCAP® Pain versus Clustered Care

Nineteen NIDCAP® movements were examined to compare the frequency of the movements occurring during Lance/squeeze versus Clustered Care Phases. Infants showed greater numbers of twitch face and flex legs during the Lance/squeeze than during Clustered Care. However, infants extended their arms, extended their tongues, finger splayed, airplaned (infant extends arms laterally), saluted (extension of the arms into mid-air in from of the infant), sat on air, and yawned more frequently during the Clustered Care Procedure (See Table 12 and Appendix VII). Because the infants showed ongoing NIDCAP® stress cues following the Clustered Care, the data was further examined to compare the frequency of the movements during the Recovery Phases of both procedures. The frequency of eye floating was statistically significantly higher during the Clustered Care Recovery Phase than during the Pain Recovery Phase ($t = -2.98$, $p < 0.004$).

7.2.5. Relationships between NFCS and NIDCAP® Behaviours and Perinatal Variables

Examining the relationship between gestational age at birth and the frequencies of NIDCAP® behaviours during Lance/squeeze showed that increased body twitches during the Lance/squeeze Phase was associated with infants who were born at earlier gestational ages (< 30 weeks). Similarly, infants who were born at earlier gestational ages showed greater frequencies of flexing of legs, finger splays and saluting during the

Table 12. Comparison of Frequencies of NIDCAP® Movements During Pain Versus Clustered Care

NIDCAP® Behaviour	**Mean Differences (sd)**	**t**	**P<**
Twitch face	0.15 (0.5)	2.2	0.03
Flex legs	1.94 (5.0)	2.5	0.02
Extend arms	- 0.85 (2.1)	- 2.9	0.005
Tongue extension	- 0.85 (1.7)	- 3.6	0.001
Hand on face	- 0.3 (1.5)	- 1.5	0.0001
Finger splay	-1.6 (3.0)	- 3.8	0.014
Airplane	- 0.4 (1.0)	- 2.6	0.001
Sitting on air	- 0.2 (0.5)	- 3.3	0.002
Yawn	- 1.1 (1.9)	- 4.2	0.0001

Clustered Care procedure. While less morphine exposure and less pain exposure was associated with increased frequencies of leg flexion during the Clustered Care Phase, infants who had been exposed to fewer pain procedures showed increased leg extensions during the Clustered Care Phase. Finally, fewer body twitches during Clustered Care were associated with infants who had been more stable on the first post natal day (SNAP 1) (See Table 13).

Table 13. Correlations Between NIDCAP® Behaviours And Perinatal Variables

Infant Characteristics	**NIDCAP® Behaviour**	**Procedure**	**r**	**P <**
Gestational age at birth	Flex legs	CC‡	- 0.40	0.003
	Finger splay	CC	- 0.30	0.03
	Saluting	CC	- 0.30	0.03
	Twitch body	P	- 0.29	0 .03
Illness severity (SNAP-II Day 1)	Twitch body	CC	0.33	0.02
Pain Exposure*	Flex legs	CC	- 0.37	0.006
	Extend legs	CC	- 0.27	0.05
Morphine exposure†	Flex legs	CC	-0.28	0.04

*Number of invasive (skin breaking) procedures from birth to the first study day.

†Morphine exposure = (daily average/kg per os dose/3 + daily average intravenous mg/kg) X days.

‡CC = Clustered Care, P = Pain

7.2.6. Heart Rate

Mean heart rate (HR) changed significantly across the 3 Phases of Blood Collection ($F [1, 49] = 69.2$; $p < 0.0001$), and across the 3 Phases of Clustered Care ($F [1, 53] = 41.7$; $p < 0.0001$). HR (mean ± SD) increased from Baseline 157.2 ± 10 to Lance/squeeze 177.2 ± 15 ($t = - 11.1$, $p < 0.0001$), and decreased during recovery 158.5 ± 13 ($t = 9.2$, $p < 0.0001$). Similarly, HR increased significantly from Baseline 155. 7 ± 13 to Clustered Care 168.6 ± 16 ($t = - 8.9$, $p < 0.0001$), and decreased significantly during Recovery 157.0 ± 14 ($t = 6.6$, $p < 0.0001$). While there were no statistically

significant differences between the Baseline Phases of the two procedures, nor in the Recovery Phases, the increase in HR was greater during Lance/squeeze compared to Clustered Care ($t=3.9$, $p < 0.0001$). This finding was further confirmed by converting the changes from Baseline to Lance/squeeze and Baseline to Clustered Care Phases to change scores (Δ heart rate [Δ HR]; Baseline – Lance/squeeze or Clustered Care)/ Baseline + Lance/squeeze or Clustered Care); this conversion takes into account the law of initial values (Lacey, 1956). The Δ HR Lance/squeeze was significantly greater than the Δ HR Clustered Care ($t = - 3.4$, $p < 0.001$).

7.2.7. Oxygen Saturation

Mean O2 sat decreased across the three Phases of Blood Collection ($F [1, 45] = 27.3$; $p < 0.0001$) and Clustered Care ($F [1, 50] = 62.9$; $p < 0.0001$). During the pain procedure, O2 sats dropped from Baseline 96.1 ± 3 to 91.3 ± 6 during the Lance/squeeze Phase ($t = 6.2$, $p < 0.0001$), and increased to 95.7 ± 6 during the Recovery Phase ($t = - 5.3$, $p < 0.0001$). Likewise, during the Clustered Care procedure, O2 sats decreased from Baseline levels of 95.4 ± 3 to 88.0 ± 7 during Clustered Care ($t= 8.2$, $p < 0.0001$), and then increased during Recovery 96.1 ± 4 ($t = - 8.2$, $p < 0.0001$). Baseline and Recovery changes in O2 sats did not differ between Pain and Clustered Care; however, O2 sats were lower during the Clustered Care procedure than during the Pain procedure ($t = 2.6$, $p < 0.01$).

7.3 Discussion

This is the first study to examine preterm infants' detailed physiological and behavioural responses to a common cluster of NICU procedures, using multidimensional assessments, including the full NIDCAP®, and to compare preterm infant responses to this tactile procedure with those responses stimulated by an acutely painful procedure. The infants in this study showed reactions to both procedures;

however, there were differences in intensity of responses and in the direction of the behavioural measures. The infants showed greater increases in behavioural state, facial reactivity (both NFCS brow bulge and NIDCAP® frown), and heart rate during the painful procedure than during clustered care. This finding is similar to those reported by Ballantyne et al who described lower "pain" scores (a composite measure including 3 facial actions, changes in HR and O2 sat) to diaper change than to lance (Ballantyne, et al. 1999). While there were similar differences in reduction of O2 sats between the pain and clustered care procedures, Clustered Care produced greater drops than did the Pain procedure. Other studies have also shown drops in O2 sats during acutely painful procedures (Schwartz & Jeffries, 1990; Bozette, 1993; Stevens & Johnston, 1994; Stevens et al., 1993; Van Cleve et al., 1995). However, our study differed from a number of others in that the drops in O2 sats were greater during the non-painful handling (Craig et al., 1993; Johnston et al., 1995). We speculate that this greater drop in oxygen saturation occurred because the infants were handled in a more vigorous way for the Clustered Care procedure.

Contrary to the increased behavioural state, facial actions and HR in response to pain, the infants in this study demonstrated not only a greater variety of body movements, but greater frequencies of those movements currently described by the NIDCAP® as stress cues during Clustered Care than during the Pain procedure. Increased body responses to a tactile procedure may have occurred for two reasons. First, the act of diapering and providing other nursing procedures used in this study, in general, required greater physical manipulation of the infant's body than did the heel lance during which the body is kept relatively still. Second, even though the infants showed less state arousal, and facial responses to diapering, the increased body movements may be an indication of sensitization to tactile stimulation. Such

sensitization has been reported in animal studies, and by Hellerud and Storm, who described increased physiological stress responses to tactile stimulation in preterm infants (Hellerud & Storm, 2002; Jennings & Fitzgerald, 1996). Finally, the infants continued to show increased stress cues following the Clustered Care including eye floating, finger splays and yawning. This result may indicate the wind-up phenomenon, the progressive build up of a response to repetitive low frequency stimulation (Woolf, 1996).

When we examined the specific movements in more detail, we found that the infants in our study responded to the Clustered Care with predominantly extensor movements such as extension of the arms and legs, finger splays, airplane, sitting on air and salute. Although the infants in this sample did not exhibit significant increases in some of these actions (e.g. extend arms, airplane, sit on air) in response to the painful procedure, we have observed such actions in a previous study of preterm infant responses to acute pain (Morison, et al., 2003). Moreover, according to the NIDCAP® model, these movements are described as stress cues. This interpretation of these movements is not only supported by our findings in this and other studies (Grunau et al., 2000; Morison, et al., 2003), it is further supported by Peters (2001) who found that increased extensor movements were associated with negative physiological changes in response to bathing. Another extensor action, tongue extension, also occurred more frequently to the Clustered Care than to the Pain procedure. Although tongue extension is not observed in term infants during pain, tongue extension has been reported to be a marker of pain response in preterm infants (Grunau et al., 1990; Grunau et al., 1998). But, given that the infants showed less intense facial and state responses to the Clustered Care, it is unlikely that the infants perceived the tactile procedure as "painful".

Rather, it is more likely that tongue extension is not a specific pain cue, but is a general stress response behaviour.

Similar to tongue extension, yawning, which increased significantly during Pain and Clustered Care, was more prevalent during Clustered Care. In addition, infants positioned in prone tended to yawn more during the Pain procedure than infants in other positions. Yawning is thought to help increase the level of arousal in preterm infants and is associated with the drowsy state (Giganti, Hayes, Akilesh & Salzarulo, 2002). Indeed, in our study, more infants were classified as drowsy during the Clustered Care (96%) than during the Lance/squeeze (57%), and more infants who were in prone during the Lance/squeeze phase were classified as being drowsy (67%) than those in supine/side lying (32%). However, according to the NIDCAP model®, yawning can be interpreted as a stress cue; therefore, one might interpret this cue not only as an indicator of a behavioural state, but also as a stress response. This interpretation is supported physiologically in that adrenocorticotropic hormone (ACTH, a stress hormone) facilitates yawning (Argiolas & Melis, 1998). Fisting also increased significantly to Clustered Care. Although it did not increase to statistically significant levels during the Pain procedure, fisting increased during the Lance/squeeze Phase and remained at higher levels during Recovery. Even though fisting may be included as a pain indicator in some assessments, like finger splays, it is more likely a general stress response behaviour rather than a pain specific cue. Finally, hand on face, a protective action whereby the infant places its hand on its face in an attempt to create a barrier between the face and the stimulus, increased to both procedures, but with greater frequency to Clustered Care.

Flexor movements were also observed during both Pain and Clustered Care procedures. In general, flexion actions are interpreted as attempts at self-regulation. In support of this interpretation, Peters (2001) demonstrated that greater numbers of NIDCAP® flexor motions were associated with positive clinical events (increases in physiological stability). Although flexor actions of the arms, such as hand to mouth, may provide increased stability, caution must be used in over-interpreting these actions with the lower extremities since flexor actions of the legs are also noted to be reflex responses to pain and tactile stimulation in preterm infants (Andrews & Fitzgerald, 1999). Finally, as we have found in our prior studies, twitches decreased during the Pain and Clustered Care Phases, further supporting our contention that they are not stress response indicators (Grunau et al., 2000; Morison et al., 2003).

In our previous study assessing acute pain responses in preterm infants, earlier born infants showed greater numbers of stress response behaviours than later born infants (Chapter 6; in press; Holsti et al., 2003; Morison et al., 2003). While these results were not replicated with the pain procedure in the present study, infants who were born at earlier gestational ages did exhibit greater numbers of stress cues to Clustered Care. However, infants who had been exposed to less pain and morphine showed greater numbers of lower extremity flexion and extension during Clustered Care. It is possible that those infants who experienced fewer painful procedures and were less ill (therefore needed less morphine) were able to mount more vigorous flexor withdrawal responses to tactile and painful stimuli. As previously mentioned, although twitches do not appear to be stress cues, their presence during a tactile procedure may be a marker of relative instability: infants who were more ill on day 1 of life (SNAP 1) had body twitches during Clustered Care. In agreement with this finding, infants born at earlier gestational ages showed greater body twitches during Lance/squeeze.

7.4 Conclusion

In conclusion, preterm infants demonstrate clear, reproducible biobehavioural responses to both tactile and noxious stimulation. Our study indicates that, in fact, depending on the level of physical manipulation involved in the routine care giving tasks, even those as simple as diaper changing, measuring abdominal girth and taking a temperature produce marked physiological and motor responses indicative of stress that persist beyond the time of the handling. This finding is critical since tactile procedures occur with much greater regularity than painful procedures. Our findings also highlight the need for further evaluation of the practice of clustering of care-giving tasks. While clustering tasks may provide longer rest periods, a balance between shorter rest periods interspersed with one or two care-giving tasks might produce less intense biobehavioural responses in some infants. Finally, it appears that facial activity, changes in sleep/wake state and heart rate are relatively the most specific indicators of painful procedures; nevertheless, body movements can add important information regarding the infants' responses to painful events. Adding observations of body movements to the assessment of pain and stress responses is particularly important for those infants born at earlier gestational ages who may show dampened facial responses after repeated pain exposure (Grunau et al., 2001a; Johnston & Stevens, 1996). Not only do further studies need to determine whether infants assessed at earlier gestational ages show similar responses to both painful and non-painful nursery procedures, but clinicians working in NICUs must continue to study the cumulative effects of *both* painful and non-painful handling as potential mechanisms for alterations in brain development in these vulnerable infants.

CHAPTER 8

Conclusion: Theoretical and Clinical Implications

The typical neonatal intensive care unit (NICU) in the 1980's was bright and loud; infants lay flat and exposed on the mattresses in their incubator; they were handled repeatedly, hurriedly and with little attention to their behavioural responses. Parents were allowed to visit only during specific times. In fact, at that time, developmental interventions focused on providing *more* stimulation to infants because they were thought deprived. Today, we find a very different situation in the NICU. Preterm infants are now shielded from the bright lights with quilts and blankets covering the infant's incubator. Several times a day, a "quiet hour" may be implemented when the lights are dimmed, and the staff is quiet. Infants are nested with soft rolls surrounding them and they are often bundled in soft blankets. They are handled only as needed, the pacing of care slow and gentle. In some units, beds are available beside each incubator so that parents to stay at the bedside as long as they want. Dramatic alterations in the NICU environment and in care giving such as these are largely due to the implementation of the synactive theory of development, the pioneering work of Dr. Heidelise Als.

Before developmental science firmly established the link between early stress and later development, Als made that link. She believed that the developmental differences observed in preterm infants were due to the mismatch between the infants' brain and the environment of the neonatal intensive care unit (NICU). In Chapters 2 and 3, I reviewed the extensive human and animal literature which supports Als contention that early exposure to stress and pain alters long-term development.

In Chapter 5, I showed that the synactive theory is a synthetic theory integrating principles from multiple lines of study including ethology, neuroembryology, organismic psychology and motor physiology (Als, 1982). Most importantly, Als generalized the

application of the principle of dual antagonistic systems, taken from neurophysiological studies of motor systems, as an overriding principle of development. Her synactive theory of development directs us to conceptualize infant development as biphasic in that an infant will work towards a more integrated and differentiated developmental level through interaction with its environment, approaching stimulation which is of appropriate intensity, complexity and timing and defending itself, or withdrawing, from stimulation which is too intense or inappropriately timed. An infant's current level of functioning can be readily observed through the evaluation of five subsystems of functioning, the autonomic, the motor, the state organizational, the attentional and self-regulatory subsystems. Development is synactive in that all five subsystems are in continuous interaction with one another; stability in one subsystem allows differentiation in another.

Als hypothesized that by applying a model of care based upon the synactive theory, the Newborn Individualized Developmental Care and Assessment Program (NIDCAP®), preterm infant developmental outcomes would improve. The NIDCAP® utilizes detailed, repeated observational assessments of preterm infant behaviours and suggests alterations in care giving and in the environment depending on the individual needs of each infant (Als, 1984). Als' primary hypothesis has been tested in number of studies, some studies showing improvements in development, few showing long-term benefits. Unfortunately, firm conclusions can not be made as to the efficacy of the NIDCAP® because many of the early intervention studies have methodological flaws which make the interpretation of results equivocal.

The concern was that the lack of conclusive evidence regarding the NIDCAP® efficacy might not be ascribed to methodological insufficiencies alone, but also to flaws in one of the overriding principles of this theory, the principle of dual antagonistic systems. This principle directs us to interpret preterm infant movements as indicators of

stress and stability, but also directs us to maintain flexibility in our interpretations. The way in which we interpret preterm infant movements directly affects the clinical care of these vulnerable infants. Infants who are too stressed or in too much pain, could be given too much medication, which may in turn, have deleterious effects.

In three previous studies, we began to explore the validity of this principle; and we found, in agreement with AIs, that some movements such as leg extension and finger splay were associated with highly intrusive and painful procedures (Grunau et al., 2000; Grunau et al., 1998; Morison et al., 2003). However, some of these initial studies did not include all the NIDCAP® movements, had short observation periods and/or involved a small sample size.

The goals of this dissertation have been both theoretical and clinical. The primary purpose was to examine the validity of the principle of dual antagonistic systems by applying the NIDCAP® assessment to a larger sample of preterm infants in controlled conditions, during procedures of varying intensities. I addressed the concurrent validity by comparing the NIDCAP® to well validated and reliable indicators of pain. In this way, I determined which movements could be associated specifically with a known stressor. The secondary purpose had a clinical focus. In order to assist in the identification of stress and pain responses in preterm infants, I determined if specific, developmentally relevant, body movements could help us distinguish between these responses.

8.1 Theoretical Implications

A series of five studies has evaluated the interpretation of preterm infant movements of the NIDCAP®, the most recent two forming chapters 6 and 7 of this dissertation (Holsti, et al. in press; Holsti, Grunau, Weinberg, Whitfield & Oberlander, under review). This series of studies has examined the responses of preterm infants across procedures of varying intensities including at rest, during nasogastric feeds,

diaper changing, chest physiotherapy, endotracheal suctioning and blood collection. I conclude that the classification of preterm infant movements in the synactive theory of development requires revision.

I maintain that a fundamental problem with the synactive theory is that the concept of "flexibility" is applied at the wrong time and in the wrong place. First, while the synactive theory directs us with general rules about interpretations of movements, we are also advised to apply moment to moment flexibility to our interpretations. One minute a movement may be a sign of stability, the next, an indicator of thresholds to stress. Even with rigorous training in the NIDCAP® system, applying such flexibility to a large catalogue of preterm infant movements leads to highly subjective assessments.

I have found that a more strict interpretation of preterm infant movements can be applied at early postnatal ages. For example, in agreement with AIs, a subset of 14 NIDCAP ® movements *are* consistently observed in preterm infants during highly intrusive and painful procedures and can be reliably identified as stress response movements. However, contrary to the model, twitches and startles are not associated with stressful procedures, are present during rest and are likely normal movements indicative of active sleep. Although moment by moment flexibility in interpretations of preterm infant movements at very early postnatal ages may be misguided, such flexibility is necessary for older infants because their movements are less influenced by reflex activity and as such interpretations are context specific. For example, in a six month old, extending an arm can be a defensive response, but it can also be reaching for a toy.

On the other hand, the synactive theory is not flexible enough in its interpretation of preterm infant movements; it does not take into account spinally mediated mechanisms of movement, or biomechanical influences of movement. Rather than

categorize some movements as indicators of stress and stability, actions such as flexing the leg are likely reflexive responses to tactile and noxious stimulation. Also, in our studies, active leg extension, a NIDCAP® stress cue, was observed during stressful procedures, but under other circumstances, such as at rest, leg extension may be a passive movement and an action influenced by gravity, as Thelen shows in studies of dynamic systems theory (e.g.Thelen, 1986; Thelen & Smith, 1994). Based on our series of studies, we now caution clinicians not to "over-interpret" preterm infant movements and to consider alternative explanations for the behaviours.

8.2 Clinical Implications

I demonstrated in Chapter 7 that, in addition to painful procedures, tactile procedures such as diaper changing, along with other minor nursing tasks, produce significant motor and autonomic responses in preterm infants. I emphasized that the accumulation of stress responses to a variety of types of handling likely contribute to altered development in preterm infants. It is critical, therefore, that we have developmentally relevant, norm referenced, objective measures to evaluate the stress responses of high risk infants.

One of the benefits of the NIDCAP® is that it is the only available multidimensional tool which measures stress responses and which is developmentally relevant for preterm infants. Instead, the development of measures of stress responses in preterm infants has focused more specifically on the development of pain assessments. Thus, there exists a huge gap in the area of assessments of stress responses in preterm infants. I suggest two strategies which would address the need for more a general stress assessment of preterm infants.

Continuing to implement the NIDCAP® does provide clinicians with a philosophy of care which is family centred and takes into account the individual developmental

needs of the infant. In order to ensure that NIDCAP® assessment truly measures stress responses, the entire catalogue of behaviours needs to be adjusted so that a standardized tool is developed with proper age norms. Two groups of researchers have begun this process in an attempt to quantify the NIDCAP® (Pressler & Hepworth, 2002; Pressler et al., 2001; Sell, Hill-Mangan & Holberg, 1992). A quantifiable tool would help remove some of the subjective nature of the interpretation of the movements and would likely provide a shorter and more efficient tool for use at differing ages.

A second approach would be to create a new "stress" scale utilizing facial, physiological and the subset of body movements we found associated with intrusive and invasive events. This approach will be lengthy since all test development requires multiple steps involving evaluation of multiple aspects of reliability, validity and clinical utility. Moreover, complexities in scaling, such as deciding how to define degrees of intensity of various stressors, make this task difficult. However, the benefit of this approach is that, in the end, clinicians and parents would have a short assessment which could be easily integrated into the clinical setting.

The synactive theory of development, and its clinical application, the NIDCAP®, have radically altered the way in which neonatal medical and nursing care are delivered to preterm infants. This theory has a worthy goal, to improve developmental outcomes in high risk newborns. Now, research is needed to provide more objective and easily implemented assessments of stress responses in these vulnerable infants.

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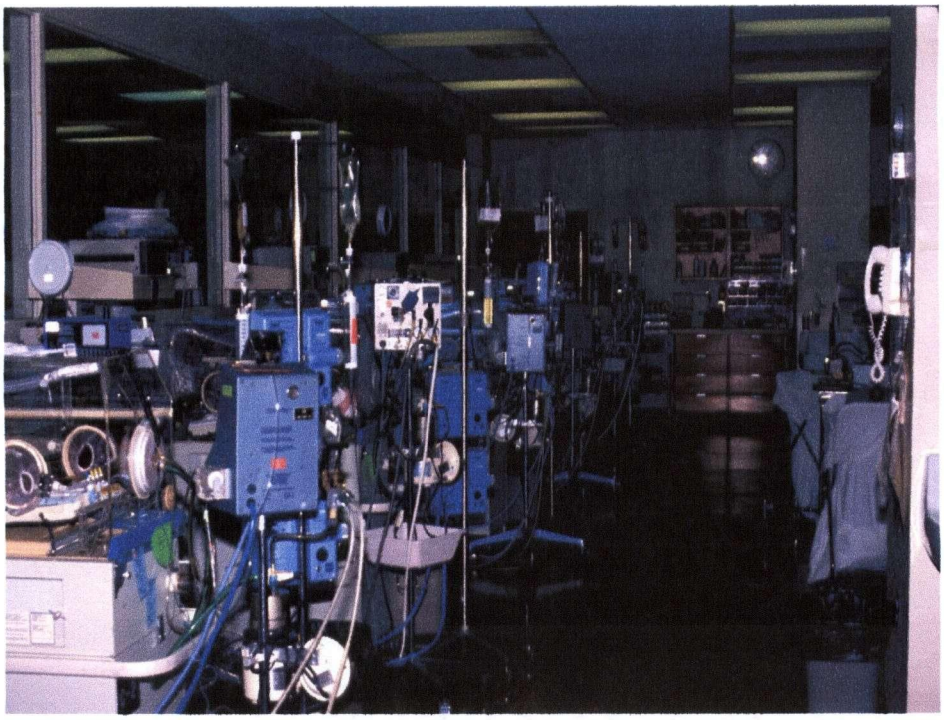
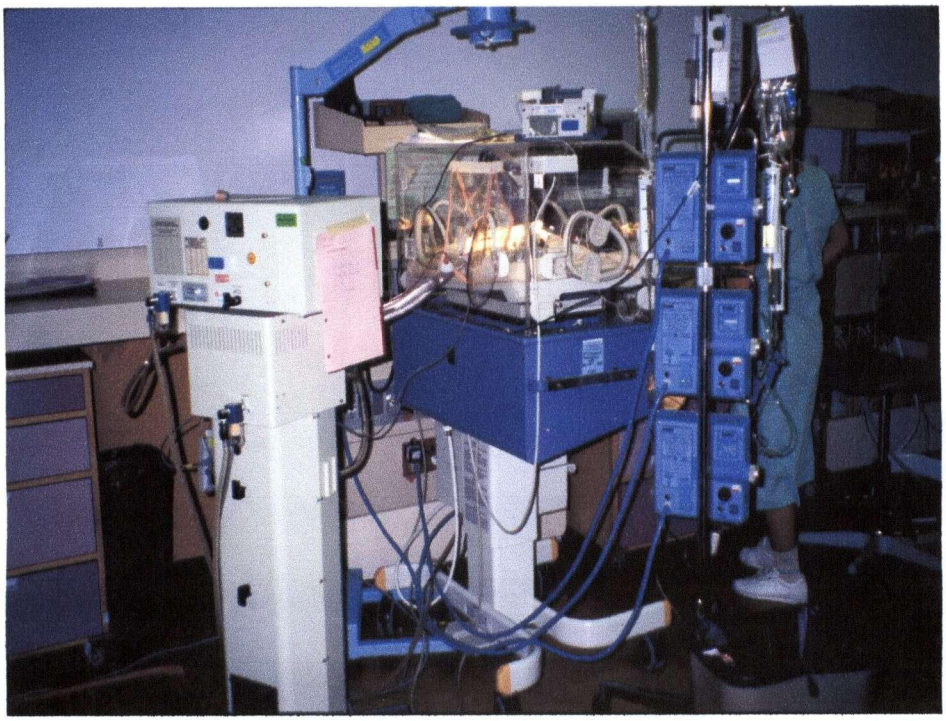
Appendix I

The Special Care Nursery (SCN) at Children's and Women's Health Centre of British Columbia in the early 1990's.

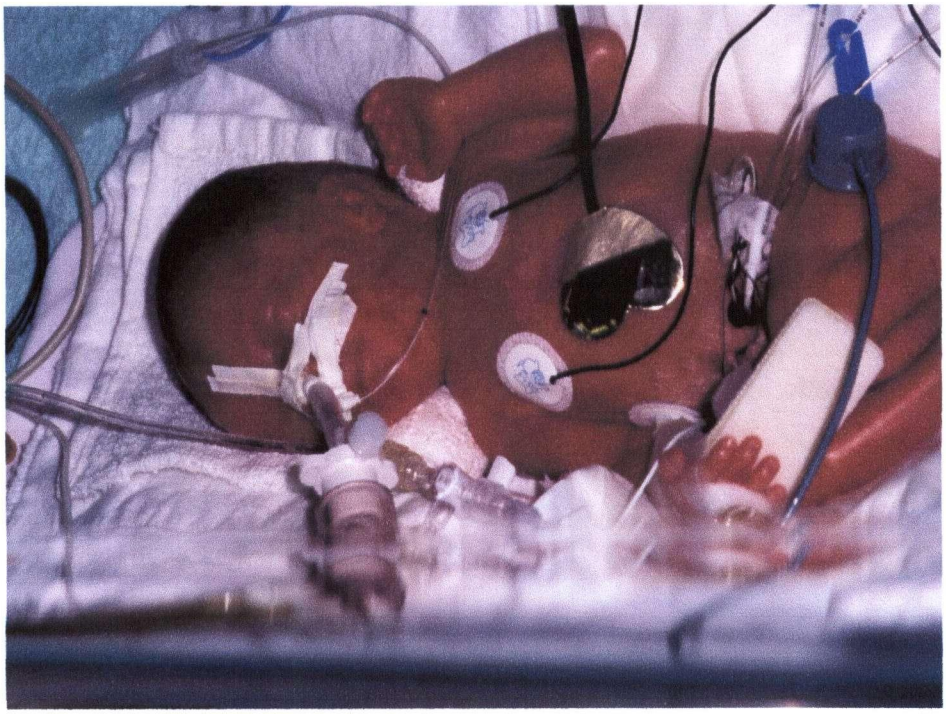
A. Room 41



B. Individual Incubator in SCN

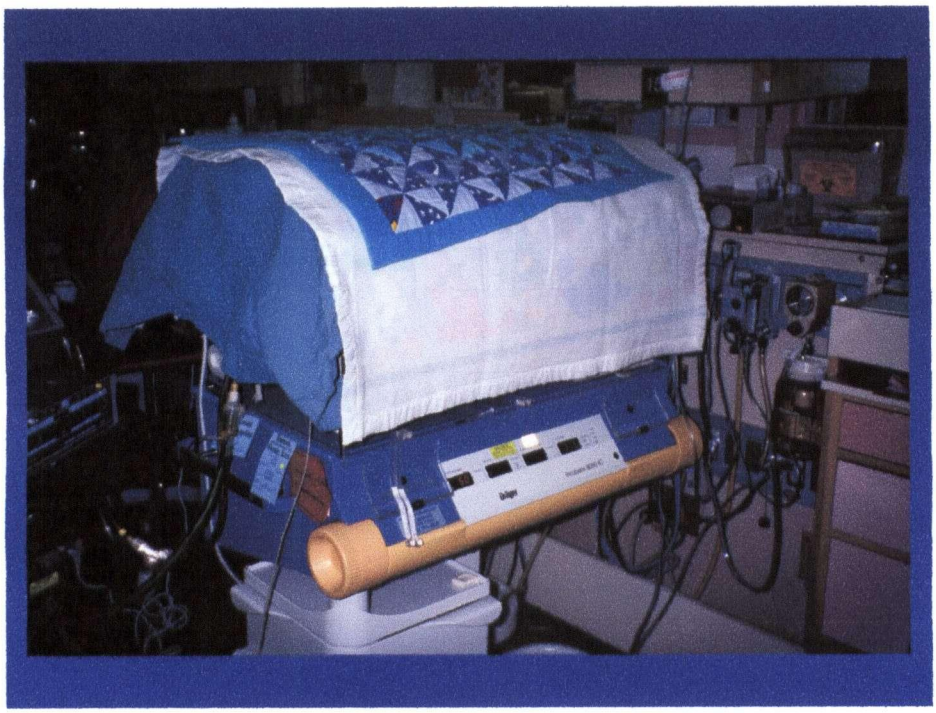


C. Preterm Infant at 25 weeks gestational age

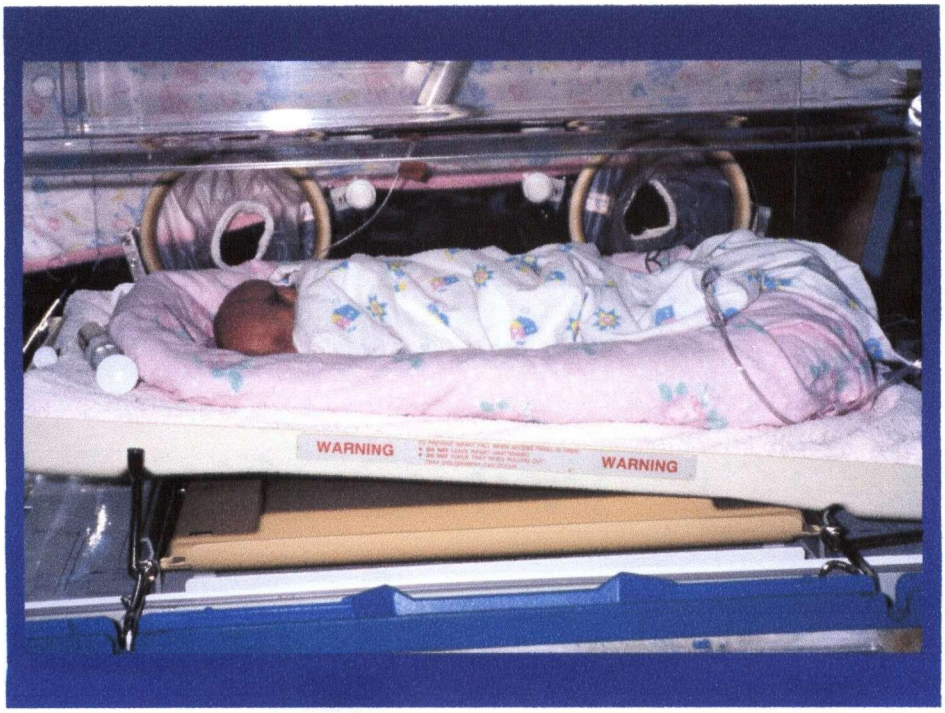


Appendix II

A. Infant isolette following implementation of the NIDCAP®

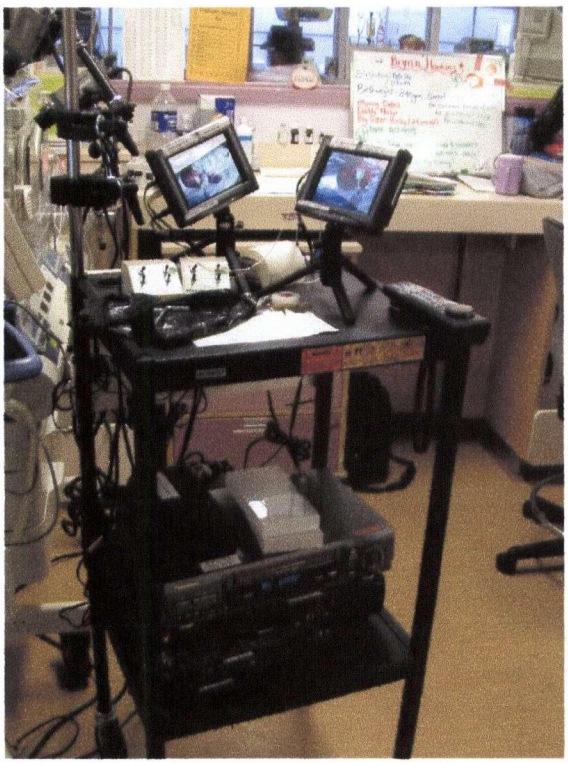


B. Infant positioning with bundling and nesting.

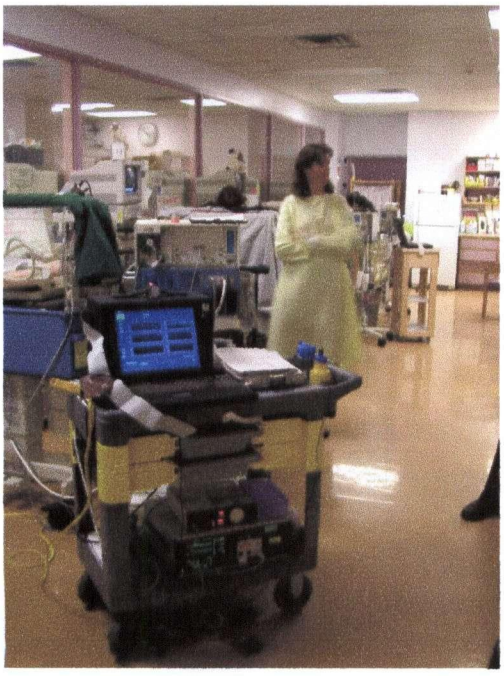


Appendix III

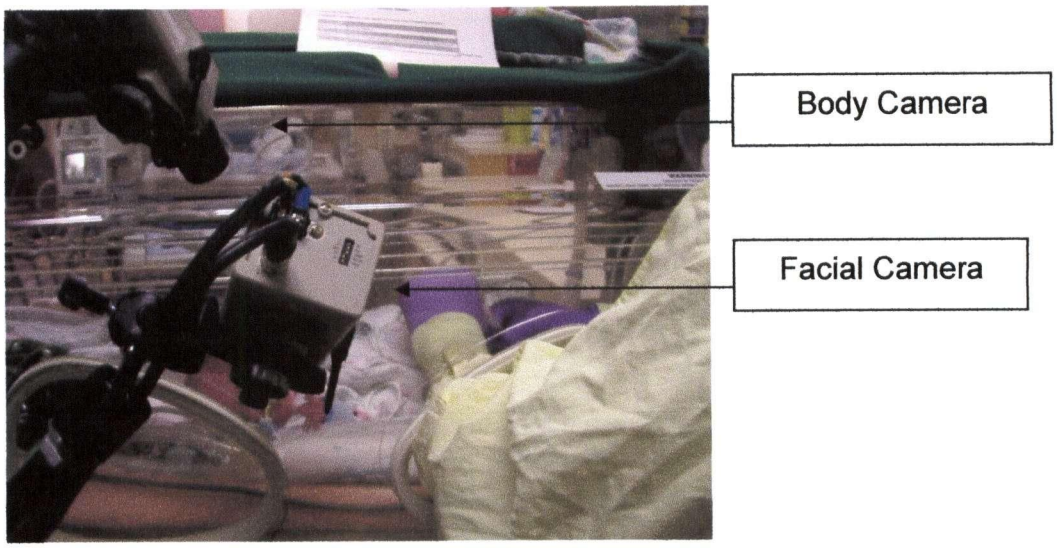
A. Video equipment for recording facial and body reactivity.



B. Computer equipment for recording physiological responses.

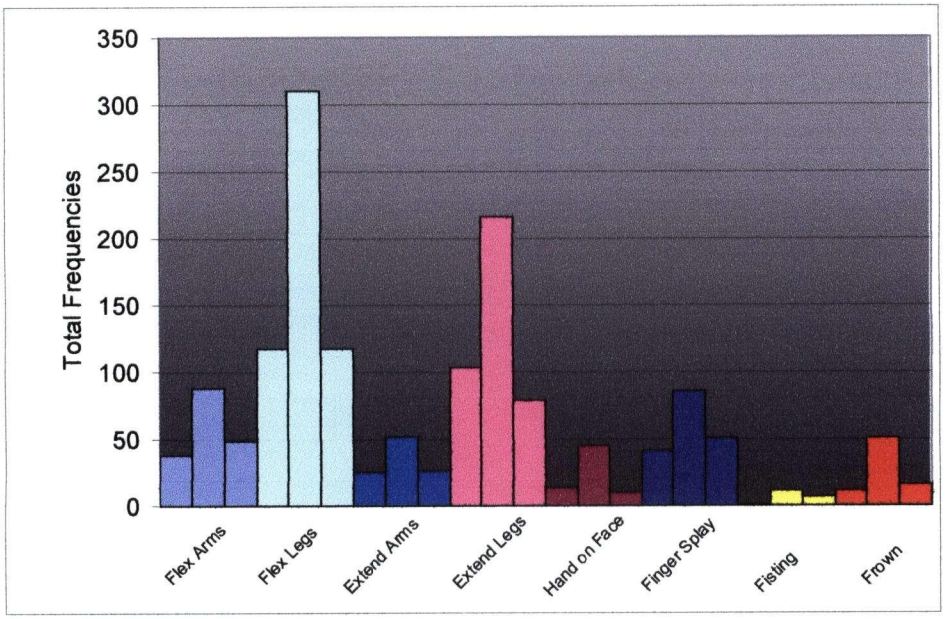


B. Placement of cameras for video recordings.

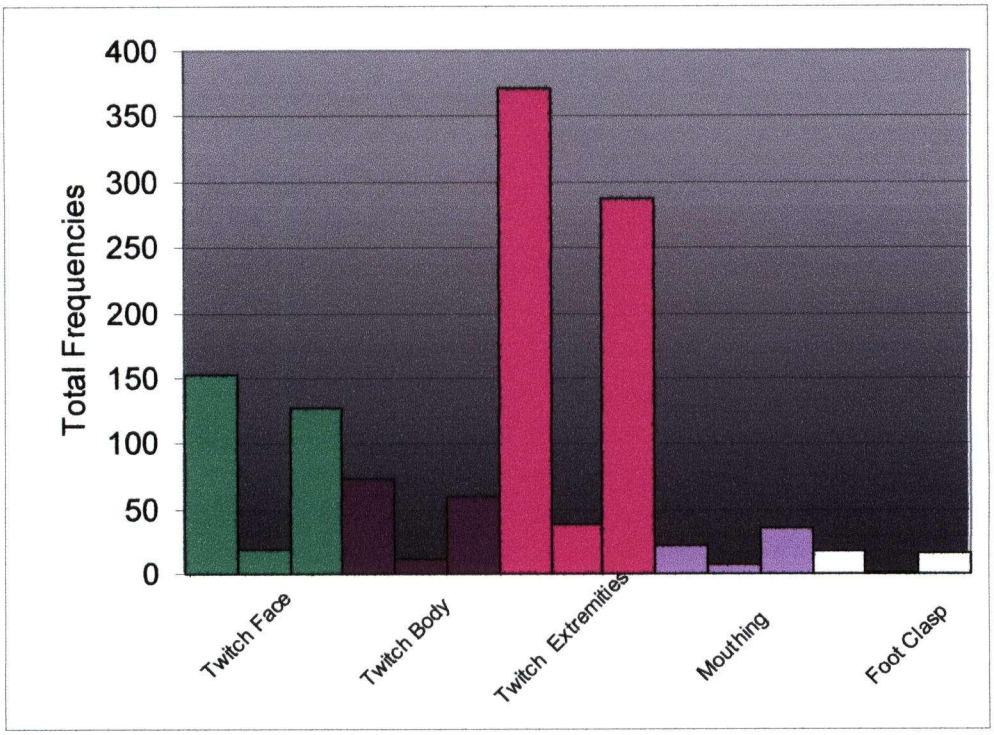


Appendix IV

A. NIDCAP® Movements which Increased During Pain (All P< 0.01)

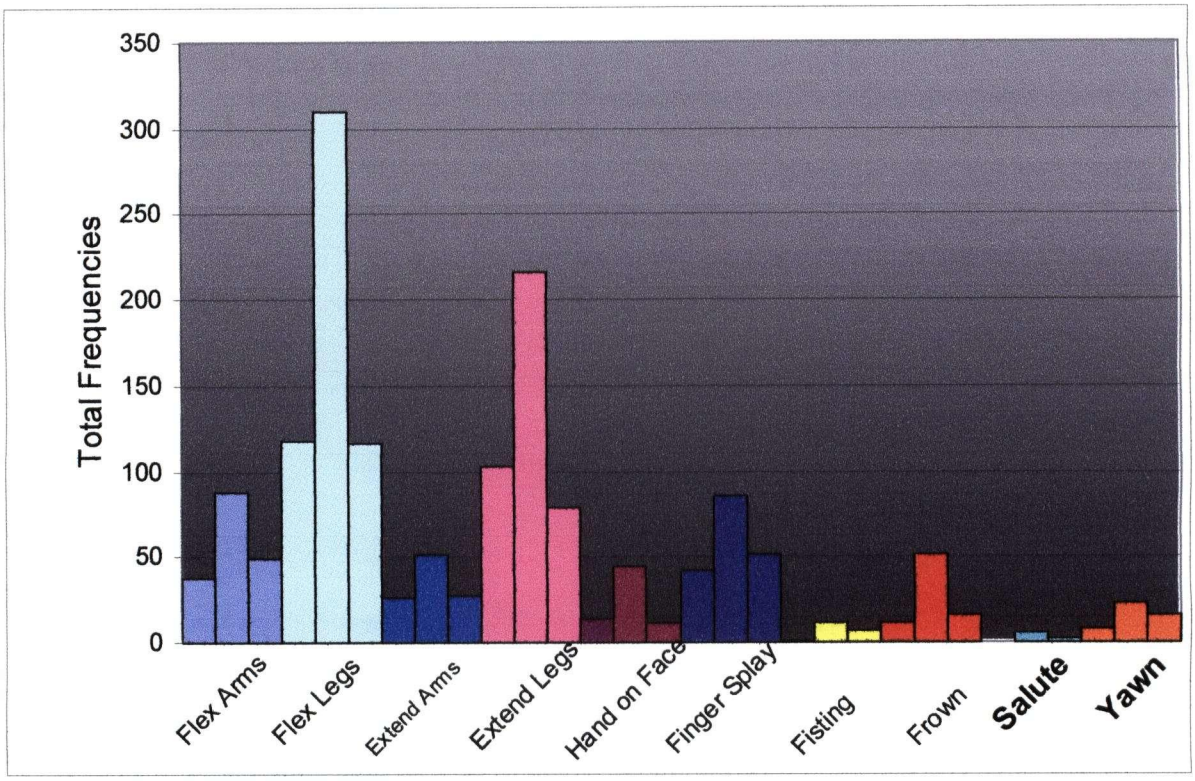


B. NIDCAP® Movements which Decreased During Pain (All P< 0.01)



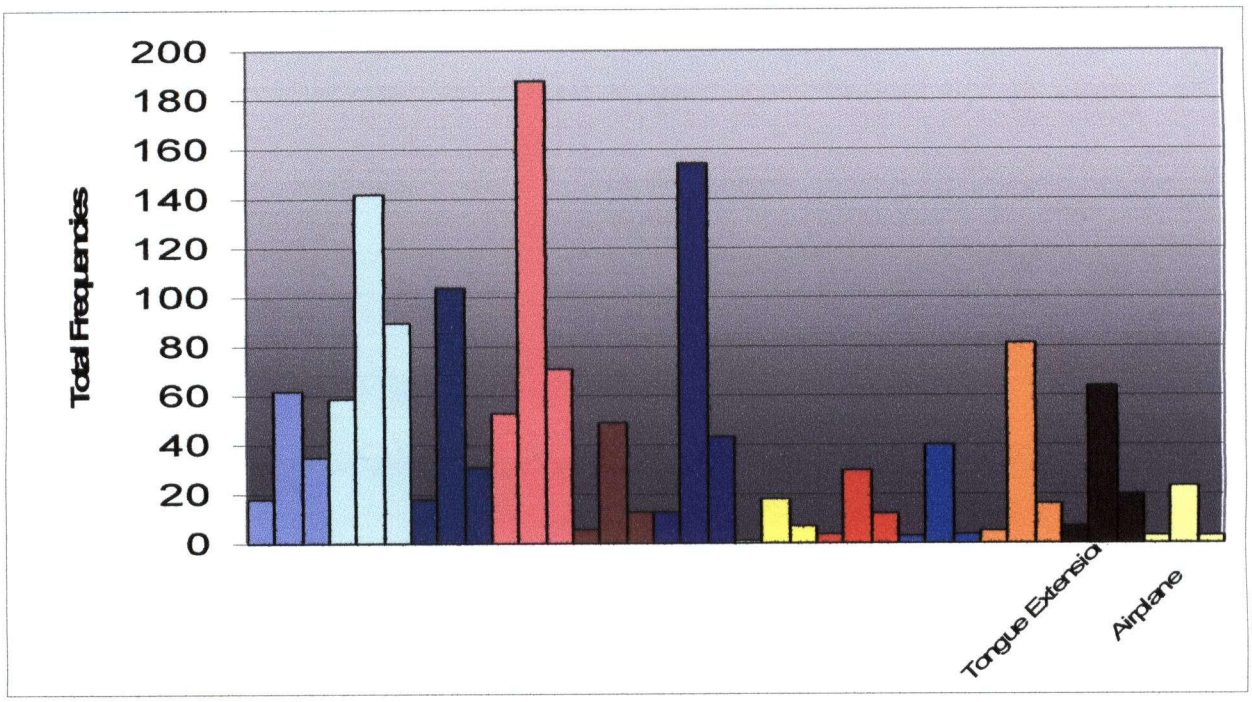
Appendix V

Additional NIDCAP® movements (salute and yawn) which increased during Pain not observed in Study 1 (Chapter 6).

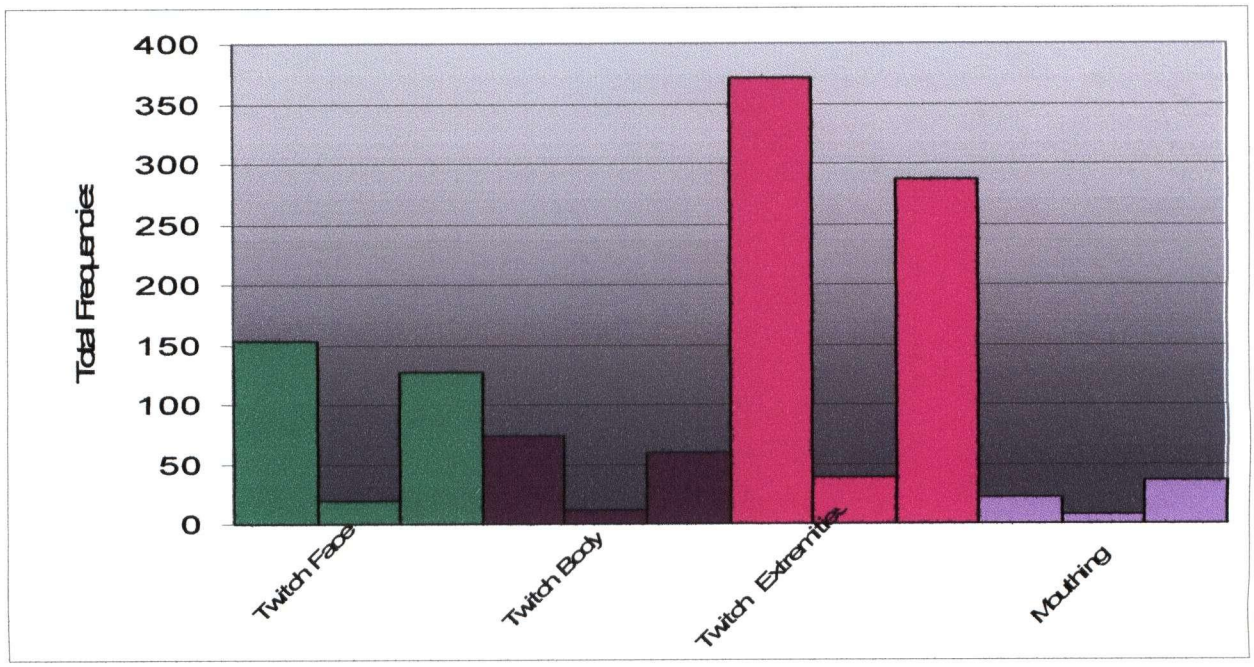


Appendix VI

A. Additional NIDCAP® movements (tongue extension and airplane) which increased during Clustered Care, but which were not observed during Pain.



B. NIDCAP® movements which decreased during Clustered Care.



Appendix VII

Comparison of Frequencies of NIDCAP® Movements during Pain versus Clustered Care.