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Effects of IV morphine on nociceptive stress responses and cardiorespiratory stability of premature neonates following surgery

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EFFECTS OF IV MORPHINE ON NOCICEPTIVE STRESS RESPONSES AND CARDIORESPIRATORY STABILITY OF PREMATURE NEONATES FOLLOWING SURGERY

by

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DISSERTATION

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ABSTRACT

EFFECTS OF INTRAVENOUS MORPHINE ON NOCICEPTIVE STRESS RESPONSES AND CARDIORESPIRATORY STABILITY OF PREMATURE NEONATES FOLLOWING SURGERY

Linda Sturla Franck, R.N., Ph.D.

University of California, San Francisco, 1995

This study evaluated the effects of a single dose of intravenous (iv) morphine on nociceptive stress responses of premature neonates following surgery. In addition, the effects of iv administration of morphine on the cardiorespiratory function of the premature neonates was evaluated. Changes in plasma norepinephrine (NE) levels, vagal tone index (VTI), flexor reflex threshold (FRT), and cardiorespiratory status were measured following administration of morphine to premature neonates within the first twelve hours following surgery.

Measures were obtained during the preoperative period and in the postoperative period immediately prior to the administration of the first postoperative dose of morphine. Measures were again obtained 20 minutes and 1 hour after the morphine dose.

No significant changes from preoperative baseline values were observed in plasma NE levels at the pre- or post-morphine measures. VTI decreased significantly from preoperative baseline levels but did not change significantly between the pre- and post-morphine measures. In the neonates who demonstrated a flexor reflex during the postoperative period, there were no significant differences from preoperative FRT levels and no changes in FRT between the pre- and post-morphine measures. Heart rate increased

significantly, respiratory rate decreased significantly, and oxygen saturation tended to decrease between the preoperative baseline and the pre-morphine measures. These differences were not clinically significant. There were no differences in blood pressure measures between the baseline and pre-morphine measures. There were no statistically significant differences in any of the cardiorespiratory measures at 20 or 1 hour following morphine administration.

Christine Miaskowski, RN, PhD

Christine Miaskowski, R.N., Ph.D., F.A.A.N. - Chair

TABLE OF CONTENTS

Chapter Page

Chapter Page

Chapter Page

Chapter Page

Chapter Page

LIST OF TABLES

Table

LIST OF FIGURES

Figure

CHAPTER 1

INTRODUCTION

Stressors are stimuli perceived by an organism as noxious, novel, or arousing and may be physical or psychological in nature. The "fight or flight" response to stress was first described by Cannon (1929). Selye expanded the concept of the human stress response to include "nonspecific response(s) of the body to any demand" (Selye, 1976a, p. 15) that promote physiological and behavioral adaptation (Selye, 1976b). Stress, therefore, is the "state of disharmony or threatened homeostasis" requiring adaptive responses (Chrousos & Gold, 1992, p. 1245). Investigation of human stress responses is complex and challenging because virtually every body system is involved in re-establishing homeostasis through complex regulatory and feedback mechanisms. Successful stress responses maintain homeostasis by activating numerous compensatory mechanisms (e.g., physiological and behavioral arousal and inhibition of vegetative functions). Because homeostasis is maintained, stress responses may not be readily observable. Stress responses are often detected only when homeostasis can not be maintained or when stress responses are of such magnitude that behavioral or physiological functions are disrupted (Gold, Goodwin, & Chrousos, 1988; Kopin, Eisenhofer, & Goldstein, 1988).

Stress responses occur in phases, namely: the initial mobilization phase, the short-term compensatory phase, the long-term adaptation phase, and the terminal decompensation phase (Asterita, 1985). However, it is often difficult to recognize which phase of adaptation the observed stress response represents because the duration and manifestation of the phases of the stress response are influenced by the duration and frequency of exposure to stressors.

Nociception associated with surgical injury has been used extensively as a

model to study of stress responses associated with large magnitude physical stressor (Bryan-Brown, 1986; Kehlet, 1984). This research led to the recognition of the harmful effects of stress response activation in patients undergoing surgery for whom normal adaptive stress responses may be maladaptive, exacerbating physiological instability (e.g., by increasing protein catabolism or increasing the work of the heart). These stress responses can result in postoperative complications (see Table I), and can prolong recovery (Bryan-Brown, 1986; Phillips & Cousins, 1986; Cousins, 1989; O'Gara, 1988).

Research conducted over the past three decades has focused on developing more precise measures of nociceptive stress responses and on evaluating methods to reduce or prevent nociceptive stress responses associated with surgical injury in adults (Kehlet, 1986, 1989). Only within the past two decades has research on the measurement and attenuation of stress responses been extended to infants and children (Franck, 1992; Zeltzer, Barr, McGrath, & Schechter, 1992).

Measurement of neonatal stress responses is particularly problematic because of the lack of descriptive information about the phenomena, lack of appropriate instruments, and inability to determine the influence of concomitant neurological development on the nature and magnitude of nociceptive stress responses. Despite these obstacles, sufficient evidence now exists demonstrating that neonates perceive nociceptive stimuli associated with surgical injury and mount stress responses similar to, but not identical to, adults (Fitzgerald & Anand, 1993). In addition, limited data suggest that chronic or excessive nociceptive stress responses present a danger to critically ill neonates undergoing surgery, and that these stress responses may be attenuated with the use of opioid analgesics (Anand & Hickey, 1992). Unfortunately, clinically appropriate assessment parameters that can be used by clinicians at the neonate's

bedside do not exist at the present time to guide interventions to reduce the deleterious consequences of nociceptive stress responses in neonates.

Significance

Nociceptive stressors are prevalent in the neonatal intensive care unit (NICU) and can increase the risk of physiological compromise in the critically ill or premature neonate (Anand, 1990; Franck & Gregory, 1993). The identification of specific interventions to reduce the deleterious effects of nociceptive stressors represents an important area of research. Recognition of the adverse, potentially life-threatening sequelae of surgical stress in critically ill, premature neonates has prompted increased interest in the use of opioids to provide postoperative analgesia to these patients. However, despite evidence supporting the need to reduce neonatal stress responses, the inability to accurately evaluate the benefits and risks of opioid analgesia often results in under-medication or inappropriate use of these drugs in neonates (Franck & Gregory, 1993; Koren & Maurice, 1989).

Neonates lack the ability to use language to communicate their perception of nociceptive stimuli. Therefore, neonatal responses to nociceptive stimuli must be inferred from neuroendocrine, physiological, and behavioral indices. In contrast, the cardiorespiratory side-effects of opioids can be easily and accurately measured in this population, yet the prevalence and severity of opioid-induced side-effects have not been clearly established.

The current paucity of information about the analgesic efficacy and side-effects of opioid analgesia in neonates results from a lack of valid, reliable, and clinically useful instruments to quantify neonatal responses to nociceptive stimuli and to evaluate interventions to reduce nociceptive stress responses. This study evaluated the effects of a single intravenous (iv) dose of morphine on nociceptive stress responses of premature neonates following surgery using neuroendocrine,

physiological, and behavioral measures. In addition, the effects of bolus iv administration of morphine on the cardiorespiratory status of critically ill premature neonates was evaluated.

Purpose of the Study

The purpose of this study was to describe changes in plasma epinephrine (E) and norepinephrine (NE) levels, vagal tone index (VTI), flexor reflex threshold (FRT), and cardiorespiratory status following administration of a single iv dose of morphine to premature neonates within the first 12 hours following thoracotomy. A secondary purpose of the study was to describe the relationship between preoperative and postoperative or post-morphine levels of E, NE, and VTI.

Specific Aims

The following specific aims were evaluated in a sample of premature neonates undergoing a thoracotomy:

1. To determine changes in plasma E levels measured preoperatively, immediately before, and 20 minutes after bolus administration of the first iv dose of morphine given within the first 12 hours following thoracotomy.
2. To determine changes in plasma NE levels measured preoperatively, immediately before, and 20 minutes after bolus administration of the first iv dose of morphine given within the first 12 hours following thoracotomy.
3. To determine changes in VTI measured preoperatively, immediately before, 20 minutes after, and 1 hour after administration of the first iv dose of morphine given within the first 12 hours following thoracotomy.
4. To determine changes in FRT measured preoperatively, immediately before, 20 minutes after, and 1 hour after administration of the first iv dose of morphine given within the first 12 hours following thoracotomy.

5. To determine changes in cardiorespiratory status (i.e., heart rate, respiratory rate, blood pressure, and oxygen saturation) measured preoperatively, immediately before, 20 minutes after, and 1 hour after administration of the first iv dose of morphine given within the first 12 hours following thoracotomy.
6. To determine the relationships between plasma E and NE levels, VTI, and FRT at the preoperative baseline, pre-morphine, 20 minutes post-morphine, and 1 hour post-morphine (VTI and FRT only) measurement time points;
7. To determine the relationship between preoperative plasma E and NE levels and the degree of change in plasma E and NE levels following surgery and before and after morphine administration.
8. To determine the relationship between preoperative VTI and the degree of change in VTI following surgery and before and after morphine administration.

Assumptions

The assumptions underlying this study were as follows:

1. Surgical injury is nociceptive and induces stress responses.
2. Postoperative pain is a stressor and induces stress responses.
3. Premature neonates are capable of responding to nociceptive stimuli.
4. Premature neonates demonstrate stress responses to surgical injury.
5. Stress responses can be measured in premature neonates through neuroendocrine, physiological, and behavioral parameters.
6. The amount of anesthesia administered during surgery attenuates the intraoperative stress responses associated with the thoracotomy procedure.

Definition of Terms

For the purposes of this study, the following definitions were used:

1. Premature neonate. A male or female newborn, less than 37 weeks gestation at birth and less than 30 days postnatal age at the time of surgery.
2. Thoracotomy. A surgical incision into the chest wall performed under general anesthesia, most commonly for the purpose of ligating the ductus arteriosus (PDA).
3. Vagal tone index (VTI). An index of the influence of the right vagus on the sinoatrial node using the method of Porges (1985).
4. Flexor reflex threshold (FRT). The minimal force required to elicit a withdrawal reflex of the leg by applying graduated von Frey filaments to the plantar surface of the foot.
5. Plasma epinephrine (E) levels. The plasma concentration (in nmol/L) of epinephrine from arterial blood, measured by high performance liquid chromatography with electrochemical detection (HPLC-ED).
6. Plasma norepinephrine (NE) levels. The plasma concentration (in nmol/L) of norepinephrine from arterial blood, measured by HPLC-ED.
7. Bolus administration of iv morphine. The injection of a dose of morphine over 5 to 10 minutes through the injection port of the iv tubing.
8. Heart Rate (HR). The number of contractions of the cardiac ventricles per minute, as measured by a cardiorespiratory monitor.
9. Respiratory Rate (RR). The number of breaths (including inspiration and expiration) per minute, as measured by a cardiorespiratory monitor. The respiratory rate includes spontaneous breaths and breaths from mechanical ventilation.

10. Blood pressure (BP). Quantification of the pressure of the blood against the walls of blood vessels during the systolic (the contraction phase) and diastolic (the relaxation phase) phases of the cardiac cycle, as measured by direct pressure transduction and displayed on a cardiorespiratory monitor.

11. Oxygen saturation (SaO_2). The ratio of hemoglobin saturated with oxygen (oxyhemoglobin) to the total hemoglobin available for binding oxygen (%), as measured transcutaneously by a Nellcor N200 pulse oximeter.

CHAPTER 2

LITERATURE REVIEW

Introduction

This chapter reviews the relevant literature on the mechanisms of adult and neonatal nociceptive stress responses and the attenuation of nociceptive stress responses by opioid analgesics. Neuroendocrine, physiological, and behavioral responses to surgical injury in adults are described first, followed by a discussion of the use of opioids to attenuate nociceptive stress responses associated with surgical injury in adults. Neonatal responses to nociceptive stressors are then discussed, prefaced by a description of the anatomical prerequisites for nociception. Similarly, the following section on opioid administration in neonates is preceded by a review of the development of opioid responsiveness and basic opioid pharmacokinetics in neonates. Factors that contribute to the variability in neonatal stress responses are highlighted.

A critical analysis of the research on neonatal nociceptive stress responses and effects of opioids presented in this chapter suggests that major refinements in the instrumentation used to measure neonatal stress responses are needed before further research on interventions to modify stress responses can be accurately evaluated for risks and benefits. Several measures that appear promising for evaluation of neonatal nociceptive stress responses are identified.

Manifestations and Attenuation of Nociceptive Stress Responses in Adults

Neuroendocrine, Physiological, and Behavioral Responses in Adults

Nociceptive stimuli associated with surgical injury evoke a multitude of non-specific neuroendocrine, physiological, and behavioral stress responses. Nociceptive information is relayed from peripheral and visceral nociceptors along

afferent neural pathways. The release of local mediators at the site of tissue injury serves to amplify afferent nociceptive transmission and also directly evokes hormonal responses, reaching the central nervous system (CNS) through the circulatory system. Nociceptive input reaches sites in the medulla, hypothalamus, and thalamus through the lateral and medial spinothalamic tracts and the spinoreticulothalamic tract. The spinoreticulothalamic tract is thought to play the greatest role in activation of hormonal responses to nociception (Cepeda & Carr, 1993). Physiological and behavioral manifestations of nociceptive stress responses are then observable.

Measurement of Neuroendocrine Responses to Nociceptive Stressors in Adults

Activation of the hypothalamic-pituitary-adrenal axis (HPA) and the sympathoadrenomedullary system occurs through the release of corticotropin releasing hormone (CRH) from the hypothalamus and NE from the locus ceruleus. Adrenocorticotropic hormone (ACTH) and beta-endorphin secretion is stimulated by CRH and begins the cascade of hormonal responses commonly observed in the classic "fight or flight" response to stress. Autonomic nervous system (ANS) activation, generally sympathetic stimulation and parasympathetic inhibition, serves to mobilize energy substrates and promote adaptation to stressors through mechanisms that are anti-anabolic, catabolic, and immunosuppressive (Table I; see Asterita, 1985; Cepeda & Carr, 1993; Chrousos & Gold, 1992; Munck, Guyre, & Holbrook, 1984; Weissman, 1990 for reviews).

The catecholamines E and NE are believed to be the primary mediators of the sympathetic response to stress (Schmeling & Coran, 1991; Slotkin & Seidler, 1989) and are responsible for many of the acute physiological and behavioral manifestations of stress, providing the most direct indication of the extent and

magnitude of stress responses. Cortisol and other steroid hormones are thought to augment or moderate rather than initiate stress responses (Schmeling & Coran, 1991; Wilmore, 1976) and specifically amplify or inhibit catecholamine effects through counter-regulatory mechanisms that are complex and not well understood (Munck et al., 1984).

Norepinephrine and E, provide the "first line of defense" against nociceptive stressors. Norepinephrine is immediately released from sympathetic nerve endings following perception of a nociceptive stressor and spills over into the systemic circulation where it exerts primarily alpha adrenergic effects on target tissues. Epinephrine, and smaller amounts of NE, are released approximately 30 seconds later from the chromaffin cells of the adrenal medulla. Epinephrine has almost equal alpha and beta adrenergic effects on target tissues. Both E and NE produce rapid effects but degenerate quickly due to reuptake or enzymatic degradation. Although the half life of catecholamines (injected) in the circulation is approximately 20 seconds, high plasma levels can be achieved when the release of catecholamines overwhelms the mechanisms for catecholamine inactivation. Conversely, tachyphylaxis and/or depletion of catecholamine stores can occur during stress. Other hormones (e.g., glucocorticoids and thyroid hormone) are needed to sustain or prolong the physiological effects of catecholamines (Tepperman & Tepperman, 1991).

In the normal resting adult, plasma levels of E and NE are approximately 0.27 nmol/L and 1.18 nmol/L, respectively (Asterita, 1985; Tepperman & Tepperman, 1991). Surgical stress can elevate plasma E and NE levels many-fold in proportion to the severity and site of surgery, with thoracic and deep abdominal procedures evoking greater increases than peripheral or superficial procedures (Chernow et al., 1987). Peak plasma E and NE levels usually occur in

the immediate postoperative period (Halter, Pflug, & Porte, 1977; Nistrup-Madsen, Fog-Moller, Christiansen, Vester-Andersen, & Engquist, 1978; Stanley, Berman, Green, & Robertson, 1980). A positive correlation between severity of injury and catecholamine levels has also been demonstrated in trauma patients (Davies, Newman, Molyneux, & Grahame-Smith, 1984; Frayn, Little, Maycock, & Stoner, 1985; Jaattela et al., 1975). In Intensive Care Unit (ICU) patients, E and NE levels were found to be four-fold higher than in healthy adults (Wortsman, Frank, & Cryer, 1984). An acute stressor, such as cardiac arrest, superimposed upon the chronic stress of the ICU environment, further increased E and NE levels up to 32-fold higher than levels in stable ICU patients (Wortsman et al., 1984).

Preoperative psychological and physical stressors are believed to influence intraoperative and postoperative neuroendocrine responses although the exact nature and underlying mechanisms are poorly understood. For example, two studies (Stanley, Isern-Amaral, & Lathrop, 1975a; Stanley, Isern-Amaral, & Lathrop, 1975b) demonstrated a small rise in urinary NE concentrations after induction of morphine anesthesia in patients with low preoperative urinary NE levels, while no rise or even decreased levels were seen in patients with high preoperative urinary NE levels.

Measurement of Physiological Responses to Nociceptive Stressors in Adults

Physiological changes associated with nociception following surgical injury are best described in terms of cardiovascular and respiratory effects. Cardiovascular and respiratory alterations are of particular interest due to: 1) the functional interaction between pain and cardiovascular regulation (Zamir & Maixner, 1986); 2) the marginal cardiovascular and respiratory function in the critically ill patient and the potential for further compromise related to noxious

stimulation; and 3) the potential for cardiovascular and respiratory compromise related to the interventions used to modulate the effects of a nociceptive stressor.

Cardiorespiratory changes occur following increases in plasma E and NE levels because of stimulation of alpha and beta adrenergic receptors located in the heart and lungs. Cardiovascular effects of nociceptive stress include arterioconstriction and vasoconstriction (alpha), increased myocardial contractility (beta 1), increased heart rate (beta 1), and selective vasodilation of coronary arteries and skeletal muscle (beta 2). Respiratory effects of nociceptive stress include increased respiratory rate, increased minute volume, and dilation of the bronchioles (beta 2; Asterita, 1985). The increased metabolic demands on the cardiovascular and respiratory systems caused by nociceptive stress can lead to increased oxygen consumption, hypoxemia, and myocardial ischemia in the patient with compromised cardiovascular and respiratory function (O'Gara, 1988; Phillips & Cousins, 1986).

The parasympathetic system is generally inhibited during the acute phases of the nociceptive stress response, altering or "resetting" the baroreceptor reflex such that both heart rate and blood pressure are elevated (Cepeda & Carr, 1993; Randall, 1991). However, in some individuals or situations, parasympathetic arousal occurs parallel to (or may predominate) sympathetic arousal, leading to decreased blood pressure, syncope, and bronchoconstriction through activation of the vagus nerve (Asterita, 1985; Carruthers & Taggart, 1973; Bohus et. al., 1987; Gellhorn, 1968).

Quantification of short term heart rate variability (HRV) and estimates of cardiac vagal tone have been used to measure sympathetic and parasympathetic influences on cardiac function (see van Ravenswaaij-Arts, Kollee, Hopman,

Stoelinga, & van Geijn, 1993 for review). Estimates of cardiac vagal tone can be derived most simply by calculating the standard deviation of beat-to-beat R-wave interval differences within a specified time period. More recently, techniques for spectral analysis have been used to permit the study of frequency-specific oscillations. Low frequency heart rate fluctuations (0.01 to 0.04 Hz) are thought to represent thermoregulatory blood flow adjustments mediated by the sympathetic nervous system. Mid-frequency heart rate fluctuations (0.04 to 0.20 Hz) represent baroreceptor activity that is believed to be under both sympathetic and parasympathetic control. High frequency (greater than 0.20 Hz) short-term heart rate fluctuations represent changes in heart rate related to respiration. The respiratory sinus arrhythmia (RSA) is a periodic process defined as the variation in heart period pattern associated with spontaneous respiration, specifically, acceleration during inspiration and deceleration during expiration (Porges, 1991, 1992; van Ravenswaaij-Arts, 1993).

One measure of RSA, vagal tone index (VTI), employs both frequency and time series techniques to estimate RSA (Porges & Borher, 1990). Pharmacological studies with drugs known to have vagal effects have demonstrated the validity of Porges' VTI method in adult humans and animals (Dellinger, Taylor, & Porges, 1987; Porges, 1988; Porges, McCabe, & Yongue, 1982; Yongue et al., 1982). Furthermore, the VTI method has been shown to be highly correlated with other methods used to estimate vagal tone (Grossman, 1992).

Vagal cardioinhibitory neurons are believed to originate in the medulla and are regulated by the central inspiratory drive, although they are also influenced by activation of intrapulmonary receptors during lung inflation (Potter, 1981; Spyer, 1988). Transmission of efferent activity to the sino-atrial node via cardioinhibitory neurons is mediated by the release of acetylcholine. Respiratory

sinus arrhythmia occurs because of inspiratory inhibition of parasympathetic vagal efferent activity and concomitant attenuation of the baroreceptor reflex (van Ravenswaaij-Arts et al., 1993).

Sympathetic influences are less important in regulating beat-to-beat variability in heart rate because of the 2 to 3 second delay between receptor activation and end-organ stimulation (Hill-Smith & Purves, 1978; Karemaker, 1987). This finding may explain the lack of relationship between changes in cardiac vagal tone and plasma catecholamine levels following cold pressor testing found by Goldstein et al. (1994).

Cardiac vagal tone decreases during emotional and physical stressors (Allan & Crowell, 1989). However, no specific studies have evaluated the influence of discreet nociceptive stressors on cardiac vagal tone in adults. Decreased vagal tone has been shown to be predictive of poorer outcomes in adults following neurosurgery, with cardiovascular disease, and with diabetes (Donchin, Constanti, Szold, Byrne, & Porges, 1992; Katona & Jih, 1975; Porges, 1983). Cardiac vagal tone measures have also been used in adults to evaluate the effects of anesthetics and opioids (see below).

Measurement of Behavioral Responses to Nociceptive Stressors in Adults

Behavioral responses to nociceptive stress following surgical injury have not been well described. Critically ill adults in the postoperative period exhibit both aroused and depressed behaviors, including: restlessness and increased muscle tension or immobilization; guarding/splinting; sleeplessness; and/or vocalization/verbalization (Cousins, 1989; Johnson & Sexton, 1990; Sternbach, 1989). These behaviors may be maladaptive for the postoperative patient, impairing respiratory effort and preventing early ambulation and sleep.

A discrete behavioral response to noxious stimuli is observable in both

humans and animals. This specific nociceptive flexor reflex occurs following stimulation of RIII (a-delta) afferent fibers of a cutaneous nerve of a lower limb by electrical stimulation. The intensity of the stimulus and the flexor reflex threshold are both linearly correlated with self-reports of pain sensation (Bromm & Treede, 1980; Chan & Dallaire, 1989; Willer, Roby & LeBars, 1984). Nevertheless, there are situations in which the flexor reflex and self-report of pain sensation may be dissociated (Coda, 1989; Price, 1989), or in which the flexor reflex response is contaminated by other factors such as a startle response (Dowman, 1992). Despite the potential importance of the measure as a more objective behavioral correlate of nociception and analgesic responsiveness, it involves nociceptive electrical stimulation and, therefore, is not appropriate for use in critically ill patients.

Opioid Attenuation of Nociceptive Stress Responses in Adults

Systemic administration of opioids is a common strategy used to alter the perception of nociceptive stimuli and to attenuate the neuroendocrine, physiological, and behavioral nociceptive stress responses of adults (Benedetti, 1990; Cousins, 1989; Hansen-Flaschen, Brazinsky, Basile, & Lanken, 1991; Kehlet, 1986; Weissman & Hollinger, 1988; Yeager, Glass, Neff, & Brinck-Johnson, 1987). Opioids are administered in anesthetic doses intraoperatively and in analgesic doses during the postoperative period.

Opioid Effects on Neuroendocrine Nociceptive Stress Responses in Adults

The effects of systemically administered opioids appear to be centrally mediated at sites within the medulla oblongata (Laubie & Schmitt, 1983). Opioids are believed to reduce the nociceptive input reaching the hypothalamus, inhibit CRH release from the hypothalamus and NE release from the locus ceruleus, resulting in a dose dependent suppression of the hormonal response to surgical

injury (Jaffe & Martin, 1990; Stanley et al., 1980; Walsh, Paterson, O'Riordan, & Hall, 1981).

Conflicting data exist on the effects of opioids on catecholamine release and other hormonal stress responses. Because of the principle role that endogenous opioids play in both the activation and modulation of neurohormonal stress responses, the effects of exogenous opioids on stress responses are context specific and may appear contradictory. For example, exogenous opioids have excitatory effects when administered to resting subjects, but inhibitory effects when administered during stress (Carr & Murphy, 1988).

Several studies have shown significant decreases in the magnitude of the surgical stress response, as measured by plasma E and/or NE levels, during the intraoperative period when high dose fentanyl anesthesia is used compared to inhalation anesthesia (Campbell, Parikh, Naismith, Sewnauth, & Reid, 1984; Kono et al., 1981; Sebel, Bovill, Schellekens, & Hawker, 1981; Stanley et al, 1980). Others (Fahmy, Sunder, & Soter, 1983), reported no changes in plasma NE levels and increases in plasma E levels following the administration of morphine during anesthesia induction, suggesting activation of the adrenal medulla by histamine. Still others (Hicks, Mowbray, & Yhap, 1981) reported an increase in catecholamine levels following anesthesia induction using a 15 mcg/kg fentanyl dose. As the dose was increased to 50 mcg/kg, catecholamine levels returned to preoperative baseline levels.

Postoperatively, opioid analgesia does not completely abolish neuroendocrine responses (Kehlet, 1984; Schulze, Roikjaer, Hasselstrom, Jensen, & Kehlet, 1988) and decreases in catecholamine levels as a result of opioid analgesia appear to be transient. Elevated levels of catecholamines can persist in the postoperative period despite complete pain relief reported by patients

receiving either intermittent iv morphine or fentanyl patient controlled analgesia (Moller, Dinesen, Sondergard, Knigge, & Kehlet, 1988; Scheinin et al., 1987).

Factors that may affect E and NE responses to surgical stress include the choice of anesthetic agent, failure to achieve adequate anesthesia, individual differences in sympathetic responsiveness, individual differences in preoperative stress level, underlying cardiac disease, use of cardiopulmonary bypass, and route and interval of postoperative opioid administration (Bovill et al., 1984; Cepeda & Carr, 1993; Kehlet, 1989).

Opioid Effects on Physiological Nociceptive Stress Responses in Adults

The physiological effects of anesthesia and postoperative analgesia on the cardiovascular and respiratory systems have been described. In animal models, both fentanyl and morphine have been shown to selectively reduce the sympathetic tone and increase the parasympathetic tone of the cardiovascular system (Feldberg & Wei, 1977, 1978; Laubie, Schmitt, Canellas, Roquebert, & Demichel, 1974; Roscow, Moss, Philbin, & Savarese, 1982). Although reduced sympathetic tone decreases the work of the heart, vasodilation and ventilatory depression may stimulate increased release of catecholamines and other hormones (Bovill et al., 1984). Morphine-associated bradycardia and hypotension are believed to be vagally mediated (Laubie & Schmitt, 1983). However, in anesthetized dogs, cardiovagal fibers were preferentially activated by fentanyl (Inoue, Samodelov, & Arndt, 1980). Vagal tone was maintained in dogs during morphine anesthesia but decreased by halothane or thiopental anesthesia (Halliwill & Billman, 1992).

The limited data from human studies contradict findings from animal studies (Kato et al., 1992; Koltry, Ebert, Vucins, Roerig, & Kampine, 1984; Latson & O'Flaherty, 1993). An overall decrease in HRV with shifts in sympathetic-

parasympathetic balance has been shown to occur during inhalation anesthesia (Kato et al., 1992; Latson & O'Flaherty, 1993). An interesting phenomenon of reversed RSA (i.e., heart rate decrease with mechanical lung inflation in contrast to heart rate increase with normal inspiration) has been reported in mechanically ventilated adults receiving inhalation anesthesia (Yli-Hankala, Porkkala, Kaukinen, & Hakkinen, 1991). Reversed RSA may occur due to the direct stimulation of the aortic baroreceptors by the force of the mechanical breaths (van Ravenswaaij-Arts, 1993). While morphine anesthesia has also been shown to depress baroreflex responses (Koltry et al., 1984), no studies have specifically evaluated cardiac vagal tone responses to opioid anesthesia.

Administration of analgesic doses of morphine resulted in a vagally mediated tachycardia (Newlin, Wong, & Cheskin, 1992; Pretorius, Wong, & Newlin, 1990). However, no studies have evaluated the effects of opioids on vagal tone in the presence of nociceptive stressors.

Discrepancies in the limited available data may be related to the absence of consistent experimental models; differences in drug, route, and species; and the fact that cardiovascular changes associated with opioids may represent both pressor and depressor effects related to both the central and peripheral actions of the drugs (Willette & Sapru, 1982).

Administration of opioids may produce ventilatory depression consisting of decreased minute volume, tidal volume, ventilatory drive, as well as hypercapnia and decreased ventilatory response to hypoxia and hypercarbia (see Shook, Watkins, & Camporesi, 1990 and Stanley, 1981 for review).

Opioid Effects on Behavioral Nociceptive Stress Responses in Adults

Opioids can alter behavior by altering the perception of and tolerance for postoperative nociceptive stimuli (Jaffe & Martin, 1990; Martin, 1983). For

example, the patient who has received an opioid analgesic may more easily perform therapeutic activities such as deep breathing, ambulation, or sleep and potentially expedite discharge (Ready, 1990).

The flexor reflex response and pain sensation decrease linearly following: 1) activation of large diameter cutaneous fibers that respond to non-painful tactile (RII, a-beta) stimulation (Willer, 1977); 2) stress-induced stimulation of descending pathways (Willer, Dehen, & Cambier, 1981); or 3) by administration of an opioid (Bromm & Seide, 1982). Decreases in flexor reflex response and pain sensation following morphine administration are dose-dependent and naloxone reversible (DeBroucker, Willer, & Bergeret, 1989; Willer & Bussel, 1980; Willer, 1985). Only one study (Willer, Bergeret, & Gaudy, 1985) describes the use of the flexor reflex response to measure the effect of morphine in patients with clinical pain. Flexor reflex threshold to electrical stimulation was measured following epidural administration of morphine in patients with postoperative pain. Significant inhibition of the reflex corresponded with self-reports of decreased pain. Report of pain relief coincided with a 40% or greater decrease in reflex threshold, beginning 25 to 30 minutes following injection.

Summary

The neuroendocrine stress responses evoked by nociceptive stimuli occurring during surgery are manifest as physiological and behavioral alterations in adult humans. Although the purpose of these complex responses is mobilization of energy stores in order to respond to perceived stressors, the resultant catabolism, hyperglycemia, and behavioral distress can be maladaptive for the critically ill patient (Table I). Thus, effective attenuation of nociceptive stress responses is an important clinical issue. Systemic administration of opioids reduces perception of and may attenuate (but not abolish) neuroendocrine stress

responses to nociceptive stressors in adults. However, many factors influence the degree to which nociceptive stress responses are modified by either intraoperative anesthesia or postoperative analgesia. Anesthesia and analgesia related side-effects, including cardiorespiratory compromise, may also occur. Maintaining a delicate balance between attenuation of nociceptive stress responses and the deleterious side-effects associated with the administration of opioid analgesics requires accurate measures of the neuroendocrine, physiological, and behavioral responses associated with nociceptive stress and antinociceptive interventions as well as a greater understanding of the factors that influence stress responses.

Manifestations and Attenuation of Nociceptive Stress Responses in Neonates

The ability of neonates to mount stress responses has only recently been recognized (Fitzgerald & Anand, 1993; Franck & Gregory, 1993). In addition, the potential negative effects of nociceptive stressors on the clinical condition of full-term and preterm neonates are beginning to receive attention (Anand, 1990; Gottfried & Gaiter, 1985; Gunnar, Connors, Isensee, & Wall, 1988; Slotkin & Seidler, 1988). A focus on nociceptive stimuli as sources of stress for neonates in the NICU is important because neonates receiving intensive care are exposed to repeated nociceptive stimuli over a prolonged period of time (Barker & Rutter, 1995).

Neonatal responses to nociceptive stressors are determined by the degree of neurodevelopment, particularly of the sensory pathways. The interaction between the developing neonate and the external environment further influences maturation of the CNS. A critical analysis of the research on the development, expression, and attenuation of neonatal nociceptive stress responses by opioid

analgesia is presented in the following sections. The specific aspects of neonatal development that influence stress responses and opioid responsiveness in premature neonates are highlighted.

Anatomical Prerequisites of Nociception

Intact sensory and perceptual systems are necessary to elicit stress responses to nociceptive stimuli. The inability to detect a potentially tissue-injuring stimulus or to perceive it as noxious precludes activation of stress response systems. Knowledge of the neuroanatomy of the human fetus and neonate and direct observations of the neonate's responses to nociceptive stressors provide strong evidence that a functional nociceptive system develops early in gestation (see Anand, 1990 and Fitzgerald & Anand, 1993 for reviews). The peripheral, spinal, and central structures necessary for transmission of nociceptive (and non-nociceptive) stimuli are present and functional between the first and second trimesters. For example, development of sensory receptors in all cutaneous surfaces is complete by 20 weeks gestation (Humphrey, 1964). Development of the spinal cord, including laminar ordering, synaptic connections, and neurotransmitter vesicles, begins before 13 to 14 weeks of gestation and is completed by 30 weeks (Rizvi, Wadhas, & Bijlani, 1987). The fetal cortex has its full complement of neurons by 20 weeks of gestation (Marin-Padilla, 1983) and complete synaptogenesis of the cortex rapidly occurs within 24 weeks of gestation (Rakic & Goldman-Rakic, 1982; Kostovic & Goldman-Rakic, 1983). Thus, newborn infants, even premature neonates 25 to 27 weeks of gestation, appear to possess the anatomic components necessary to perceive and respond to nociceptive stimuli.

Studies using animal models have provided further elaboration of the functional development of the peripheral (Fitzgerald, 1987) and central nervous

systems (Fitzgerald, 1988, 1991a; Ralston, 1984). These studies support the assertion of an early maturation of these systems in the human fetus. They also suggest that there may be differences in synaptic transmission and receptive fields resulting in the increased sensitivity of neonates to nociceptive and non-nociceptive stimuli as compared with adults (Fitzgerald, 1991a, 1991b).

The increased sensitivity of premature neonates to most sensory stimuli has been well documented (Gorski, Hole, Leonard, & Martin, 1987). Sensory stimuli, such as light and noise in the NICU, which are perceived as non-nociceptive in the adult or older child, appear to be nociceptive in the premature neonate and elicit dramatic physiological and behavioral stress responses (Grauer, 1989; Long, Lucey, & Philip, 1980). In addition, handling and care giving procedures cause significant physiological distress to the premature neonate, including blood pressure and heart rate instability as well as hypoxia (Long et. al., 1980; Norris, Campbell, & Brenkert, 1982; Perry et al., 1990; Porter, Miller, Cole, & Marshall, 1991). The cardiorespiratory effects of handling may result from increased circulating catecholamines, particularly NE, and cortisol (Gunnar, Connors, & Isensee, 1989; Lagercrantz, Nilsson, Redham, & Hjemdahl, 1986). For the premature neonate, social interaction (e.g., the human voice and face) can cause physiological and behavioral distress similar in magnitude to that occurring with chest physiotherapy (Gorski et al., 1983).

Neuroendocrine Responses to Nociceptive Stressors in Neonates

Neonatal neuroendocrine stress responses develop early in gestation and consist of complex patterns of neural activity and hormone release similar to what is observed in adults. Plasma NE has been detected as early as 16 weeks gestation and levels increase significantly with increasing gestational age. Epinephrine is produced by the fetus by 23 weeks of gestation (Greenough,

Nicolaides, & Lagercrantz, 1990). Increases in E, NE, cortisol, growth hormone, and endorphins, as well as suppression and/or increased peripheral resistance to insulin are commonly observed in both the fetus and neonate in response to stressors (Schmeling & Coran, 1991). Perhaps the most significant activation of neonatal neuroendocrine responses occurs with the transition from the intrauterine to the extrauterine environment during birth.

Neuroendocrine Response to Birth

Birth is a significant stressor that clearly demonstrates the ability of the fetus to mount a neuroendocrine stress response. At birth, plasma catecholamine levels, predominantly NE, greatly exceed those of normal or stressed adults (Bistoletti, Nylund, Lagercrantz, Hjemdahl, & Strom, 1983; Faxelius, Lagercrantz, & Yao, 1984; Lagercrantz & Bistoletti, 1973; Slotkin & Seidler, 1988) and then rapidly decrease to near resting adult levels by 12 hours of age (Eliot, Lam, Leake, Hobel, & Fisher, 1980). Non-neurogenic mechanisms unique to the neonate (i.e., extra-adrenal chromaffin cells) contribute to the surge of catecholamines that occurs with birth or hypoxic insult (Slotkin & Seidler, 1988; 1989). It has been proposed that the catecholamine surge at birth prepares the neonate for extrauterine life, promoting respiratory, cardiovascular, and metabolic adaptation (e.g., thermogenesis, resorption of lung water, etc.) to the demands of a new environment (Phillippe, 1983; Slotkin & Seidler, 1988).

Neonates who were asphyxiated at birth with a poor prognosis had significantly lower cerebrospinal fluid (CSF) NE levels compared to normal neonates. Asphyxiated neonates with a good prognosis had significantly higher NE levels in CSF than normal neonates (Blenow, Zeman, Dahlin, & Lagercrantz, 1994). Cocaine-exposed neonates also demonstrate significantly higher plasma NE levels, but no difference in plasma E levels compared to normal neonates

(Mirochnick et al., 1994).

The catecholamine response to birth may be greater in premature neonates than in full-term neonates (Newnham et al., 1984). Recent data suggest that E and NE levels remain elevated longer after birth in premature neonates, due to either slower clearance or because of the continued stress of the NICU environment (Mehandru, Assel, Johnston, Fanaroff, & Kalhan, 1991). In another study of premature neonates less than 5 days of age in the NICU (Nitsche et al., 1994), plasma E and NE levels varied widely and were not affected by catecholamine infusion (i.e., dopamine or dobutamine). One small study of NICU neonates demonstrated a correlation between blood pressure waves and NE levels (drawn at the peak and the trough of the waves) suggesting that the peak of the wave is associated with an NE surge (McIntosh, Stephen, Smith, & Cunningham, 1994).

Neuroendocrine Response to Surgical and Non-Surgical Stressors in Neonates

Surgical procedures performed on premature neonates, such as thoracotomy for PDA ligation, induce dramatic hormonal and metabolic stress responses (Anand, Brown, Causon, et al., 1985; Anand & Aynsley-Green, 1985) that may contribute to postoperative morbidity and mortality (Anand, Sippell, & Aynsley-Green, 1987; Anand & Aynsley-Green, 1988a). In one study (Anand, Brown, Causon, et al., 1985), plasma E and NE levels increased dramatically by the end of surgery but returned to baseline levels within 6 hours postoperatively. Although catecholamine levels may not remain elevated for as many hours postoperatively in the neonate as compared with the adult, the increase is believed to play a significant role in stimulating the extreme metabolic aberrations noted postoperatively in neonates (Anand, Brown, Bloom, & Aynsley-

Green, 1985).

Norepinephrine and E levels are also closely correlated with the intensity and duration of the surgical stimuli (Anand & Aynsley-Green, 1988b) and the catecholamine response to surgical stress appears similar in both preterm and full-term neonates (Anand, Brown, Causon, et al., 1985). However, increases in catecholamine levels are not specific to surgical stressors. Other noxious procedures such as chest physiotherapy and endotracheal suctioning are also associated with large increases in plasma E levels and smaller increases in NE levels (Greisen, Fredericksen, Hertel, & Christiansen, 1985). Hypoxia and acidosis also lead to substantial increases in plasma E and NE levels in neonates (Cabal et al., 1985; Greenough, Lagercrantz, Pool, & Dahlin, 1987).

Table II summarizes the results of studies that have measured E and NE levels in premature and full-term neonates following nociceptive and non-nociceptive stressors (Anand, Brown, Bloom, et al., 1985; Anand, Brown, Causon, et al., 1985; Anand et al., 1987; Anand & Aynsley-Green, 1988a; Anand & Aynsley-Green, 1988b; Anand & Hickey, 1992; Baumgartner, Ritsch, Luz, Schneeberger, & Hammerer, 1992; Blenow et al., 1994; Cabal et al., 1985; Greenough et al., 1987; Greisen et al., 1985; Lagercrantz et al., 1986; McIntosh, et al., 1994; Mirochnick et al., 1994; Nitsche et al., 1994; Quinn et al., 1992). Although neonates clearly demonstrate catecholamine responsiveness to nociceptive stressors, there is marked variability in both the baseline values and the degree of change in plasma E and NE levels associated with nociceptive stress. The sources of the variability in responses have not been identified.

The measurement of catecholamines in neonates remains limited by difficulties in obtaining blood samples, as well as the lack of assay availability in most hospitals. Despite these limitations, measurement of E and NE levels appear

to be the most sensitive and reliable method for determining the neonate's neuroendocrine response to nociceptive stressors.

Physiological Responses to Nociceptive Stressors in Neonates

Profound physiological changes in all major organ systems occur as a result of the neonate's neuroendocrine responses to nociceptive stressors. The cardiovascular and respiratory effects of stress are of great clinical significance and have been most thoroughly studied in neonates immediately after birth (Faxelius et al., 1984; Slotkin & Seidler, 1988). The major cardiovascular manifestation of the neonatal stress response is tachycardia. Regulation of heart rate is the most effective mechanism available to improve blood flow to vital organs, since the neonatal myocardium has a limited ability to increase contractility as compared to the adult (Friedman, 1972; Teitel et al., 1985). Respiratory effects of neuroendocrine stress response activation include increased lung compliance, increased bronchiolar dilation, resorption of lung fluid, and release of surfactant (Slotkin & Seidler, 1989).

Measurement of neonatal physiological responses to nociceptive stimuli include changes in heart rate, respiratory rate, blood pressure, transcutaneous oxygen and carbon dioxide levels, oxygen saturation, intracranial pressure, cardiac vagal tone, and palmar sweat (Harpin & Rutter, 1983; Porter, Porges, & Marshall, 1988; Rawlings, Miller, & Engel, 1980; Schwartz & Jeffries, 1990; Stevens & Johnston, 1994). Research evaluating physiological responses to nociceptive stimuli has generally included measurement of multiple physiological parameters. However, the accuracy and sensitivity of these measures, particularly in the ill or premature infant, have been difficult to establish due to the influence of other non-nociceptive stimuli (Cabal, Siassi, & Hodgman, 1992).

In contrast to adults, short-term HRV has been used extensively as a

measure of stress responses in the fetus and newborn. Loss of fetal HRV is a sensitive but not specific indicator of fetal compromise (Maulik, Saini, & Zigrossi, 1983). Loss of fetal HRV is associated with fetal compromise and poor outcome (Huey, Paul, Hadjiev, Jilek, & Hon, 1979; Martin, Siassi, & Hon, 1974). Reliable methods for quantifying HRV and identification of RSA within the fetal heart rate are recent developments (Brown, Gee, Olah, Docker, & Taylor, 1992; Groome, Mooney, Bentz, & Singh, 1994; Swartjes, van Geijn, Mantel, & Schoemaker, 1992). Functional development of the autonomic control of heart rate is believed to change during gestation with sympathetic tone predominating earlier in gestation and parasympathetic tone predominating later in gestation (near term), and in the early neonatal period (Assali, Brinkman, Woods, Dandavido, & Nuwayhid, 1977). The period between 28 to 29 weeks gestation may be the age at which control of fetal heart rate changes from predominantly sympathetic to parasympathetic (Yoshizato et al., 1994).

Vagal tone changes have been anecdotally described during delivery (Porges, 1991), with decreased vagal tone occurring during the more stressful phases of delivery (i.e., during maximum dilation and delivery of the head). Vagal tone returns to baseline at the completion of delivery and progressively increases over the first 24 hours after birth.

In preterm neonates, short-term HRV increases as gestational and postnatal age increase (Fox, 1983; Katona, Frasz, & Egbert, 1980). Factors associated with persistent decreases in short-term HRV include respiratory distress syndrome (RDS), intraventricular hemorrhage (IVH), and sedative administration (Hornchen, Betz, Kotlarek, & Roebruck, 1983; Prietsch, Knoepke, & Obladen, 1994; van Ravenswaaij-Arts et al., 1991), possibly indicating higher sympathetic tone (Fox, 1983). Short-term HRV was inversely correlated with

PCO_2 in 16 neonates (mean gestational age 35.3 weeks) with RDS (Aarimaa, Kero, & Valimaki, 1985). The degree of HRV also changes with sleep state, with greater RSA seen in full-term neonates during quiet sleep with a regular breathing pattern (Thompson, Brown, Gee, & Taylor, 1993). In contrast, between-state differences in HRV were not seen in 12 premature neonates reaching normal term (Eiselt et al., 1993).

Vagal tone index (Porges, 1985) is normally distributed in healthy full-term neonates, with a mean of 3.8 and standard deviation of 1.2 $ln/msec^2$. Vagal tone index is decreased in preterm neonates less than 32 weeks gestation and in ill neonates as compared with healthy full-term neonates (Porges, 1983, 1988, 1992). Ill neonates (both full-term and premature neonates at term) appear uniformly to have lower VTI than normal neonates (Porges, 1992), with a mean VTI less than 2 $ln/msec^2$. Vagal tone index appears to be normally distributed in premature neonates receiving intensive care, ranging from 0.75 to 2.5 $ln/msec^2$ (Porges, 1988; 1992). The correlation between VTI and gestational age reported in two studies (Porges, 1988; Porges et al., 1982) was .70 and .82, respectively. The correlation decreased to .40 when only neonates less that 36 weeks gestation were included (Porges, 1983, 1988).

Pancuronium bromide and chloral hydrate have been reported to eliminate VTI in ill neonates (Porges, 1991). Decreased VTI was observed in preterm neonates in response to both gavage feeding and handling (DiPietro, Cusson, Caughy, & Fox, 1994). The decreases in VTI were not moderated by the provision of a pacifier during the gavage feeding.

Vagal tone index appears sensitive to differences in stimulus intensity and may discriminate between nociceptive and non-nociceptive stress responses in neonates. For example, in full-term neonates, decreases in VTI following

restraint were smaller than the decreases following circumcision (Porter et al., 1988). In addition, pre-circumcision VTI was predictive of the magnitude of the change in VTI following the procedure whereas heart period was not. Neonates with higher baseline VTI values had larger decreases in VTI following circumcision. Thus, in neonates, VTI may provide better specificity and sensitivity in detecting the parasympathetic response to nociceptive stimuli than either heart rate or heart period variability (Porter, 1989). Further research is required in order to determine if VTI is a more sensitive measure of the effect of nociceptive stimuli than heart rate or heart period in this population.

Measurement of VTI should provide accurate estimates of RSA amplitude for neonates receiving assisted ventilation as long as the total respiratory rate (i.e., spontaneous and mechanical breaths) is within the respiratory frequency specified in the VTI algorithm (S. W. Porges, personal communication, 1992). However, few studies have been conducted to evaluate VTI or other measures of short-term HRV in mechanically ventilated neonates (Porter et al, 1989; Preitsch et al., 1994; van Ravenswaaij-Arts et al., 1991).

Van Ravenswaaij-Arts et al. (1995) recently described RSA in 20 mechanically ventilated premature neonates during the first 12 hours of life. The RSA observed in these neonates was similar to that in normal neonates and the synchrony between the spontaneous and ventilator breaths appeared to represented entrainment of the central respiratory drive with the ventilator breath rate. The authors proposed that the strong influence of mechanical ventilation on RSA, as well as the entrainment of heart rate fluctuations caused by mechanical ventilation with baroreflex-related heart rate fluctuations, indicated an adequately functioning parasympathetic nervous system in these neonates.

Behavioral Responses to Nociceptive Stressors in Neonates

Neonatal behavioral responses of full-term and premature neonates to nociceptive stressors include gross motor activity, facial expression, and vocalization (Franck, 1986; Stevens, Johnston, & Horton, 1994). Endogenous opioids, cortisol, and catecholamines are elevated during crying (Gunnar, Connors, & Wall, 1988; Panksepp, Meeker, & Bean, 1979). However, similar elevations in plasma cortisol levels were measured in neonates who did not cry or display behavioral distress during a physical examination (Gunnar et al., 1988). While behavioral responses are consistently associated with increases in neuroendocrine measures, elevations in neuroendocrine parameters are not always accompanied by increases in behavioral responses.

The neonate's ability to demonstrate behavioral responses to stressors is strongly influenced by neuromuscular maturation and severity of illness (Coll, 1990; Tronick, Scanlon, & Scanlon, 1990). Premature neonates demonstrate decreased behavioral responsiveness to nociceptive stressors (Stevens & Johnston, 1994). The behavior of neonates becomes more organized and under inhibitory control as the neonate matures and health improves (Als, 1984).

Unfortunately, lack of definition of response parameters, lack of standardization of methods, and lack of control over confounding variables limit the usefulness of most behavioral measures to evaluate neonatal nociceptive stress responses (Berry & Gregory, 1987). Methods to reliably account for the differences in behavioral responses to nociceptive stimuli due to illness or prematurity have not been developed. Furthermore, changes in behavior may reflect responses to nociceptive stimuli, caution must be used in inferring the absence of a nociceptive stress response from the absence of behavioral responses.

One behavioral measure, flexor reflex threshold (FRT), shows promise as a potentially valid and reliable measure of a behavioral responses to nociceptive stressors. The flexor reflex of the neonate can be evoked by a cutaneous mechanical stimulus to the heel that produces a clear, distinct withdrawal of the leg, similar to that observed in adults in response to electrical stimulation, as described above. The FRT is discrete and reproducible in both the neonatal human and animal, but is evoked by stimuli of much lower intensity than in the adult (Fitzgerald, Shaw, & McIntosh, 1988).

The FRT response in healthy and moderately ill neonates, 25 to 42 weeks gestation, has been described (Andrews & Fitzgerald, 1994; Fitzgerald, Shaw, & McIntosh, 1988). In these two studies, the FRT ranged from 0.2 to 1.0 and 0.5 to 2.0 grams, respectively. The FRT increased significantly with gestational age but was not associated with postnatal age. Repeated stimulation (with the same force administered at 5 second intervals) produced sensitization in the premature infant less than 30 weeks gestation such that rhythmic flexion and extension or chronic flexion of the limb occurred and movement was also noted in the other leg and torso. Sensitization decreased with increasing gestational age and habituation (i.e., diminished responsivity to repeated stimulation) was observed in most infants at 32 to 35 weeks gestation (Andrews & Fitzgerald, 1994; Fitzgerald, Shaw, & McIntosh, 1988).

The effect of tissue damage to the heel (induced by repeated heelsticks over a period of 1 to 4 weeks) on the FRT of premature neonates was described and compared to the FRT of the uninjured heel of the same neonates (Fitzgerald, Millard, & McIntosh, 1988, 1989). Although there was wide variability in FRT from day to day, a lower FRT was consistently demonstrated in the injured heel as compared with the intact heel (Fitzgerald, Shaw, & McIntosh, 1988; Fitzgerald et

al., 1989). The hypersensitivity of the injured heel was reversed with application a topical local anesthetic (Fitzgerald et al., 1989). In contrast to tissue damage, contralateral tactile stimulation resulted in a significant increase in FRT in the injured heel (Andrews & Fitzgerald, 1994). These data suggest a clear organization of spinal sensory processing and a high level of excitability within the developing spinal cord of the premature neonate.

Although FRT has only been used in a few studies, measurement of FRT shows promise as a quantitative behavioral measure of neonatal responsiveness to cutaneous, potentially nociceptive, stimuli. FRT is sensitive to changes in gestational age, stimulus characteristics (i.e., intensity), and tissue injury.

Variability in Neonatal Responses to Nociceptive Stressors

Although specific neonatal data are lacking, factors that influence nociceptive stress responses in adults (i.e., specificity, magnitude, intensity, and duration of stressors) presumably influence the mode of expression and magnitude of the neonatal nociceptive stress responses. In addition, expression of neonatal stress responses is further modulated by: 1) the increased sensitivity of premature neonates to sensory stimuli; 2) the initial behavioral state of the neonate; and, 3) the neonate's ability to habituate to stressors. In the healthy neonate, stress responses may diminish with repeated nociceptive stimuli indicating adaptation to the stressor. These mechanisms are not well developed in the premature neonate resulting in a relative hypersensitivity of the premature neonate to nociceptive and normally non-nociceptive stimuli (Fitzgerald, 1991b). Furthermore, lack of stress responses in ill neonates may indicate exhaustion. The inability to reliably distinguish between habituation and exhaustion is a significant challenge in the investigation of neonatal stress responses.

It has been suggested that the factors described above contribute to

variability in the neuroendocrine, physiological, and behavioral responsiveness of individual neonates to the same nociceptive stressors at different times of the day or on successive days (Arendt, Halpern, MacLean, & Youngquist, 1991; Fitzgerald, Shaw, & McIntosh, 1988; Gunnar, Hertsgaard, Larson, & Rigatuso, 1992). In contrast, other authors contend that, despite large variability among neonates, stress responses of individual neonates are relatively stable in the early months of life (Izard et al., 1991; Lewis, Thomas, & Worobey, 1990). Some of the intra-individual variability seen in neonatal responses to nociceptive stimuli may be due to differences in baseline behavioral state during the study periods which may influence the neonate's degree of responsiveness to stimuli. Thus, any evaluation of stress responses requires careful examination of the influence of the individual's baseline state and reactivity, as well as the context of the stressful stimuli (Boyce, Barr, & Zeltzer, 1992; Manuck, Kasprowicz, & Muldoon, 1990; Porges, 1992).

Neonates often exhibit the same stress responses to nociceptive and non-nociceptive stimuli. The degree of responsiveness to stressors may also be predictive of later behaviors and health status (Brazelton, 1973; Gunnar et al., 1989; Izard et al., 1991; Lewis, 1992; Lewis et al., 1990; Porges, 1992; Tronick et al., 1978). Lewis (1992) suggests that it is a combination of genetic determinants of temperament and environmental conditions that accounts for the differences in responsiveness to stressors among neonates. Porter (1989) believes that the inter-individual variability in behavioral responses to nociceptive stressors may indicate the early development of individual coping styles. Large inter-individual variability in neonatal responses to nociceptive stimuli suggests that in order to detect changes in responses to nociceptive stimuli, neonates should be used as their own controls.

Summary

Although the body of literature on the ontogeny and expression of neonatal nociceptive stress responses is limited, it appears that neonates: 1) have the necessary neurologic substrates to perceive nociceptive stressors; 2) exhibit neuroendocrine, physiological, and behavioral responses to nociceptive stressors; 3) display great intra and inter-individual variability in their responses to stressors that may be due to maturational and/or environmental factors and may be influenced by habituation and behavioral state; and 4) may be more sensitive to both nociceptive and non-nociceptive stressors than adults.

Neonatal responses to nociceptive stimuli have been measured by a variety of techniques within three classes of responses (i.e., neuroendocrine, physiological, and behavioral). Plasma E and NE assays provide the most accurate and sensitive measures of neonatal neuroendocrine responses to nociceptive stimuli. However, catecholamine assays are difficult to obtain in the clinical setting. Physiological responses are potentially the most clinically relevant measures. However, while changes in heart rate are commonly assessed in clinical practice, VTI may be a more sensitive measure of nociceptive stress responses. Behavioral measures have been most commonly used to evaluate neonatal responses to nociceptive stimuli, but generally lack sensitivity and specificity. Flexor reflex threshold may provide a more valid and reliable behavioral measure of the neonatal response to nociceptive stimuli.

Opioid Administration in Neonates

Opioids are frequently administered to critically ill neonates to attenuate stress responses induced by frequent nociceptive stimuli associated with mechanical ventilation, invasive monitoring, and surgical procedures (Bell & Ellis, 1987; Koren & Maurice, 1989; Levene & Quinn, 1992; Yaster & Deshpande,

1988). Cardiorespiratory side-effects, including bradycardia, hypotension, and respiratory depression, are known and anticipated pharmacologic effects of opioids. However, surprisingly little data exist regarding the prevalence and severity of cardiorespiratory side-effects associated with opioids in premature and full-term neonates. This section reviews the evidence for the attenuation of the neonatal stress response by opioids, pharmacokinetic considerations, and the prevalence of opioid-induced cardiorespiratory side-effects in neonates.

Development of Opioid Responsiveness in the Fetus and Neonate

Data regarding the development of opioid responsiveness in the human fetus and neonate are extremely limited and knowledge of the development of the opioid system is primarily derived from investigations using neonatal rats. The organization of the nervous system of the full-term human neonate is thought to be comparable to the 12 to 13 day old rat pup (Romijn, Hofman, & Gramsbergen, 1991). The nervous system of the 24 week gestation premature neonate is believed to be similar to the newborn rat pup (Fitzgerald, 1991a). Thus, the ontogeny of opioid responsiveness in the human neonate may be cautiously inferred from studies using neonatal rat pups, recognizing that there may be significant (but as yet unknown) differences between the species with regard to the development of opioidergic mechanisms.

General agreement exists that opioid peptides begin to appear in the rat brain between days 12 to 16 of fetal life (Bayon, Shoemaker, Bloom, Mauss, & Guillemin, 1979; Kent, Pert, & Herkenham, 1982; Leslie, Tso, & Hurlbut, 1982) and between days 16 to 17 in the substantia gelatinosa of the spinal cord (Kirby, 1981). The development of the main opioid receptor subtypes (i.e., mu, delta, and kappa) coincide with the appearance of the opioid peptides (Clendeninn, Petraitis, & Simon, 1976). Opioid receptor density increases in the rat brain

between days 5 to 15, then decreases until day 20, when the receptor density stabilizes (Auguy-Valette, Cros, Gouarderes, Gout, & Pontonnier, 1978). The opioid receptor subtypes develop independently and may play different roles in the development of behavioral responses to environmental stimuli and responsiveness to exogenous opioids (Kehoe, 1988). For example, high affinity mu receptors appear to be associated with analgesia while low affinity mu receptors appear to be associated with respiratory depression in neonatal rats (Pasternak, Zhang, & Tecott, 1980).

Early functional development of the opioidergic system is believed to facilitate conditioned responses to environmental stimuli that are adaptive for the immature animal, promoting recognition of social/physical cues for survival. Many studies have documented the responsiveness of the neonatal rat to opioid agonists and antagonists, but results are conflicting with regard to the relative sensitivity of the neonatal animal to the antinociceptive effects of opioids (see Kehoe, 1988 for review). For example, in one study, neonatal (2 day old) rats were found to be less sensitive to morphine's antinociceptive effects than older rats, despite having higher brain concentrations of morphine (Zhang & Pasternak, 1981). In another study, the antinociceptive effects of morphine increased in rats from day 5 to day 15 and then decreased rapidly, stabilizing at day 30 (Auguy-Valette et al., 1978).

One reason for the variation in response to opioids may be related to the presence of other stressors prior to opioid administration. For example, antinociceptive effects in stressed rats, both neonatal and adult, were associated with an increased number of opioid receptors and enhanced morphine-induced antinociception (Kehoe & Blass, 1986; Sherman, Strub, & Lewis, 1984; McLaughlin, Lichtman, Fanselow, & Cramer, 1990; Torda, 1978).

Scant data exist regarding the development and function of opioid systems in the human fetus. Opioid peptides and receptors have been observed in the spinal cord of the human fetus at around 12 weeks gestation (Charnay, Paulin, Dray, & Dubois, 1984). However, no studies have been conducted to determine opioid peptide and receptor development in the human fetal brain (Anand, 1990). Opioids administered to mothers during labor have been show to exert a depressive effect on fetal HRV in full-term fetuses (Petrie et al., 1978). Meperidine produced a dose dependent decrease in fetal HRV, with a slight transient increase in variability occurring with lower doses (Petrie, 1993).

Similar degrees of respiratory depression following morphine administration have been demonstrated in younger and older rats (Pasternak et al., 1980). However, the dose of morphine that was lethal to 50% of the animals in the sample (LD50) was found to be five times less in neonatal animals than in adults (Goldenthal, 1971). Data from other studies using neonatal rats (Kupferberg & Way, 1963; Domek, Barlow & Roth, 1960; Sanner & Woods, 1965), suggest that neonates are more sensitive to opioids and other sedatives due to increased permeability of an immature blood-brain barrier. In the rat, permeability of the blood-brain barrier to morphine decreases between days 15 to 30 (Auguy-Valette et al., 1978).

Although many parallels can be drawn between the human fetus and the newborn rat pup, techniques must be developed to investigate the development and function of opioid peptides and receptors in the human fetus and neonate to validate the findings from rat studies. The paucity of information on the development of the opioidergic system in the human fetus and neonate makes the interpretation of studies on opioid pharmacokinetics and effects very difficult.

Opioid Pharmacokinetics in Neonates

Opioids such as morphine and fentanyl are administered to neonates. However, data on the kinetics of these opioids are limited. Morphine and fentanyl pharmacokinetic parameters have been measured in neonates after a single dose of opioid (Bhat et al., 1990; Bhat, Abu-Harb, Chari, & Gulati, 1992; Bhat, Chari, Iver, 1994; Collins et al., 1985; Gauntlett et al., 1988; Koehntop, Rodman, Brundage, Hegland, & Buckley, 1986) or during a continuous opioid infusion (Barrett, Elias-Jones, Rutter, Shaw, & Davis, 1991; Choonara, McKay, Hain, & Rane, 1989; Elias-Jones, Barrett, Rutter, Shaw, & Davis, 1991; Farrington, McGuiness, Johnson, Erenberg, & Leff, 1993; Hartley, Green, Quinn, & Levene, 1993; Koren et al., 1985; Lynn & Slattery, 1987; Walker, Chay, & Duffy, 1991).

In neonates, mean elimination half-life following a single dose administration of fentanyl is reported to range between 6 to 32 hours (Collins et al, 1985; Gauntlett et al., 1988; Koehntop et al., 1986; Christie, Santiero, Stromquist, & Torres, 1995). Mean elimination half-life following a single dose morphine is reported to range between 2.6 to 14 hours (Bhat et al., 1990; Bhat et al., 1994; Walker et al., 1991). While some of these studies included both full-term and premature neonates, recent evidence suggests that neonates exhibit increased clearance and decreased half-life of opioids as gestational age increases (Bhat et al., 1994). There is also evidence to suggest rapid maturation of opioid metabolic mechanisms during postnatal life, with clearance levels approaching those of adults by six months of age (Lynn & Slattery, 1987). In one study (Bhat et al., 1994), all neonates (ranging from 24 to 40 weeks gestational age and 1 to 60 days postnatal age) excreted more than 20% of the morphine administered as a continuous infusion in an unmetabolized state. One third of the neonates in the study did not metabolize any morphine. However, those neonates greater than 5

days of age metabolized morphine, primarily to morphine-3-glucuronide. The pharmacokinetics of multiple intermittent opioid dosing in neonates have not been evaluated.

The finding of significant individual variation in pharmacokinetic parameters in preterm neonates warrants additional investigation. None of the studies of opioid pharmacokinetics included quantitative measures of analgesic effects or side-effects in neonates. No data exist on the plasma opioid concentrations associated with analgesia or cardiorespiratory side-effects in neonates or on the influence of previous exposure to opioids (e.g., prenatally) on analgesia or cardiorespiratory side-effects.

Opioid Anesthesia/Analgesia Effects on Neonatal Neuroendocrine and Physiological Responses

Opioid effects on neuroendocrine and physiological responses have been investigated in neonates in studies of surgical and non-surgical nociceptive stressors. In one study, plasma E levels remained low in neonates who received fentanyl anesthesia as compared with neonates who received halothane anesthesia, while plasma NE levels were elevated in both groups in the immediate postoperative period (Anand et al., 1987), at 24 hours, NE levels were decreased in the fentanyl group as compared with the halothane group. In another study, neonates who received fentanyl analgesia during broviac catheter insertion had lower (although not statistically significant) E and NE levels compared to neonates who received secobarbital sedation (Cordero, Gardner, & O'Shaughnessy, 1991). Increases in plasma E levels were attenuated by phenobarbitone sedation while NE levels were unaffected.

The studies evaluating the effects of opioids during surgery (Anand et al., 1987; Anand & Hickey, 1992; Collins et al., 1985; Cordero et al., 1991; Friesen &

Henry, 1986; Robinson & Gregory, 1981; Yaster, 1987) suggest that intra-operative neuroendocrine and physiological responses to surgical stress are attenuated by opioid anesthesia. However, differences in anesthetic technique, surgical procedure, length of procedure, age of the neonates (gestational or postnatal), underlying pathology, and sampling interval limit a comparison of the findings across these studies. Interpretation of the data on attenuation of the stress response in the postoperative period (Anand et al., 1987; Anand & Hickey, 1992) is confounded by differences in postoperative analgesics and the variability in postoperative medical management. However, overall, the results of these studies support the use of opioids for both preterm and full-term neonates during surgery and suggest that opioids are efficacious in attenuating hemodynamic, hormonal, and metabolic effects of surgical stress. Based on these data, some clinicians now advocate use of 50 to 100 mcg/kg of fentanyl anesthesia and continuation of a 10 to 15 mcg/kg fentanyl infusion during the early postoperative period to prevent neuroendocrine stress responses and hemodynamic instability in infants undergoing cardiac surgery (Wessel, 1993).

Several studies have attempted to evaluate the use of opioids to reduce neuroendocrine stress responses, improve cardiorespiratory stability, and provide analgesia and/or sedation in mechanically ventilated neonates. In a large randomized control trial (Quinn et al., 1992), 95 premature neonates receiving mechanical ventilation were randomly assigned to receive a continuous morphine infusion, intermittent pancuronium, or continuous morphine infusion plus intermittent pancuronium. Heart rate, blood pressure, and ventilatory parameters were measured prior to drug administration and after six hours, while plasma E and NE levels were obtained prior to drug administration and after 24 hours. Blood pressure increased significantly in the morphine and pancuronium

group and ventilatory requirements increased in all groups, with no significant differences between groups. Norepinephrine levels in the morphine group were significantly lower compared to baseline. No statistically significant differences were found among the other groups, although E and NE levels tended to decrease in all groups. Although this study contained a large sample, interpretation of the results is limited by the large degree of variability in catecholamine levels within the groups and the administration of two different doses of morphine (50 mcg/kg/hr and 100 mcg/kg/hr) to neonates in the morphine group. In addition, correlations between the cardiorespiratory and catecholamine data cannot be made because these data were obtained at different times.

In a subsequent study (Quinn et al., 1993), 41 mechanically ventilated premature neonates received a loading dose of morphine followed by a continuous infusion of morphine or saline. A significant reduction in plasma E and a non-significant decrease in NE was observed during the first 24 hours.

In another study (Vacanti et al., 1984), a continuous fentanyl infusion (3 mcg/kg/hr) was administered to neonates following the repair of a diaphragmatic hernia. Neonates also received pancuronium and rapid ventilation (i.e., rates of 120 to 150 breaths per minute) and demonstrated decreased pulmonary arterial vasoconstriction as compared with historical controls who had not received fentanyl.

More recently, Goldstein and Brazy (1991) compared the effects of morphine, fentanyl, and pancuronium on blood pressure fluctuations associated with spontaneous breathing in premature, mechanically ventilated neonates. A similar decrease in blood pressure variation was demonstrated equally as well following administration of a single dose of morphine (0.1 mg/kg), fentanyl (5-10 mcg/kg), or pancuronium (0.1 mg/kg). However, both opioids allowed the

neonates to breathe in synchrony with the ventilator while avoiding the side-effects associated with pancuronium. The neonates receiving morphine or fentanyl exhibited a wide range of spontaneous respirations following opioid administration. The variability in spontaneous respiratory rate may be explained by the wide range of doses administered (fentanyl) and the variable length of time between drug administration and measurement of respiratory rate.

The effects of opioid anesthesia or analgesia on vagal tone in neonates have not been studied. Two studies (Oberlander, Berde, Lam, & Saul, 1993; Oberlander, Berde, Lam, Rappaport, & Saul, 1994) demonstrated a decrease in sympathetic as opposed to parasympathetic tone in former premature neonates during halothane anesthesia and recovery of sympathetic tone in the postoperative period. Tetracaine spinal anesthesia was found to decrease overall HRV, with the parasympathetic-sympathetic balance remaining stable (Oberlander et al., 1994).

Opioid Analgesia Effects on Neonatal Behavioral Responses

Changes in FRT have been reported in neonates receiving opioids in only one abstract (Morse & McIntosh, 1994). In this study, FRT was measured in mechanically ventilated neonates with (n = 18) and without (n = 18) a continuous morphine infusion (with morphine levels greater than 12 ng/ml) during the first 12 hours of life. Neonates receiving the continuous infusion demonstrated a non-significant increase in FRT compared to controls. Twelve hours following discontinuation of the infusion, the opposite effect was seen; the neonates who had received morphine demonstrated a significant decrease in FRT compared to the control group. The differences in FRT between the two groups disappeared within 24 hours of discontinuation of the infusion.

Summary

Clinical studies using opioids in neonates have increased our knowledge of the effects of these agents during surgery and mechanical ventilation. However, in many of these studies, the sample sizes were small and clinical conditions were not well-controlled. Comparisons between studies are difficult due to the differences in drugs, doses, and sample characteristics. As with the pharmacokinetic studies, many of the clinical studies suggest that opioids can be administered safely and can effectively attenuate stress responses in neonates.

Opioids are frequently used to sedate, promote respiratory synchrony, improve ventilation, and relieve pain or discomfort in neonates (Bell & Ellis, 1987; Koren & Maurice, 1989). The paucity of data regarding the effectiveness of this common practice is disappointing. The results of the studies done to date (Goldstein & Brazy, 1991; Irazuzta, Pascucci, Perlman, & Wessel, 1993; Quinn et al., 1992; Vacanti et al., 1984), suggest that opioids can be used to improve the cardiorespiratory status of mechanically ventilated neonates. Unfortunately, the investigators did not measure the effectiveness of the opioid analgesics or quantify the sedative effects of the opioids in mechanically ventilated neonates. Further research is needed to evaluate the relative risks and benefits of using opioids as analgesics to attenuate the stress response to nociceptive stimuli, particularly in critically ill neonates. Additionally, the prevalence and magnitude of side-effects following opioid administration have not been adequately measured in neonates.

Cardiorespiratory Side-Effects of Opioids in Neonates

Opioid analgesics are known to produce numerous side effects. Cardiorespiratory side-effects, specifically hypoventilation (i.e., respiratory depression), bradycardia, and hypotension are of greatest concern in neonates

because of the belief that neonates are more sensitive to the cardiorespiratory effects of opioids than adults or older children (Gregory, 1994). Despite the emphasis in the literature on the risks of opioid-induced cardiorespiratory side-effects, very little data are available on the prevalence of these side-effects.

Cardiovascular Side-Effects in Neonates

In neonates, opioids can depress heart rate and blood pressure (Gregory, 1994). As with the respiratory effects, cardiovascular effects vary depending on the specific opioid administered as well as on the route and rate of administration. Hydration status is also a key factor in the maintenance of cardiovascular stability, with opioid-induced hypotension more likely to occur in the hypovolemic neonate than in the normovolemic neonate (Gregory, 1992; Yaster & Deshpande, 1988). Therefore, comparison of post-opioid measures to baseline parameters for individual neonates provides the most accurate method to evaluate opioid-induced changes in heart rate and blood pressure.

Respiratory Side-Effects

Although the definition of respiratory depression in neonates is not precise, it is generally measured by a decrease in lung expansion/volume; a decrease in ventilatory control (i.e., respiratory rate); alterations in gas exchange (i.e., increased pH, decreased partial pressure of oxygen (PO_2); and an increased partial pressure of carbon dioxide (PCO_2). Apnea, with cessation of respirations for 15 seconds or greater accompanied by hypoxia or bradycardia, is common in neonates of 34 weeks gestation or less (Barrington & Finer, 1991).

Only one study (Purcell-Jones, Dormon, & Sumner, 1987) documented the incidence of opioid-induced respiratory depression in neonates. This retrospective chart review of 933 neonates during the postoperative period, revealed a 13.5% incidence of opioid-induced respiratory depression in

spontaneously breathing neonates. Four of 83 ventilated neonates (4.5%) who received codeine or papaveretum experienced delays in weaning from ventilatory support due to opioid-induced respiratory depression as judged by clearly defined criteria, although none of the 10 neonates who received morphine had delays in weaning. In contrast, two other studies (Koren et al., 1985; Farrington et al., 1993) reported no cases of respiratory depression in neonates receiving up to 40 mcg/kg/hr of morphine, with serum morphine levels as high as 90 ng/ml.

The respiratory effects of opioids have been directly measured in two studies (Lynn, Nespeca, Opheim, & Slattery, 1993; Way, Costley & Way, 1965). In the first study (Way et al., 1965), 10 normal full-term neonates demonstrated a 22% decrease in responsiveness to carbon dioxide (CO_2) after an intramuscular morphine injection compared to a control group who received an injection of saline. No changes in CO_2 responsiveness were seen following the administration of meperidine in an equianalgesic dose. Administration of both drugs resulted in the loss of a regular sigh that normally occurs as part of the newborn's respiratory pattern.

More recently, Lynn et al. (1993), measured $PaCO_2$ during spontaneous respiration and CO_2 response during rebreathing in neonates, infants, and children receiving continuous morphine infusion following cardiac surgery. Steady state serum morphine levels above 20 ng/ml were associated with a greater occurrence of hypercarbia and depressed CO_2 responsiveness. There was no correlation between age and morphine serum levels or CO_2 responsiveness. Three neonates with cyanotic heart disease retained CO_2 responsiveness despite high serum morphine levels.

Concomitant administration of morphine and pancuronium was found to

decrease functional residual capacity (FRC) in premature neonates with hyaline membrane disease (Miller et al., 1994). However, the relative contribution of the two drugs to the decreased FRC could not be determined because of limitations in the study design.

Because of the significant variation in the respiratory function of premature neonates related to immaturity and illness, infants must serve as their own controls for all measures of opioid-induced changes in respiratory function. Determination of opioid-induced hypoventilation in neonates receiving mechanical ventilation is difficult. Decreased respiratory depth and rate of spontaneous respirations may indicate relaxation or sleep rather than respiratory depression. The respiratory effects of opioids can only be inferred from measurement of the change from baseline of respiratory parameters following the administration of the drug. Continuous pulse oximetry and spontaneous respiratory rate trending combined with periodic arterial blood gas measurements appear to be the most sensitive, reliable, and clinically useful methods to measure opioid-induced respiratory depression in premature neonates.

Opioid-induced decreases in heart rate, respiratory rate, and blood pressure may reach statistical significance but be well within normal physiological limits (Hartley et al., 1993). Provision of analgesia may in fact improve ventilation and cardiovascular status through depressant effects on cardiorespiratory parameters (Elias-Jones et al., 1991; Irazuzta et al., 1993; Robinson & Gregory, 1981; Vacanti et al., 1984). Conversely, failure to adequately relieve pain could lead to hypoventilation. For example, Pokela (1994), administered 1 mg/kg meperidine or saline to premature neonates prior to endotracheal suctioning and routine procedures (weighing, bathing, temperature measurement, chest x-ray). Neonates in both groups experienced a similar

number of episodes of hypoxemia. However, these episodes were of significantly shorter duration in the neonates who received meperidine prior to the procedures.

In order to discriminate between inadequate analgesia and opioid-induced cardiorespiratory side-effects, vital signs must be evaluated within the context of the clinical situation and timing of opioid administration.

Summary

Fear of cardiorespiratory side-effects is a major consideration in decisions to use opioid analgesics in premature neonates. Yet the prevalence and severity of these side effects has not been well described. The use of valid and reliable methods for measuring cardiorespiratory side-effects of opioids in neonates should be included in any evaluation of opioid analgesics in this population.

Conclusions

Research data suggest that the use of anesthesia during surgery (Anand et al., 1987; Anand & Aynsley-Green, 1988a) and anesthesia combined with postoperative analgesia (Anand & Hickey, 1992) may significantly decrease neuroendocrine stress responses and improve clinical outcomes in neonates. Although some of the cardiorespiratory effects of morphine in full-term infants have been described (Way et al., 1965) and some data exist on the pharmacokinetics of morphine (e.g., Bhat et al., 1990; Bhat et al., 1994; Koren et al., 1985), no studies have been conducted to determine the efficacy of opioids in providing analgesia or reducing postoperative stress responses in neonates.

Because of the degree of variability in neonatal responses and the large number of uncontrolled stressors in the NICU, studies to evaluate the validity and reliability of measures of neonatal responses to nociceptive stimuli must use neonates as their own controls. Furthermore, given the inadequacies of current

measurement methods in differentiating between nociceptive and non-nociceptive responses, triangulation of methods across all three measurement classes (i.e., neuroendocrine, physiological, and behavioral) may provide more accurate and clinically useful information to determine neonatal responses to nociceptive stimuli.

Unfortunately, the concern regarding adverse cardiorespiratory effects of opioids on neonates has yielded very little research to determine the prevalence and severity of these side-effects. Clearly, opioids can cause hemodynamic instability and respiratory depression. However, the specific parameters (e.g., age, drug dose, medical condition) which may affect the occurrence of these side-effects remain poorly understood.

Despite fears of opioid-induced side-effects, their use is now common in the NICU for modulation of behavioral and physiological responses to mechanical ventilation and for analgesia. Rational risk-benefit analyses to guide the use of opioids to attenuate the stress response, improve ventilatory management, and provide analgesia cannot be accomplished until methods are developed to better evaluate opioidergic effects and to describe the prevalence and severity of cardiorespiratory side-effects. An investigation of methods to measure neonatal nociceptive stress responses is an important research priority. Until better methods are available to accurately monitor drug effects and associated toxicities, underutilization of opioids in the neonate will continue to be a significant clinical problem.

CHAPTER 3

METHODOLOGY

Research Design

The purpose of this descriptive study was to compare plasma E and NE, VTI, FRT, and cardiorespiratory status in premature neonates prior to surgery and at 20 minutes and 1 hour following bolus administration of the first iv dose of morphine given within the first 12 hours following thoracotomy.

Research Setting

Neonates were recruited from the NICU at Children's Hospital Oakland (CHO) and from the NICU at the University of California, San Francisco (UCSF). At CHO, neonates were transported from the NICU to the operating room for surgery. The neonates were anesthetized with iv fentanyl or, infrequently, with a combination of inhaled anesthesia and iv fentanyl. They were paralyzed with equivalent doses of either pancuronium or vecuronium. Neonates were returned to the NICU for recovery and postoperative care. At UCSF, PDA ligation was performed in the NICU and neonates were anesthetized with iv fentanyl and paralyzed with pancuronium.

Surgical Procedures and Care

Thoracotomy for PDA ligation involves a left posterolateral incision about 3 cm in length over the third or fourth intercostal space, retraction of the lung, incision of the mediastinal pleura, and dissection of the ductus. The ductus is then permanently occluded using either a clip or suture (Canarelli et al., 1994; Karwande & Rowles, 1992; Kirkin, 1990). A chest tube is sometimes placed in the left pleural cavity. The ribs and muscles are approximated and sutured and the skin is closed.

Total operative time for the neonates in this study was generally 20 to 30

minutes in length. Operative times tended to be longer at CHO due the need to stabilize the neonates after transfer to the operating room.

A thoracotomy was performed in one neonate with a tracheoesophageal fistula in order to mobilize the esophagus to approximate the upper pouch. The thoracotomy technique and length of surgery were similar to a thoracotomy for PDA ligation.

For all neonates, postoperative analgesia was ordered as 0.1 mg/kg of iv morphine, every 3 to 4 hours, as needed. The actual frequency of administration was determined by the neonate's nurse. The first dose was usually administered 4 to 8 hours following the completion of the surgery.

Sample

Twenty-five premature neonates, with gestational ages less than 32 weeks and birth weights less than 1.5 kg, scheduled for thoracotomy were recruited to participate in the study. Neonates were excluded from the study for any of the following reasons: 1) refusal of consent by parent(s); 2) severe intraventricular hemorrhage (Grade III or IV); 3) severe congenital anomalies; 4) administration of cholinergic drugs prior to surgery; 5) restricted movement of both legs; or 6) lack of indwelling iv access for blood sampling (i.e., no umbilical artery, umbilical vein, or radial artery catheter).

Instruments/Measurements

Data Collection Form

Description: A two-part, 124 item data collection form was used to obtain demographic data and record specific measurements to be analyzed in this study (Appendix A).

Validity and Reliability: Content validity was obtained by review and revision of the instrument by a panel of experts.

Epinephrine and Norepinephrine Assays

Purpose: To measure the plasma levels of E and NE preoperatively and before and after morphine administration.

Equipment: ESA Coulochem LC/EC System (Model 5200 detector; Model 5011 analytic cell; ESA, Bedford, MA). The potentials applied to the first electrode, the working electrode, and the guard cell were +20 mV, +320 mV, and +400 mV, respectively. Separation of electroactive compounds was achieved on an ESA HR-80 column (3 um, 4.6 X 15.0 mm, C18), maintained at 30-35°C with a column heater (Waters-Millipore, Milford, MA). Standards and samples were injected (100 ul) into the system with a CMA/200 autosampler (BAS, Inc., West Lafayette, IN). The mobile phase consisted of 75 mM sodium dihydrogen phosphate monhydrate; 0.5 mM sodium docdecyl sulfate; 25 uM EDTA; 20% acetonitrile; 5% methanol, final pH 5.6, and was recycled at flow rates of 0.9-1.0 ml/min with an ESA 420 pump.

Description: Using an ESA plasma catecholamine analysis kit, the catecholamines were extracted by absorption onto alumina at a pH of 8.6 and then further eluted with dilute acid. An internal standard, dihydroxybenzylamine (DHBA) 10^{-8}M, was included with each extraction to monitor recovery and aid in quantification.

Specificity and Sensitivity: HPLC-ED measures the current generated by oxidation-reduction occurring at the hydroxy groups of the catecholamines in the acidic solution during exposure to an electrical field (Goldstein, Feurerstein, Izzo, Kopin, & Keiser, 1981; Hallman, Farnebo, Hamberger, & Jonsson, 1978). HPLC-ED is highly sensitive and selective for catecholamines and is simpler, more rapid, and less expensive than

radioimmunoassay (Hallman et al., 1978; Stulik & Pacakova, 1986; Zeigler, Woodson, & Kennedy, 1986). However, plasma E levels were lower than the limit of detection of the HPLC-ED system of 0.27 nmol. The limit of detection for NE was 0.18 nmol.

Numerous factors in the collection and handling of the blood samples influence the sensitivity and reproducibility of assays. Blood samples were obtained from a consistent site (i.e., arterial). The samples were stored in heparinized vials, frozen within one hour of collection, kept at -80°C, and not repeatedly thawed and refrozen (Falconer, Lake, & MacDonald, 1982; Goldstein, 1986).

Procedure:

1. Whole arterial blood samples (0.5 ml) were collected from indwelling lines (i.e., umbilical artery or radial artery catheters) into lithium-heparin tubes and placed on ice. The samples were centrifuged and the plasma frozen at -80°C within 1 hour of collection for later analysis by HPLC-ED.
2. HPLC-ED assays were performed with the ESA Coulochem LC/EC System using the extraction procedures recommended by the manufacturer (ESA, Inc., Bedford, MA). Due to the precise quantitative nature of the assays, it was not necessary for the individual performing the assays to be blinded to the origin of the samples.
3. Duplicate measures to determine intra-assay variation were not performed due to inadequate plasma volume. Inter-assay variation was less than 10%.

Vagal Tone Index:

Purpose: To quantify the magnitude of the rhythmic shifts in heart rate mediated by the vagus nerve within the frequency band of respirations.

Equipment:

1. Cardiorespiratory monitor (Corometrics model 506 or 515; Hewlett Packard Merlin) with RS232 output port and cable.
2. Personal computer 386, 33 MHz with analog to digital data acquisition board (MetraByte, Tauton, MA).
3. Computer program (Dr. Richard Powers, Children's Hospital Oakland) to detect the peak of the R-wave spike for each cardiac cycle, sampling every 1000 Hz with a 0.2 Hz refractory period. The sequential R-R intervals (i.e., heart period or inter-beat interval) is calculated to the nearest millisecond for heart rates up to 300 beats per minute (bpm).
4. MXedit (Delta Biometrics, Bethesda, MD) software program to calculate VTI.

Description: VTI is derived from continuous electrocardiogram (ECG) data recorded from the neonate's cardiorespiratory monitor.

Specificity and Sensitivity: VTI has been shown to be a specific and sensitive measure of vagal tone in both healthy and ill neonates (Fox & Porges, 1985, Porges, 1992). In this study, ECG data were collected by the primary investigator or research assistant using a standardized procedure. The primary investigator calculated VTI after receiving training in the use of MXedit. In a random sample of 15 measurements from this study, there was 100% agreement in the calculation of VTI between the primary investigator and another experienced investigator.

Procedure:

1. A cable was connected from the RS232 output jack on the cardiorespiratory monitor to the computer. The ECG was displayed on the computer screen between the upper and lower voltage detection thresholds (0.1-0.5 V). The ECG amplitude and lead placement was adjusted so that only the R-wave spike crossed the upper threshold.
2. The Vgtone program was initiated and ECG data were recorded continuously for 3 minutes. This created the inter-beat interval data file formatted for MXedit.
3. At a later time, outside the NICU, the file was edited, if necessary, and analyzed using the MXedit software program developed by Porges (1985). MXedit allows editing of the data for elimination of artifact, isolates the pattern of variability occurring within the frequency range of the neonate's respiratory rate (i.e., 0.3 to 1.3 Hz), and calculates VTI. Due to the automatic calculation of VTI by the MXedit program, it was not necessary for the individual using the program to be blinded to the time point the data were obtained.

Flexor Reflex Threshold

Purpose: To quantify the degree of force of a point-pressure tactile stimulus that elicits a withdrawal reflex in the neonate.

Equipment: Calibrated von Frey filaments (Semmes-Weinstein Monofilaments, Stoelting, Co., Wood Dale, IL).

Description: von Frey filaments are devices with nylon filaments of a graded diameter that when pressed onto the skin apply a force ranging from 0.008 to 280 grams. Fifteen of the filaments were used, representing

forces of approximately 0.01 to 16.80 grams.

Specificity and Sensitivity: Using a standardized procedure, FRT is reported to be a specific and sensitive measure of the relative threshold of cutaneous sensation for an individual neonate at a point in time (Fitzgerald et al., 1988). The pressure exerted by the filaments varies with environmental conditions. Periodic measurement of the forces exerted was performed over the course of the study by pressing the filaments to the bending point on a gram scale. The average variation between measures was 17%. The variation in force of filaments less than 2.0 grams was 1 to 4%. The variation in force of the stiffer filaments exerting the greatest force was 88% (i.e., forces ranged from 16.80 to 28.90 grams).

Procedure:

1. The neonate was lying supine.
2. Starting with a fine filament (0.01 gram) and working upwards in force, each filament was applied to the plantar surface of the neonate's unrestricted foot until a clear distinct flexion withdrawal of the entire leg (or in the absence of full leg withdrawal, withdrawal of foot or toes) was observed (Fitzgerald, Shaw, & McIntosh, 1988), or the maximum stimulus (16.80 grams) was reached. A value of 16.80 grams was assigned if a neonate reached the maximum stimulus. The maximum stimulus represents loss of protective sensation in adults (Tomancik, 1987).
3. The von Frey filaments were cleaned with isopropyl alcohol between each use.

Heart Rate, Respiratory Rate, and Blood Pressure Monitor

Purpose: To provide continuous, non-invasive measures of heart rate, respiratory rate, and direct intra-arterial measurement of blood pressure.

Equipment: Corometrics model 506 or 515 or Hewlett Packard Merlin, three lead wires and electrodes, disposable pressure transducer, cardio-respiratory and transducer cables.

Description: The cardiorespiratory monitor is a non-invasive device which provides continuous heart rate, respiratory rate, and arterial blood pressure monitoring. The wave forms and numerics of the parameters are displayed on the screen.

Accuracy and Sensitivity: The monitors' sensitivity can be determined by the operator or adjusted automatically depending on the mode of operation selected.

1. Heart Rate: Range: 20 to 250 bpm. Accuracy: ± 3% of displayed value (above 20 bpm). Sensitivity: will not trigger on less than a 0.15 mV R-wave (Corometrics, 1982). Heart rate is displayed numerically in beats per minute and as a continuous wave form on the cardiorespiratory monitor. Accurate measurement of heart rate and detection of bradycardia is also dependent upon correct placement of electrodes and adequate skin contact (Hunt 1991; Cabal et al., 1992).

2. Respiratory Rate: Range: 0 to 170 breaths per minute. Accuracy: ± 3% of displayed value. Sensitivity: will not detect breath amplitudes of less that 0.15 ohm. Respiratory rate is measured by transthoracic impedance and is displayed numerically as breaths per minute and as a continuous wave form on the cardiorespiratory monitor. While most

monitors have sophisticated signal processing filters to minimize inaccuracy, artifact related to the placement of the electrodes on the neonate and other sources of electrical impedance can decrease the accuracy of respiratory rate detection (Neuman, 1992). In addition, respiratory rate is affected by gestational age, behavioral state, and clinical condition (Cohen, Xu, & Henderson-Smart, 1991; Wallen, 1992).

3. Blood Pressure: Range: 0 to 255 mmHg. Accuracy: ± 3% of displayed value. Sensitivity: 5 microvolt/V/mmHg. Systolic, diastolic, and mean arterial blood pressure are displayed numerically in mmHg and as a continuous wave form on the cardiorespiratory monitor. Accurate blood pressure recording is dependent upon patency of the catheter and correct calibration and placement of the transducer (Neuman, 1992). Because of the potential for error with direct measurement of blood pressure, values are periodically compared with measurements using an automated indirect oscillometric technique which measures air pressure oscillations associated with systolic, diastolic, and mean blood pressure. The accuracy and reliability of the indirect oscillometric technique is dependent upon proper cuff size and minimal movement.

Procedure:

1. The heart rate, respiratory rate, and blood pressure from the cardiorespiratory monitor were compared with manually obtained values recorded by the neonate's nurse. If there were large differences between the manually obtained values, electrodes were repositioned and the transducer recalibrated as necessary.

2. Heart rate, respiratory rate, and arterial blood pressure were read from the digital display on the monitor and recorded on the data collection sheet.

Oxygen Saturation Monitor

Purpose: To provide continuous non-invasive measurement and display of arterial oxygen saturation and pulse rate.

Equipment: Nellcor N200 Pulse Oximeter and sensor.

Description: The Nellcor N200 utilizes sensors containing two light emitting diodes (LEDs) of a given wavelength (660 and 920 nm). A photodetector, placed on the patient opposite the LEDs, measures the intensity of light that passes through and is not absorbed by blood and tissue components. The difference in intensity of transmitted light between the two LEDs is caused by differences in the absorption of light by oxygenated and deoxygenated hemoglobin species contained within the vascular bed. The determination of arterial hemoglobin saturation is computed by the pulse oximeter from the relative amount of light transmitted to the photodetector occurring during pulsatile flow as compared with nonpulsatile flow. Pulse rate is calculated by measuring the time interval between detected peaks of the infrared light wave form.

The N200 is calibrated to display "functional" saturation, represented by the amount of oxyhemoglobin as a percentage of the hemoglobin that can be oxygenated: Functional saturation = (HbO_2 X 100)/[100 - (COHb + MetHb)], where: HbO_2 = Fractional hemoglobin; COHb = Carboxyhemoglobin; MetHb = Methemoglobin.

Accuracy and Sensitivity:

1. Oxygen saturation: Range: 0 to 100%. Accuracy: ± 3 digits for neonatal saturation between 70 to 95% (unspecified for saturations less than 70% or greater than 95%).

2. Pulse Rate: Range: 20 to 250 bpm. Accuracy: ± 2 bpm. Accuracy is affected by motion artifact, decreased perfusion secondary to hypothermia, vasoactive drugs, shock, severe peripheral edema, presence of abnormal hemoglobins (i.e., high fetal hemoglobin, high methemoglobin), and interference with light transmission and absorbance (i.e., phototherapy, lipid particles, and dyes). The most significant accuracy limitations of pulse oximetry in preterm infants are due to motion artifact and fetal hemoglobin. Motion of the pulse oximeter probe is associated with false alarming of the monitor. Another limitation is the inability of pulse oximetry to distinguish between adult and fetal hemoglobin. Therefore, in the immediate newborn period and in the absence of large volume transfusions, large PO_2 changes (i.e., 6 to 12 mmHg or more) are associated with small SaO_2 changes (1 to 2%). For example, SaO_2 by pulse oximeter may indicate 100% saturation, while the actual PO_2 may be much greater. Conversely, SaO_2 readings in the range of 85% to 90% may be present despite an actual PO_2 of only 35 to 45 mmHg (Hay, Brockway, & Eyzaguirre, 1989; Severinghaus & Kelleher, 1992).

Pulse oximetry is not adequate for evaluation of hyperoxia, but does provide accurate and reliable detection of hypoxemia. Ventilatory support for most premature neonates is adjusted in order to maintain SaO_2 within a range of 88 to 92% (Phillips, McQuitty, &

Durand, 1988).

Procedure:

1. The sensor was placed on the left or right foot, sometimes on the left or right palm. No warm-up or calibration is required. The pulse oximeter was considered accurate when the pulse rate displayed correlated with the pulse rate displayed on the cardiorespiratory monitor.
2. Oxygen saturation was read directly from the digital display on the pulse oximeter and recorded on the data collection sheet.

Behavioral State Scale

Purpose: To measure the neonate's level of arousal.

Equipment: Behavioral State Scale (Appendix B).

Description: The Behavioral State Scale used by Prechtl (1974) and Brazelton (1973) consists of five levels (i.e., deep sleep, light sleep, drowsy, awake, and crying). Neonates are rated using four characteristics (i.e., respiratory pattern, eye movements, gross motor activity, and vocalization). Depending on the presence or absence and the quality of these characteristics, the neonate receives a behavioral state score along the sleep-wake continuum.

Validity and Reliability: Numerous studies have demonstrated the validity and reliability of the Behavioral State Scale (see Prechtl, 1974 for review). Determination of behavioral state can be made accurately by experienced neonatal clinicians using the Behavioral State Scale and good inter-rater reliability has been demonstrated (Gill, Behnke, Conlon, McNeely, & Anderson, 1988).

Procedure:

1. The respiratory pattern, eye movements, and gross motor activity of each neonate were visually assessed for at least three minutes and classified at the specified intervals.
2. Each characteristic must remain stable for three minutes to be considered representative of a particular state.
3. The behavioral state was recorded on the data collection sheet.

Procedures

Preoperative Selection of Neonates

Identification of neonates who were to undergo surgical ligation of the PDA was made in collaboration with NICU physicians and nurses. The physicians and/or nurses caring for the neonate were consulted about the ability of the neonate to tolerate the amount of blood needed for this study without requiring subsequent transfusion. After parental consent for surgery was obtained, parents were contacted and requested to have their infant participate in the study. Written informed consent was obtained in English or Spanish in accordance with the standards of the Human Research Committee at UCSF and Children's Hospital Oakland (see Appendix C for consent forms). A hospital interpreter facilitated the communication between the investigator and the parents during the consent process for all Spanish speaking parents.

Overview of Data Collection Procedures

Data collection was done at four time points during the study: 1) preoperatively (baseline); 2) postoperatively, immediately prior to morphine administration (pre-morphine); 3) 20 minutes after the first postoperative dose of morphine which was administered over a 10 minute period (post-morphine, time 1); and 1 hour following morphine administration (post-morphine, time 2). The

measurement sequence is described below and in Appendix D.

Baseline Measures of Stress Responses and Cardiorespiratory Status

Prior to surgery, at a time when the neonate had not experienced noxious stimuli within the previous 30 minutes and the neonate was in a light or deep sleep state, baseline measures of vital signs (i.e., heart rate, respiratory rate, blood pressure, and oxygen saturation) and ECG data for calculation of VTI were recorded. Ventilator settings were also recorded. Flexor reflex threshold was then measured. Lastly, a blood sample for E and NE levels was obtained. When possible, the blood sample for the study was obtained at a time when blood was being obtained for other laboratory tests.

Postoperative Measures of Stress Responses and Opioid-Induced Cardiorespiratory Side-Effects

Following the completion of surgery, the investigator or research assistant was notified by the neonate's nurse when morphine was to be administered. While the nurse was preparing the medication, the investigator obtained ECG data; documented behavioral state, vital signs, oxygen saturation, and ventilator settings; and measured FRT. A blood sample (0.5 ml) was drawn from the umbilical artery catheter or radial artery catheter immediately before the nurse administered the morphine. Twenty minutes after the nurse injected the dose of morphine, behavioral state was assessed, ECG data were recorded, vital signs and oxygen saturation were read from the monitors, FRT was measured, and a 0.5 ml blood sample was obtained. One hour following administration of morphine, all measures, except the blood sample, were again obtained. Based on available pharmacokinetic data (Bhat et al., 1990), the time frame of 20 minutes following the completion of a slow morphine injection was believed to be adequate to observe changes in stress response parameters and cardiorespiratory effects of morphine

in the premature neonate. When possible, the study blood samples were obtained at the same time as blood was obtained for other laboratory tests.

Data Analysis

Data were analyzed using the CRUNCH statistical software package (version 4, Crunch Software Corporation, Oakland, CA). Missing data were handled in CRUNCH by leaving the variable blank and allowing the program to assign an internal missing value. Descriptive statistics were done to summarize sample characteristics and measures of central tendency for each variable. One-way, repeated measures analysis of variance (ANOVA) tests were done only on those subjects who had a complete set of data (i.e., data for all time points) for the variable under consideration. The Scheffe test for multiple comparisons was selected when post hoc contrasts were indicated because it is the most conservative of all the multiple comparisons procedures (Rossner, 1995). Differences between the demographic and dependent variables were evaluated using student's t-tests. Correlations between the variables were evaluated using Pearson Product-Moment correlations. Due to the presence of multiple dependent variables in this study, which may be correlated, the Type I error for each ANOVA test was set at the more conservative 0.01. A p-value of less than .05 was considered statistically significant for all other tests.

CHAPTER 4

RESULTS

Preliminary Data

A pilot study was conducted to refine the methodology and data collection techniques for each of the main measures (i.e., plasma E and NE, VTI, and FRT) in critically ill neonates. The pilot study was conducted in two phases using a sample of neonates from the NICU at Children's Hospital Oakland. The purpose of phase 1 was to determine if a 1.0 ml sample of whole blood was sufficient for detection of plasma E and NE levels in critically ill neonates using HPLC-ED. The purpose of phase 2 was to measure E, NE, VTI, and FRT before and after a bolus injection of morphine. A secondary aim of phase 2 was to determine if a 0.5 ml sample of whole blood was sufficient for detection of plasma E and NE levels in critically ill neonates using HPLC-ED. The pilot study demonstrated that measurement of E and NE levels, VTI, and FRT was feasible in critically ill neonates in the NICU setting. Only NE was detectable in the 0.5 ml whole blood sample. The entire phase 1 and 2 results are summarized in Appendix E.

Study Results

This descriptive study compared plasma NE levels, VTI, FRT, and cardiorespiratory status prior to surgery, prior to the first postoperative dose of morphine, and at 20 minutes and 1 hour following the administration of morphine in premature neonates. Demographic data, results for each specific aim, and additional findings are presented in the following sections.

Demographic Data

Characteristics of the Pregnancy

Table 3 provides a summary of the characteristics of the pregnancy for the neonates in the study. Sixty percent of the mothers of the neonates (N = 15) in

this study received complete prenatal care, while 16% (N = 4) received late prenatal care (i.e., after 23 weeks gestation), and 24% (N = 6) received no prenatal care. Seventy-six percent (N = 19) of the pregnancies were singletons and 24% (N = 6) were twin gestations. However, fetal demise of a twin occurred in three of the cases.

There was no maternal drug use during pregnancy by self-report or neonatal toxicology screen in 80% (N = 20) of the pregnancies. Those mothers who did report drug use during pregnancy used opioids (N = 1), alcohol and marijuana (N = 1), alcohol and tobacco (N = 1), marijuana (N = 1), or reported polydrug use, including cocaine and amphetamines (N = 1).

The major complications of the pregnancies included infection (N = 9) and/or premature rupture of membranes (N = 8), placenta previa (N = 2), and hypertension (N = 2). Steroids were administered prior to delivery in 40% (N = 10) of the cases. Neonates were delivered by spontaneous vaginal delivery in 52% (N = 13) of the cases, while the remainder (N = 12) were delivered by cesarean section.

There was only one parent who refused consent for their infant to participate in the study (i.e., refusal rate = 3.8%).

Neonatal Preoperative Characteristics

The study sample consisted of 25 neonates. Nineteen neonates were recruited from CHO and 6 neonates were recruited from UCSF. Table 4 provides a summary of the neonatal preoperative characteristics.

All neonates were premature with a mean gestational age of 26.3 weeks (SD = 2.35) and a mean birth weight of 0.87 kg (SD = 0.35). The majority of neonates (N = 16) were female. The study sample was varied in ethnicity with 36% (N = 9) African American, 32% (N = 8) Hispanic, 24% (N = 6) Caucasian, and 8% (N = 2)

Asian neonates.

One minute Apgar scores at birth were 3 or less in 32% (N = 8) of the neonates. At 5 minutes, only 8% (N = 2) of the neonates had Apgar scores of 3 or less. All neonates had a primary diagnosis of respiratory distress syndrome, except for 1 neonate with a primary diagnosis of tracheoesophageal fistula. Surfactant was administered immediately after birth to 84% (21) of the neonates.

Many of the neonates received treatment for hypotension (N = 14) and/or infection (N = 10) during the period prior to surgery. Some of the neonates received steroids (N = 7), and/or high frequency ventilation (N = 5) during the presurgical period. Comorbid conditions occurring in the presurgical period included bronchopulmonary dysplasia (N = 3), decreased renal function (N = 3), small intraventricular hemorrhage (N = 2), pulmonary interstitial emphysema (N = 2), and imperforate anus (N = 1). Three neonates had another surgical procedure performed prior to the thoracotomy.

Sixty-four percent (N = 16) of the neonates received at least one dose of an opioid in the presurgical period. Only 1 neonate received a dose of fentanyl within 4 hours of surgery and 2 neonates received a dose of morphine between 4 and 12 hours prior to surgery. The remaining 88% of the neonates (N = 22) did not receive any opioids within 12 hours of surgery. Most of the neonates (N = 19) received at least one course of indomethacin prior to surgery.

Neonatal discharge characteristics are shown in Table 5. Eighty percent (N = 20) of the neonates were alive at the time of discharge, while 20% (N = 5) of the neonates had expired. Medical diagnoses at the time of discharge requiring ongoing treatment are shown in Table 5 for the neonates who survived. The most common discharge diagnoses included bronchopulmonary dysplasia (N = 19), retinopathy of prematurity (N = 6), and status post bowel resection due to

necrotizing enterocolitis (N = 5).

Surgical Characteristics

Table 6 lists the surgical characteristics for the neonates in this study. The mean postnatal age at the time of surgery was 13.56 days (SD = ±8.01). The weight at the time of surgery (0.93 ± 0.39 kg; M ± SD) was only slightly higher than the birth weight (0.87 ± 0.35 kg; M ± SD). In the 19 neonates who received indomethacin prior to surgery, a mean of 5.68 days (range = 1 to 18 days) had elapsed since the last dose of indomethacin. Of the 17 neonates who received opioids prior to surgery, the mean duration of treatment was 3.94 days (range = 0 to 10 days). The blood urea nitrogen (BUN) and creatinine levels were elevated in most neonates at the time of surgery (mean BUN 29.47 mg/dl, normal range 8-25 mg/dl; mean creatinine 1.36 mg/dl, normal range = 0 to 0.6 mg/dl).

The majority of neonates (N = 21) received fentanyl as the single anesthetic agent. Three neonates received isoflurane in addition to fentanyl. One neonate received a local infiltration of marcaine at the thoracotomy site in addition to fentanyl anesthesia. The dose of iv fentanyl ranged from 16 to 149 mcg/kg (55.12 ± 37.97 mcg/kg, M ± SD). All neonates received a nondepolarizing neuromuscular blocking agent during surgery, with 84% (N = 21) of the neonates receiving pancuronium and 16% (N = 4) receiving vecuronium. The mean dose of neuromuscular blocking agent was 0.25 mg/kg. One neonate received a dose of atropine for intraoperative bradycardia. One neonate had pericardial fluid removed during surgery. In 48% (N = 12) of the neonates, a chest tube was inserted at the thoracotomy site during surgery.

Study Characteristics

Baseline measures were obtained preoperatively 3.91 ± 6.13 hours (M ± SD) prior to surgery and 2.07 ± 1.66 hours (M ± SD) after the last noxious procedure

(e.g., endotracheal tube suctioning). Concurrent therapies at the time that baseline measures were obtained included dopamine infusion in 32% (N = 8) of the neonates, insulin infusion in 8% (N = 2) of the neonates, and packed red blood cell transfusion in 8% (N = 2) of the neonates. Additional therapies and other descriptive characteristics of the neonates at the time of the measures, such as ventilator settings and arterial blood gas values, are listed in Table 7.

Morphine was administered within 12 hours after surgery in all neonates (4.96 $\pm$ 2.45 hours, $\underline{M} \pm \underline{SD}$). The most common reason (N = 9) for the nurse to administer morphine was an increase in vital signs (usually heart rate and blood pressure) with or without increased behavioral activity. In 28% (N = 7) of the cases, the nurses stated that they observed no specific signs indicating the neonate was in pain, but felt that it had been "long enough" after surgery that morphine should be given to prevent postoperative pain. For 36% (N = 9) of the neonates, the nurse did not state a reason for the administration of morphine.

At the time pre-morphine measures were obtained, 40% of the neonates (N = 10) were receiving packed red blood cell (PRBC) transfusions, 32% of the neonates (N=8) were receiving dopamine infusions, 12% of the neonates (N = 3) were receiving insulin infusions, and/or 8% of the neonates (N = 2) were receiving fluid boluses. At the time that post-morphine measures were obtained, only 12% of the neonates (N = 3) were still being transfused.

Specific Aims

Specific Aim 1: To determine changes in plasma E levels measured preoperatively, immediately before, and 20 minutes after bolus administration of the first iv dose of morphine given within the first 12 hours following thoracotomy.

Epinephrine was not detectable in any of the blood samples. Due to the small sample volume, the amount of E in the sample was below the limits of detection of the HPLC-ED system (i.e., 0.27 nmol).

Specific Aim 2: To determine changes in plasma NE levels measured preoperatively, immediately before, and 20 minutes after bolus administration of the first iv dose of morphine given within the first 12 hours following thoracotomy.

Plasma levels of NE for the preoperative baseline, pre-morphine, and 20 minute post-morphine measurements are shown in Table 8 and Figure 1 for the 18 neonates for whom complete data were obtained. A one-way, repeated measures ANOVA demonstrated no statistically significant differences between baseline plasma NE levels and pre-morphine and post-morphine plasma NE levels ($F(2, 34) = 2.73$, $p = .076$).

Specific Aim 3: To determine changes in VTI measured preoperatively, immediately before, 20 minutes after, and 1 hour after administration of the first iv dose of morphine given within the first 12 hours following thoracotomy.

Vagal tone index values for preoperative baseline, pre-morphine, 20 minute post-morphine, and 1 hour post-morphine measurements are shown in Table 8 and Figure 2 for the 22 neonates for whom complete data were obtained. A one-way, repeated measures ANOVA demonstrated statistically significant differences in VTI ($F(3, 63) = 7.18$, $p = .0001$) between the measures. Post hoc contrasts using

the Scheffe test revealed a statistically significant decrease in VTI between the preoperative baseline VTI and the pre-morphine, 20 minute post-morphine, and 1 hour post-morphine measures. There was a non-significant decrease in VTI between the pre- and post-morphine measures.

Seven neonates had a baseline VTI of zero. No statistically significant differences were found in age or weight of the neonates who had a VTI greater than zero at baseline (N = 15) compared to neonates who had a VTI of zero (N = 7) at baseline.

Specific Aim 4: **To determine changes in FRT measured preoperatively, immediately before, 20 minutes after, and 1 hour after administration of the first iv dose of morphine given within the first 12 hours following thoracotomy.**

Flexor reflex threshold values for preoperative baseline, pre-morphine, 20 minute post-morphine, and 1 hour post-morphine measurements are shown in Table 8 and Figure 3 for the 21 neonates for whom complete data were obtained. The expected brisk full-leg withdrawal response was obtained in 64% of the neonates (N = 14) preoperatively, and in only 1 to 3 neonates during the subsequent measurements. The remainder of the neonates responded with foot flexion or toe flexion. For this study, the greatest response obtained for each neonate at each measurement was included in the analyses. Neonates who did not respond were assigned a value of 16.80 grams.

Ten neonates had no flexor reflex response to the highest level of force (16.80 grams) used in this study at the pre-morphine and post-morphine measurements. Using Student's t-test, these neonates were significantly younger ($\underline{M} \pm \underline{SD}$ = 25.00 $\pm$ 1.25 vs. 27.17 $\pm$ 2.54 weeks; t = 2.83, p = .01) and smaller ($\underline{M} \pm \underline{SD}$ = 0.67 $\pm$ 0.09 vs. 1.00 $\pm$ 0.39 kg; t = 3.12, p = .007) at birth than the neonates who demonstrated a flexor reflex at the pre-morphine and post-

morphine measurements. The baseline FRT was not statistically significantly different between the two groups.

A one-way, repeated measures ANOVA was performed for the 11 neonates who demonstrated a flexor reflex response at the pre-morphine measurement. There were no statistically significant differences in FRT values between the preoperative baseline, pre-morphine, 20 minute post-morphine, and 1 hour post-morphine measures ($F(3, 30) = 0.75$, $p = .53$). Further comparisons of the neonates who did and did not demonstrate postoperative flexor reflex responses are presented in the last section of this chapter.

Specific Aim 5: **To determine changes in cardiorespiratory status (i.e., heart rate, respiratory rate, blood pressure, and oxygen saturation) measured preoperatively, immediately before, 20 minutes after, and 1 hour after administration of the first iv dose of morphine given within the first 12 hours following thoracotomy.**

The mean heart rate, respiratory rate, systolic blood pressure, diastolic blood pressure, mean blood pressure, and oxygen saturation values for preoperative baseline, pre-morphine, 20 minute post-morphine, and 1 hour post-morphine measurements are shown in Table 9 and Figure 4 (heart rate and blood pressure only) for the 24 neonates for whom complete data were obtained.

A one-way, repeated measures ANOVA demonstrated statistically significant differences in heart rates between the four time points ($F(3, 69) = 4.273$, $p = .008$). Post hoc contrasts using the Scheffe test revealed a statistically significant increase in pre-morphine and 20 minute post-morphine heart rate compared to baseline heart rate. There was no statistically significant differences in heart rates between the baseline and 1 hour post-morphine measures or between the pre- and post morphine measures.

A one-way, repeated measures ANOVA demonstrated statistically significant differences in respiratory rates between the four time points ($F(3, 66) = 8.470$, $p = .0001$). Post hoc contrasts using the Scheffe test revealed a statistically significant decrease in respiratory rate at the pre-morphine, 20 minute post-morphine, and 1 hour post-morphine time points compared to baseline respiratory rate. There were no statistically significant differences in respiratory rates between the pre- and post morphine time points.

A one-way, repeated measures ANOVA demonstrated no statistically significant differences in systolic, diastolic, or mean arterial blood pressure values between preoperative baseline, pre-morphine, 20 minute post-morphine, and 1 hour post-morphine measurements.

A one-way, repeated measures ANOVA demonstrated no statistically significant differences oxygen saturations between the four time points ($F(3, 69) = 3.126$, $p = .031$). Oxygen saturation tended to decrease slightly at the pre-morphine and 20 minute post-morphine time point compared to baseline oxygen saturation (mean decrease 2%). There were no differences between the baseline and 1 hour post-morphine values or between the pre-morphine and post-morphine measurements.

Specific Aim 6: To determine the relationships between plasma NE levels, VTI, and FRT at the preoperative baseline, pre-morphine, 20 minutes post-morphine, and 1 hour post-morphine (VTI and FRT only) measurement time points.

The correlation matrix for the study variables is shown in Table 10. No statistically significant correlations were found using Pearson product-moment correlations between 1) preoperative baseline NE and FRT, NE and VTI, VTI and FRT; 2) pre-morphine NE and FRT, NE and VTI, VTI and FRT; 3) 20 minutes post-morphine NE and FRT, NE and VTI, VTI and FRT; or 4) 1 hour post-

morphine VTI and FRT. Correlations between FRT at the pre- and post-morphine time points included only those neonates who demonstrated flexor reflex below the maximum value (i.e., 16.80 grams). There were no correlations between VTI and heart rate at any of the time points.

Specific Aim 7: **To determine the relationship between preoperative plasma NE levels and the degree of change in plasma NE levels following surgery and before and after morphine administration.**

Using Pearson Product-Moment correlations, no statistically significant correlations were found between baseline NE levels and the degree of change in NE levels from baseline to pre-morphine measures or from pre-morphine to post-morphine measures.

Specific Aim 8: **To determine the relationship between preoperative VTI levels and the degree of change in VTI levels following surgery and before and after morphine administration.**

Using Pearson Product-Moment correlations, a statistically significant negative correlation was found between baseline VTI values and the degree of change in VTI values from baseline to the pre-morphine time point ($r = -.83$, $p = .0001$). A statistically significant negative correlation was also found between the pre-morphine VTI values and the degree of change in VTI values between the pre-morphine and the 20 minute post-morphine and 1 hour post-morphine time points ($r = -.74$, $p = .0001$ and $r = -.81$, $p = .0001$, respectively). Therefore, higher preoperative baseline VTI values were strongly associated with a greater decrease in VTI following surgery. Higher pre-morphine VTI values were also strongly associated with a greater decrease in VTI at 20 minutes and 1 hour following morphine administration.

Influence of Demographic and Surgical Characteristics on Study Measures

Previous literature suggests that some of the demographic and surgical characteristics of the neonates in this study may influence plasma NE levels, VTI, FRT, or heart rate. Statistical analyses were performed using Pearson Product-Moment correlations to determine any associations between these characteristics and the study measures. Student's t-tests were used to determine differences in the study measures based on the presence or absence of specific characteristics. The results of these analyses are presented below.

Gestational Age, Birth Weight, and Postconceptual Age

The gestational ages of the neonates in the study were not associated with baseline plasma NE levels ($r = .24$, $p = .30$), VTI ($r = -.03$, $p = .09$), FRT ($r = -.19$, $p = .30$), or heart rate ($r = -.18$, $p = .38$). The birth weights of the neonates in this study were not associated with baseline plasma NE levels ($r = .15$, $p = .53$), VTI ($r = .15$, $p = .49$), FRT ($r = -.09$, $p = .70$), or heart rate ($r=-.03$, $p = .90$). The postconceptual ages of the neonates in this study were not associated with baseline plasma NE levels ($r = .27$, $p = .23$), VTI ($r = -.01$, $p = .96$), FRT ($r = -.05$, $p = .82$), or heart rate ($r = -.07$, $p = .72$).

Behavioral State

There were no statistically significant differences between the neonates who were in a deep sleep state as compared to the neonates who were in a light sleep state with respect to pre-morphine VTI (0.19 ± 0.37 vs. 0.55 ± 0.78, $t = 1.40$, $p = .19$), 20 minute post-morphine VTI (0.23 ± 0.49 vs. 0.56 ± 0.50, $t = -1.19$, $p = .30$), or 1 hour post-morphine VTI (0.19 ± 0.37 vs. 0.43 ± 0.77, $t = -0.60$, $p = .59$).

Dose of Fentanyl Anesthesia and Dose of Neuromuscular Blocking Agent

The dose of fentanyl anesthesia received by the neonates during surgery was not statistically significantly associated with pre-morphine plasma NE levels

(r = -.17, p = .44, VTI (r = .12, p = .56), FRT (r = .14, p = .50), or heart rate (r = .01, p = .97). The dose of neuromuscular blocking agent (i.e., pancuronium or vecuronium) received by the neonates during surgery was not statistically significantly associated with pre-morphine plasma NE levels (r = -.18, p = .41, VTI (r = -.15, p = .47), FRT (r = .21, p = .30), or heart rate (r = .01, p = .95).

Previous Opioid Exposure

There were no statistically significant differences between the neonates who had been exposed to opioids prior to surgery and the neonates who had no prior opioid exposure with respect to pre-morphine plasma NE levels (8.14±9.01 vs. 5.53±4.14, t = -0.73, p = .49), VTI (0.79±0.93 vs. 0.36±0.60, t = -1.13, p = .29), or FRT (1.00±0.95 vs. 1.89±3.84, t = 0.68, p = 0.50).

Length of Time between the End of Surgery and the Pre-Morphine Measurements

The length of time between the completion of surgery and the time that pre-morphine measures were obtained varied in this study. There were no statistically significant correlations between the time since surgery and pre-morphine plasma NE levels (r = -.07, p = .77, VTI (r = .16, p = .43), FRT (r = .25, p = .24), or heart rate values (r = -.16, p = .46).

Dopamine Infusion or PRBC Transfusion

There were no statistically significant differences between the neonates who received a dopamine infusion and the neonates who did not have an infusion with respect to baseline plasma NE levels (5.36±4.91 vs. 3.78±3.20, t = -0.77, p = .46) or pre-morphine plasma NE levels (7.26±4.45 vs. 5.85±6.85, t=-0.58, p = .57). There were no statistically significant differences between the neonates who received a dopamine infusion and the neonates who did not have an infusion with respect to baseline VTI (1.07±0.83 vs. 1.19±1.31, t = -0.25, p = .81) or pre-morphine VTI (0.44±0.81 vs. 0.50±0.69, t = .16, p = .57).

There were no statistically significant differences between the neonates who were receiving PRBC transfusions between the end of surgery and the time of the pre-morphine measures and the neonates who were not transfused with respect to pre-morphine plasma NE levels (7.33±8.46 vs. 5.69±3.79, t = -0.55, p = .60).

Additional Findings

When the study variables for each individual neonate were plotted, subgroups of neonates displaying distinctly different patterns of responses were identified. These subgroups were: 1) neonates who did not demonstrate a flexor reflex at the time of the pre-morphine measure and 2) neonates who expired prior to discharge from the NICU. The additional findings for these subgroups are presented below.

Absence of Flexor Reflex at the Time of the Pre-Morphine Measures

Ten neonates displayed no spontaneous movement or flexor reflex response at the pre-morphine and post-morphine measures (see Figure 3). Using Student's t-tests, there were no statistically significant differences in the amount of anesthesia or neuromuscular blocking agent per kilogram administered to the neonates who exhibited a flexor reflex response in the postoperative period compared to those who did not exhibit a flexor reflex response.

The plasma NE levels of the neonates who demonstrated a flexor reflex in the postoperative period were compared to the plasma NE levels of the neonates who did not exhibit a response. Using a two-way repeated measures ANOVA, there were no statistically significant group or time effects ($F(1, 16) = 0.61$, $p = .45$; $F(2, 32) = 2.45$, $p = .10$, respectively; Table 11). The VTI values of the neonates who demonstrated a flexor reflex in the postoperative period were compared to the VTI values of the neonates who did not exhibit a response.

Using a two-way, repeated measures ANOVA, there was a statistically significant group effect ($F(1, 20) = 7.95$, $p = .01$), statistically significant time effect ($F(3, 60) = 6.71$, $p = .0006$), but no statistically significant interaction effect (see Table 11 and Figure 5). These findings indicated that there were significant differences in VTI values between the two groups, that VTI changed over time, but that the differences in VTI between the two groups was not dependent on time.

Neonatal Mortality

The 5 neonates who expired prior to discharge from the NICU had significantly higher baseline heart rates ($M \pm SD = 164.58 \pm 8.08$ bpm) than the 20 neonates who were alive at discharge ($M \pm SD = 149.04 \pm 10.65$ bpm) using independent Student's t-test ($t = -3.0$, $p = .006$). There was no statistically significant difference in baseline VTI or NE between the neonates who expired or were alive at discharge. There was a near-significant trend toward a lower gestational age and a lower birth weight among the neonates who expired prior to discharge (Table 12).

The correlations between preoperative baseline VTI and the change in VTI from baseline to pre-morphine, and between the pre-morphine values and 20 minute post-morphine and 1 hour post-morphine values were not seen in the neonates who expired as were found in the neonates who survived. Visual inspection of the plot of the mean VTI values (Figure 7) showed differences in both baseline VTI (1.23 ± 1.31, $M \pm SD$ vs. 0.83 ± 0.85, $M \pm SD$) and degree of change in VTI (-0.86 ± 1.30, $M \pm SD$ vs. -0.12 ± 0.70) between the baseline and pre-morphine measures between the neonates who survived and the neonates who expired. However, these differences were not statistically significant.

CHAPTER 5

DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

This descriptive study compared plasma NE levels, VTI, FRT, and cardiorespiratory status prior to surgery, prior to the first postoperative dose of iv morphine, and at 20 minutes and 1 hour following the administration of morphine in premature neonates. It is the first study to attempt to triangulate neuroendocrine, physiological, and behavioral measures of nociceptive stress responses in premature neonates. In addition, it is the first study to evaluate changes in nociceptive stress response measures in neonates following a single iv dose of morphine. The use of the more powerful repeated measures design (Glantz & Slinker, 1990) also distinguishes this study from previous literature. This chapter presents a discussion of the study results, conclusions of the study, recommendations for further research, and nursing implications.

Discussion

This study provides further descriptive data regarding the stress responses of extremely premature neonates prior to and following surgery and new information regarding the neuroendocrine, physiological, and behavioral responses to the first dose of morphine administered during the postoperative period. The findings are discussed below in relationship to the effects of surgery and the administration of morphine on plasma NE, VTI, FRT, and cardiorespiratory responses. Other factors that may have affected the measures, potential confounding variables, and the significance of additional findings are also discussed. Lastly, the limitations of the study are reviewed.

Changes in Neonatal Nociceptive Stress Responses

Norepinephrine Responses to Surgery and Morphine Administration

At all time points in the study, the mean plasma NE levels of neonates were within the ranges reported in other studies of premature neonates undergoing surgery (Anand, Brown, Bloom, et al., 1985; Anand, Brown, Causon, et al., 1985; Anand et al., 1987). No statistically significant differences were found between the baseline plasma NE levels and the pre-morphine plasma NE levels (measured at approximately 5 hours after surgery), although there was a nonsignificant tendency for NE levels to be slightly increased from the preoperative baseline at the postoperative time points ($p = 0.08$). These findings are consistent with the results of previous studies (Anand, Brown, Bloom, et al., 1985; Anand, Brown, Causon, et al., 1985; Anand et al., 1987) that showed that plasma NE levels in premature neonates were significantly elevated at the end of surgery, but returned to near baseline levels by 6 hours following surgery.

No correlation was found in this study between preoperative baseline plasma NE levels and the degree in change in plasma NE levels between the baseline and pre-morphine time points. Previous research in adults (Stanley et al., 1975a, 1975b) suggests that the degree of change in NE levels during surgery is negatively associated with preoperative baseline NE levels. Further research is needed to determine if the measurement of NE levels at other time points (i.e., intraoperatively or at the end of surgery) would reveal a relationship between preoperative baseline NE levels and the NE response to surgery in premature neonates.

In this study, a single iv dose of morphine had no effect on plasma NE levels measured 20 minutes after the administration of the dose. Findings from previous studies evaluating the effect of opioid analgesia on plasma NE levels are

equivocal. Significantly decreased plasma NE levels were reported in one large study after 24 hours of continuous infusion of morphine (Quinn et al., 1992). However, no significant changes in plasma NE levels were observed in two smaller studies in which neonates received a continuous morphine infusion (Quinn et al., 1993) or a single iv injection of fentanyl (Cordero et al., 1991). Taken together with the findings from the present study, these data suggest that analgesic doses of opioids may not significantly alter plasma NE levels in neonates. Additional studies are needed to determine if plasma NE levels are altered based on the mode of opioid administration (i.e., continuous infusion or iv bolus) or if the levels of NE changed if measured at different time points than were done in this or previous studies.

Plasma E levels have been shown to decrease in neonates following opioid administration (Cordero et al., 1991; Quinn et al., 1993). Plasma E levels should be measured in future studies because changes in plasma E levels represent activation of other sympathetic pathways (i.e., sympathoadrenomedullary) that may better reflect responses to surgical stress or opioid analgesia than plasma NE levels (Frayn et al., 1985; Udelsman & Chrousos, 1988).

The Influence of Other Factors on Norepinephrine Responses

Plasma NE levels at the pre-morphine time point were not correlated with the amount of anesthesia administered in this study. This is in contrast to the findings from another study (Anand et al., 1987), that suggested that the amount and type of anesthesia administered to premature neonates may affect postoperative plasma NE levels. There are two possible explanations for the different findings between the studies: 1) differences in the anesthetic drug and dosage, and 2) differences in the timing of blood sample collection. In the Anand study (Anand et al., 1987), neonates who received inhalation anesthesia (nitrous

oxide) were compared to neonates who received fentanyl (10 mcg/kg) in addition to nitrous oxide. In this study, the neonates all received fentanyl anesthesia, although doses varied widely (16.39 to 149.25 mcg/kg). In the Anand study, significant differences in plasma NE levels between the two groups of neonates were found only at the end of surgery and at 24 hours postoperatively. Plasma NE levels were not measured at those time points in this study, due to the excessive amount of blood required. Additional studies that compare low-dose versus high-dose fentanyl anesthesia (i.e., 15 mcg/kg vs. 50 mcg/kg) and that measure plasma catecholamine levels for at least 24 hours postoperatively are needed in order to determine whether opioid anesthesia affects postoperative plasma NE levels in premature neonates.

No significant correlations were found between the dose of neuromuscular blocking agent and pre-morphine plasma NE levels, measured approximately 5 hours following the completion of surgery. Previous research (Cabal et al., 1985) suggests that neuromuscular blockade is associated with significantly increased NE levels 30 minutes after the administration of the drug in neonates. However, no studies have measured the effects of neuromuscular blocking agents on catecholamine levels beyond 30 minutes following the dose.

No statistically significant differences in plasma NE levels were found between neonates of different gestational or postconceptual ages. This finding is consistent with previous work that suggests that preterm neonates receiving intensive care have catecholamine levels equal to or greater than full-term neonates (Lagercrantz et al., 1986). Since other studies have demonstrated that tolerance and dependence to opioids can occur in the neonatal period (Doberczak, Kandall, & Willets, 1991; Van Praag & Frenk, 1991), differences in plasma NE levels between the neonates who had previous opioid exposure and those who

were not previously exposed to opioids were examined. However, no statistically significant differences in plasma NE levels were found based on the presence or absence of previous opioid exposure in the neonates in this study.

While dopamine infusion does not interfere with the plasma NE assay using HPLC-ED (Weissman & Jamdar, 1992), it may have had an indirect effect on plasma NE levels by stimulating the release of NE from nerve terminals (Hoffman & Lefkowitz, 1990). However, there is no evidence that dopamine infusion affected plasma NE levels in this study.

Since some of the neonates received transfusion of packed red blood cells during the postoperative period, plasma NE levels were compared to determine if the transfusions had a dilutional effect on plasma NE levels. No statistically significant differences were found between the plasma NE levels of neonates who received a PRBC transfusion compared to the plasma NE levels of the neonates who did not receive a transfusion during the postoperative period.

Vagal Tone Index Responses to Surgery and Morphine Administration

The mean baseline VTI values for neonates in this study were consistent with previous studies of premature neonates requiring intensive care (Porges 1988; Porges et al., 1982). Seven neonates had a preoperative baseline VTI of zero. Zero VTI may reflect the extreme immaturity of the CNS or CNS dysfunction in these neonates (Porges, 1982; 1988). Following surgery, VTI decreased significantly from baseline, with 13 of the neonates having a VTI of zero at the pre-morphine time point. While in 7 of the neonates the absence of VTI is consistent with their appearance of being under the effects of anesthesia and/or neuromuscular blockade (i.e., without spontaneous movement or flexor reflex response), the remaining 6 neonates with zero VTI displayed spontaneous respirations and movement as well as a flexor reflex response. Furthermore, 4 of

the neonates who appeared paralyzed had detectable VTI values. No statistically significant differences were found in any of the demographic or surgical characteristics of the neonates who exhibited VTI prior to the administration of morphine compared to those who did not, at any of the measurement time points. Because of these inconsistent findings, it cannot be determine whether the postoperative decreases in VTI were related to persistence of anesthetic effects, persistence of neuromuscular blockade, or surgical and postoperative stress. However, it is likely that the effects of fentanyl anesthesia on baroreceptor function may have persisted and confounded measurement of cardiac vagal tone (Duncan, Gregory, & Wade, 1981; Wear, Robinson, & Gregory, 1982). These observations require further study using substantially larger sample sizes and the use of other measures of cardiac function to draw any meaningful conclusions.

The nonsignificant tendency for VTI to decrease between the pre-morphine and post-morphine time points may represent the central depressant effect of morphine on cardiac vagal tone. Further research is needed in a sample of neonates in an non-operative context to evaluate the effects of opioid on VTI without the influence of anesthesia or neuromuscular blocking agents.

The Influence of Other Factors on Vagal Tone Index Responses

Vagal tone index is derived from RSA, representing the high frequency component of the total heart rate power spectrum. The lack of correlation between heart rate or ventilator rate and VTI is inconsistent with previous studies of VTI in neonates (DiPietro & Porges, 1991; Porges, 1988; Porter et al., 1988; van Ravenswaaij et al., 1995), but has been recently described in adults (Grossman & Kollai, 1993). The lack of correlation suggests that other determinants of heart rate that are not reflected in RSA were predominant in this sample of neonates. Additional analyses can be performed on the heart period

data collected in this study and may provide additional information on HRV that is not apparent using VTI because of the very low RSA in these extremely premature and ill neonates. For example, examination of the total heart rate power spectrum or of changes in the ratio of the high frequency and low frequency components of the power spectrum may provide more definitive information about the parasympathetic and sympathetic status of these neonates (Gordon et al., 1988; Oberlander et al., 1995).

In this study, there were no significant correlations between baseline VTI values and the gestational or postconceptual ages of the neonates. This is inconsistent with previous reports of a weaker, but statistically significant, correlation of .40 between VTI and gestational age in premature neonates as compared to full-term neonates (Porges, 1983, 1988). The lack of correlation in this sample of neonates may be due to the severity of illness, the extreme degree of prematurity, and/or the degree of mechanical ventilatory support required by the neonates in this study.

No statistically significant differences in VTI values were found based on differences in behavioral state. This finding is consistent with a previous study that showed measures of short-term HRV only distinguish deep and light sleep states in full-term neonates (Eiselt et al., 1993).

No other studies have examined VTI in neonates with previous opioid exposure or during the infusion of an inotropic agent. In this study, no statistically significant difference were found in VTI values based on previous opioid exposure or the infusion of dopamine.

There were no correlations found between the degree of change in VTI from the preoperative baseline to the pre-morphine measures and any of the above mentioned demographic or surgical characteristics. However, the magnitude of

the change in VTI following surgery and following morphine administration was strongly negatively correlated with the initial values (i.e., baseline or pre-morphine VTI, respectively). A relationship between baseline VTI values and the degree of change in VTI in response to a stressor may bias estimation of the true change in VTI related to surgery or morphine administration (Jemerin & Boyce, 1992). To correct for this potential bias, VTI values for the pre-morphine time point were regressed on baseline VTI and the VTI values at each post-morphine time point were regressed on pre-morphine VTI for each neonate. The residuals (i.e., deviations from the predicted mean) were then used as baseline-free estimates of the degree of change in VTI following surgery and morphine administration (Manuck et al., 1990). However, use of the residuals yielded no significant relationships between the degree of change in VTI and with any of the demographic or surgical variables.

Flexor Reflex Threshold Responses to Surgery and Morphine Administration

The mean preoperative baseline FRT responses of premature neonates in this study were elicited with forces within the ranges reported in two previous studies (Fitzgerald et al., 1988; Fitzgerald et al., 1989), but required approximately twice the force (0.44 vs. 0.20 grams) reported in the most recent study of neonates of similar gestational age (Andrews & Fitzgerald, 1994). Although large inter- and intra-individual variability in responses has been noted in all the studies to date, the mean difference between the results of the present study and those conducted by Fitzgerald and colleagues may be related to differences in the von Frey filaments (e.g., sharp or blunt tip) or the application technique used in the studies. The amount of exposure to or time since last heelstick could not be measured or controlled for in this study and may have

influenced the degree of force required to elicit a flexor reflex response (Fitzgerald et al., 1989).

Ten neonates displayed no spontaneous movement, movement in response to human touch, or flexor reflex in response to stimulation with von Frey filaments at the time pre-morphine measures were obtained. Interestingly, these neonates were significantly younger, smaller, and had significantly lower VTI at the preoperative baseline and pre-morphine time points than the neonates who demonstrated a flexor reflex response in the postoperative period. The absence of a flexor reflex response in these neonates may indicate that the effects of the neuromuscular blocking agent administered during surgery persisted for over 5 hours in these 10 neonates. Of note, there was no differences in dose of anesthesia or neuromuscular blocking agent administered to the neonates who demonstrated a flexor reflex and those who did not. Therefore, an alternative explanation for the lack of behavioral responsiveness in this subgroup of neonates may be activation of the endogenous opioid-mediated analgesia system (OMAS) in response to the stress of surgery and postoperative care. While data from human neonates are limited, numerous studies have found that the OMAS is readily activated in the neonatal rat by painful and non-painful stressors (see Kehoe, 1988 for review).

In the neonates who exhibited a flexor reflex prior to the administration of morphine, neither the surgery nor the administration of morphine had a significant effect on the flexor reflex responses. However, there was a non-significant tendency for FRT to be increased at the pre-morphine and post-morphine time points. In addition, the magnitude of the flexor reflex responses decreased from predominantly full, brisk, leg flexion to predominantly foot flexion only. This has not been previously reported. Decreases in the magnitude

of the responses may reflect direct spinal inhibition by opioid anesthesia/analgesia (Willer & Bussel, 1980) or activation of descending inhibitory pathways (Willer et al., 1981).

The Influence of Other Factors on Flexor Reflex Threshold Responses

Other factors that may have influenced FRT responses in the neonates in this study include gestational or postconceptual age, behavioral state, and previous opioid exposure. In this study, FRT was not significantly correlated with postconceptual age as has been previously reported (Andrews & Fitzgerald, 1994). This difference may be due to the severity of illness or extreme degree of prematurity of neonates in this study.

Previous exposure to opioids prior to surgery was also considered as a possible influence on FRT. However, there were no differences in FRT between the neonates who had previous opioid exposure and the neonates who were not exposed to opioids prior to surgery.

One technical concern in using the FRT method was the variability in the force exerted by the von Frey filaments during the course of the study. Frequent calibration of von Frey filaments using a gram scale was required to ensure accurate measurement of actual forces exerted. A better method might be to use a technique similar to that used in animal studies (e.g., Kinnman & Levine, 1995) in which the relative sensitivity of the animal is determined, by measuring the response frequency to a standardized series of stimulations with graded von frey filaments. Alternatively, research is currently being conducted using electromyography instead of von Frey filaments to more precisely quantify the flexor reflex responses of premature neonates (K. Andrews, personal communication, June, 1994).

Cardiorespiratory Responses to Surgery and Morphine Administration

In this study, heart rate tended to increase (mean increase approximately 6%; p=0.02) between the preoperative baseline and the pre-morphine measures in the neonates in this study. However, there were no statistically significant changes in any of the blood pressure values between the preoperative baseline and the pre-morphine measures. In addition, no correlations were found between heart rate and any of the blood pressure parameters. The lack of association between heart rate and blood pressure values may be explained by the fact that heart rate is the primary mechanism by which the premature neonate alters cardiac output in response to stress (Friedman, 1972; Teitel et al., 1985). In addition to the immaturity of baroreceptor function in premature neonates, even light levels of anesthesia abolish the baroresponse in premature neonates (Gregory, 1982).

Heart rate and blood pressure did not decrease significantly following the administration of morphine. These finding support recent research indicating that morphine can be safely administered to extremely premature neonates (e.g., Quinn et al., 1993) and that cardiovascular compromise following the administration of opioids is a significant risk only in hypovolemic neonates (Gregory, 1994). These findings are important because fear of bradycardia or hypotension associated with morphine administration in the extremely premature and critically ill neonate persists and leads clinicians to withhold opioid analgesics from premature neonates during the postoperative period.

Overall respiratory rate, defined as the rate of both spontaneous and ventilator-assisted breaths, decreased significantly between the preoperative baseline and the pre-morphine time points. It appears that the decrease in overall respiratory rate is due to a decrease in the spontaneous respiratory rates

of the neonates. In fact, there was a slight but significant increase in ventilator breath rate (mean increase 3.56 breaths per minute) between the baseline and pre-morphine time points. Furthermore, ventilator rate was highly correlated with respiratory rate at the pre-morphine and post-morphine time points, but not at baseline (baseline: $r = -.02$, $p = .93$; pre-morphine: $r = .76$, $p = .00001$; 20 minute post-morphine: $r = .79$, $p = .00001$; 1 hour post-morphine: $r = .61$, $p = .002$). However, ventilator rate was not correlated with baseline VTI ($r = -.40$, $p = .17$), pre-morphine VTI ($r = -.10$, $p = .74$) or 20 minute or 1 hour post-morphine VTI ($r = -.15$, $p = 0.06$, and $r = -.28$, $p = .36$, respectively). This is in contrast to a recent study of slightly older mechanically ventilated premature neonates (van Ravenswaaij et al., 1995).

These findings are consistent with the overall neurobehavioral depression observed in the neonates in this study during the postoperative period. Unfortunately, no conclusions can be drawn about the effects of morphine on respiratory rate because most of the neonates in the study were not breathing above the ventilator rate at the time of the pre- and post-morphine measures.

A nonsignificant decrease in oxygen saturation was observed between the baseline and pre-morphine time points in this study and administration of morphine had no effect on oxygen saturation. In fact, the mean oxygen saturation at all of the time points in the study was maintained above the range of 88 to 92% recommended for the prevention of oxygen toxicity in the premature neonate (Phillips et al., 1988).

Confounding Effects of Persistent Anesthesia and/or Neuromuscular Blockade

Persistence of the effects of fentanyl anesthesia during the postoperative period may have confounded the measurement of postoperative nociceptive stress responses and of the effects of morphine analgesia on nociceptive stress

responses. Little is known about the duration of the anesthetic or analgesic actions of fentanyl in neonates, although it is believed to have a short duration of action (Yaster, 1987). The elimination half-life of fentanyl in premature neonates undergoing PDA ligation is prolonged and variable, ranging from 6 to 32 hours, with a mean of 17.7 $\pm$ 9.3 hours ($\underline{M} \pm \underline{SD}$; Collins et al., 1985). Collins et al. (1985) proposed that the reason for the brief duration of action of fentanyl despite its long elimination half-life in premature neonates is the result of rapid redistribution of the drug from the brain into pharmacodynamically inert compartments such as fat and muscle, as has been demonstrated in animals (Hug & Murphy, 1979). A short duration of action and longer elimination half-life is also reported in adult humans, where the duration of action of fentanyl is 1 to 2 hours, while the elimination half-life is 3 to 4 hours (Jaffe & Martin, 1990).

The adequacy of the depth of fentanyl anesthesia is commonly determined by the degree of change in heart rate in response manipulation during surgery, most particularly, skin incision and skin closure (Collins et al., 1985; Robinson & Gregory, 1981; Yaster, 1987). Based on previous studies (Collins et al., 1985; Robinson & Gregory, 1981; Yaster, 1987), the neonates in this study would be expected to be responsive to noxious stimuli by the end of surgery or early in the postoperative period. For example, in one of the studies (Robinson & Gregory, 1981), the neonates were moving and breathing spontaneously within 1 hour after surgery, having received 30 to 50 mcg/kg fentanyl anesthesia, and required sedation within 1 hour of returning to the NICU.

While fentanyl anesthesia would not be expected to blunt perception of painful stimuli by the time of the pre-morphine measures (approximately 5 hours after the end of surgery), perturbations of the ANS control of the heart related to fentanyl anesthesia may have persisted in the neonates (Duncan et al., 1981;

Gregory, 1982; Wear et al, 1982). Thus, abnormal heart rate modulation related to anesthesia could have altered the VTI of neonates in this study.

There was no correlation between the amount of anesthesia administered and pre-morphine plasma NE, VTI, or FRT levels. Both the dosage and time between the end of surgery and the pre-morphine measures were uncontrolled and varied in this study. Interestingly, a significant positive correlation was found between the dose of fentanyl and the length of time from the end of surgery to the time of the first administration of morphine ($r = .70$, $p = .0001$) which may have prevented detection of a relationship between anesthetic dose and the study measures.

Persistence of the effects of neuromuscular blockade during the postoperative period may also have confounded measurement of postoperative nociceptive stress responses or the effects of morphine analgesia on nociceptive stress responses. Pancuronium (and vecuronium) can increase heart rate, blood pressure, and plasma catecholamines in neonates (Cabal, et al., 1985). These agents also possess cardiac vagolytic actions (Porges, 1991). The effects of these drugs on heart rate and blood pressure have been shown to be of short duration, with heart rate and blood pressure returning to resting values within 1 hour (Cabal et al., 1985). Meretoja and Luosto (1990) reported spontaneous recovery of movement in neonates from neuromuscular blockade within 72 minutes following a single dose of pancuronium. A similar duration of action was found for vecuronium (Fisher, Castagnoli, & Millere, 1985). However, in premature neonates receiving continuous paralysis for a mean of 4 days with a mean dose per day of 0.3 mg/kg, neuromuscular blockade persisted for a mean of 9 hours and was more difficult to reverse than in older neonates (Goudsouzian, Crone, & Todres, 1981). Thus, it is probable that the effects of the neuromuscular

blocking agents persisted in some of the neonates in this study. At the time pre- and post-morphine measures were obtained, use of train-of-four peripheral nerve stimulation (Ali, 1993) would have been useful in order to determine the degree of residual neuromuscular blockade in this sample of neonates.

Significance of Additional Findings

An interesting observation, not directly related to the study aims, was the finding that a rapid heart rate in the preoperative period was associated with a significantly greater incidence of neonatal mortality of the neonates in this study. This finding is supported by previous research on fetal and neonatal heart rate conducted in the 1960's and 1970's (Martin et al., 1974; Rudolph, Valbona, & Desmond, 1965). For example, in a study of premature and full term neonates (Kero, 1974), heart rate was found to be slightly but significantly higher in neonates who expired compared to those who survived.

More recently, HRV has been suggested as a more sensitive predictor of mortality than heart rate (Cabal, Siassi, Zanini, Hodgman, & Hon, 1980; Goldstein et al., 1993; Jenkins, Reid, & McClure, 1980). However, in this study, mortality was associated with tachycardia and was not associated with HRV as measured by VTI. The different findings may be related to differences in the gestational age or greater severity of illness of the neonates in the studies. Further research is needed to determine whether heart rate or heart rate variability is a better predictor of mortality or morbidity in extremely premature and critically ill neonates.

A larger sample size may have revealed a statistically significant difference between the neonates who survived and the neonates who expired in baseline VTI, or in the degree of change in VTI from the baseline to the pre-morphine measure. The tendency for lower baseline VTI and decreased degree of change in

VTI related to surgery in the neonates who expired suggests an association between loss of VTI reactivity and higher risk of mortality. Further investigation using a larger sample of neonates is warranted.

Limitations of the Study

Interpretation of the findings of this study are limited by a number of factors. First, neonates of similar age, weight, medical diagnosis, and surgical procedure were recruited for the study in order to obtain a study sample as homogeneous as possible. However, the actual sample was heterogenous with regard to severity of illness as evidenced by the variation in the amount of ventilatory support and other concurrent therapies at the time of the study (see Table 7). Unfortunately, few measures exist to accurately quantify and compare the severity of illness of neonates. Future studies should include a measure of severity of illness, such as the Score of Neonatal Acute Physiology (SNAP; Richardson, Gray, McCormick, Workman, & Goldmann, 1993).

A second limitation of the study is the large inter-individual variability in the data. A larger sample size would have provided additional power to determine statistically significant differences for some measures, compensated for small effect sizes that might have been associated with some of the measures, and/or allowed for stratification of the sample into more homogeneous subgroups.

Third, the determination of changes in plasma catecholamine levels was limited by the small amount of blood that could be obtained from critically ill and premature neonates and by the relatively large amount of blood required to assay for the plasma catecholamines or to obtain data on blood levels of other relevant substances such as fentanyl or morphine. At least 0.5 ml of whole blood is required for each measure of NE and 1.0 ml of blood from an indwelling intra-arterial catheter is required to detect both E and NE using HPLC-ED. Another

1.0 ml of blood would be required to assay for plasma fentanyl or morphine.

Fourth, measures of stress responses in the postoperative period may have been confounded by prolonged effects of fentanyl anesthesia or neuromuscular blockade, as described above. The lack of any other "gold standard" measure of nociception or analgesia in neonates limits interpretation of these data. While train-of-four testing may be useful in determining degree of neuromuscular blockade, no validated measure of depth of anesthesia or degree of analgesia exists for premature neonates at this time.

Lastly, interpretation of the findings from this study are limited by the fact that changes in the measures due to the stress response may occur in the same direction as changes in the measures due to analgesic agents. For example, VTI may decrease during nociceptive stress and may also decrease in response to the central depressant effect of morphine. Thus, differentiation of the effects of stress from those of analgesia is difficult.

Conclusions

The following conclusions can be made from this study:

1. Plasma NE levels were not significantly altered following surgery at approximately 5 hours postoperatively, nor were they altered by the administration of the first postoperative dose of morphine.
2. Surgery produced a significant decrease in VTI in the postoperative period that was not altered by the administration of morphine.
3. Neither surgery nor the administration of morphine significantly altered the FRT in neonates who were displaying spontaneous movements at the time of morphine administration.
4. There was a subgroup of neonates who did not exhibit a flexor reflex in the postoperative period. These neonates were significantly younger and

smaller than the neonates who had a flexor reflex postoperatively. They also demonstrated significantly lower preoperative and post-morphine VTI, and a nonsignificant tendency for higher NE levels postoperatively.

5. Morphine administered to critically ill extremely premature neonates during the early postoperative period had no statistically significant or clinically adverse effects on cardiorespiratory stability.
6. There was no relationship among any of the measures of stress response in this study.
7. A rapid heart rate during the preoperative period may be associated with greater neonatal mortality.

Directions for Future Research

This study has generated many questions for further research. Recommendations of future research studies include:

1. Repeat this study in full-term neonates undergoing surgery, measuring stress responses at additional time points during the postoperative period (e.g., 12, 24, 48, 72 hours postoperatively) and following additional doses of opioid analgesia.
2. Describe changes in plasma E levels preoperatively, and before and after the first postoperative dose of morphine in premature neonates.
3. Describe changes in E, NE, VTI, FRT, and vital signs before and after morphine administered for non-surgical analgesia or before and after brief surgical procedures (e.g., central line insertion) in premature neonates.
4. Examine the HRV data set from this study using other spectral analysis techniques to determine the relative contribution of parasympathetic and sympathetic systems to nociceptive stress and analgesia.
5. Retrospectively examine the relationship between rapid heart rate and

neonatal mortality in a large sample of premature neonates, including neonates who underwent surgery and those who did not.

6. Develop a neonatal animal model of postoperative pain to evaluate measures and to further test hypotheses regarding the role of the autonomic nervous system in nociceptive stress responses, perhaps using specific tests of autonomic reactivity not feasible in critically ill neonates.
7. Examine nursing decisions to administer morphine postoperative in the absence of specific physiological or behavioral indications of pain.

Nursing Implications

Nurses are responsible for the assessment of postoperative pain in premature neonates and for providing appropriate pharmacologic treatment. These responsibilities require accurate methods of assessment of postoperative pain and of the relief of pain. Such assessment methods are not currently available for critically ill and or extremely premature (i.e., less than 30 weeks gestation) neonates undergoing surgery.

This study evaluated three methods of measuring nociceptive stress responses in premature neonates in order to detect changes following the administration of morphine during the postoperative period. Unfortunately, changes in the stress response measures were not observed following the administration of morphine, possibly due to the magnitude of change in the measures related to the surgery, the variability in responses among the neonates, and/or the effects of prolonged anesthesia or neuromuscular blockade. Nevertheless, nurses caring for premature neonates must make judgements about the neonate's need for analgesia. This study provides further evidence that morphine can be safely administered to extremely premature and critically ill neonates without compromising their cardiovascular status. In the absence of

objective measures, nurses caring for neonates in this study determined the need for analgesia by evaluating the dose of anesthesia received during surgery, the duration of time since surgery, and postoperative changes in vital signs and behavior.

Nurses must continue to be actively involved in research to develop sensitive and specific measures for assessment of pain and pain relief that are feasible to perform at the bedside of the critically ill neonate in the NICU.

References

Aarimaa, T., Kero, P., Valimaki, I. (1985). Simultaneous monitoring of transcutaneous blood gases and heart rate variability in neonates. Critical Care Medicine, 13(1), 9-11.

Ali, H. (1993). Monitoring neuromuscular blockade. In M. Rogers, J. Tinker, B. Covino, & D. Longnecker (Eds.), Principles and practices of anesthesiology (pp. 27-845). St. Louis: Mosby.

Allan, M. T., & Crowell, M. D. (1989). Patterns of autonomic response during laboratory stressors. Psychophysiology, 26, 603-614.

Als, H. (1984). Newborn behavioral assessment. In W. J. Burns & J. V. Lavigne (Eds.), Progress in Pediatric Psychology (pp. 1-46). New York: Grune & Stratton.

Anand, K. J. S. (1990). The biology of pain perception in newborn infants. In D. C. Tyler & E. C. Krane (Eds.), Advances in pain research and therapy: Vol. 15. Pediatric pain (pp. 113-122). New York: Raven Press.

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

Anand, K. J. S., & Aynsley-Green, A. (1985). Metabolic and endocrine effects of surgical ligation of patient ductus arteriosus in the human preterm neonate: Are there implications for further improvement of postoperative outcome? Modern Problems in Paediatrics, 23, 143-157.

Anand, K. J. S., & Aynsley-Green, A. (1988a). Does the newborn infant require potent anesthesia during surgery? Answers from a randomized trial of halothane anesthesia. In R. Dubner, G. F. Gebhart, & M. R. Bond (Eds.), Proceedings of the Vth world congress on pain (pp. 329-335). New York:

Elsevier.

Anand, K. J. S., & Aynsley-Green, A. (1988b). Measuring the severity of surgical stress in newborn infants. Journal of Pediatric Surgery, 23(4), 297-305.

Anand, K. J. S., Brown, M. J., Bloom, S. R., & Aynsley-Green, A. (1985). Studies on the hormonal regulation of fuel metabolism in the human newborn infant undergoing anesthesia and surgery. Hormone Research, 22, 115-125.

Anand, K. J. S., Brown, M. J., Causon, R. C., Christofides, N. D., Bloom, S. R., & Aynsley-Green, A. (1985). Can the human neonate mount an endocrine and metabolic response to surgery? Journal of Pediatric Surgery, 20(1), 41-48.

Anand, K. J. S., & Hickey, P. R. (1992). Halothane-morphine compared with high dose sufentanil for anesthesia and post-operative analgesia in neonatal cardiac surgery. New England Journal of Medicine, 326(1), 1-9.

Anand, K. J. S., Sippell, W. G., & Aynsley-Green, A. (1987). Randomized trial of fentanyl anaesthesia in preterm babies undergoing surgery: Effects on the stress response. Lancet, 1, 243-248.

Andrews, K., & Fitzgerald, M. (1994). The cutaneous flexor reflex in human neonates: Sensitization, receptive fields, and the effects of contralateral stimulation. Pain, 56, 95-101.

Arendt, R. E., Halpern, L. F., MacLean, W. E., & Youngquist, G. A. (1991). The properties of V in newborns across repeated measures. Developmental Psychobiology, 24(2), 91-101.

Asterita, M. F. (1985). The physiology of stress. New York: Human Sciences Press.

Assali, N. S., Brinkman, C. R., Woods, J. R., Dandavino, A., &

Nuwayhid, B. (1977). Humoral control of fetal, neonatal, and adult cardiovascular function. American Journal of Obstetrics and Gynecology, 129, 748-759.

Auguy-Valette, A., Cros, J., Gouarderes, C., Gout, R., & Pontonnier, G. (1978). Morphine analgesia and cerebral opiate receptors: A developmental study. British Journal of Pharmacology, 63, 303-308.

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

Barrett, D. A., Elias-Jones, A. C., Rutter, N., Shaw, P. N., & Davis, S. S. (1991). Morphine kinetics after diamorphine infusion in premature neonates. British Journal of Clinical Pharmacology, 32, 31-37.

Barrington, K., & Finer, N. (1991). The natural history of the appearance of apnea of prematurity. Pediatric Research, 29(4), 372-375.

Baumgartner, H., Ritch, R., Luz, O., Schneeberger, J., & Hammerer, I. (1992). Capillary versus arterial plasma catecholamines as markers for sympatho-adrenal activity. Pediatric Research, 31(6), 579-582.

Bayon, A., Shoemaker, W., Bloom, F., Mauss, A., & Guillemin, R. (1979). Perinatal development of the endorphin and enkephalin-containing systems in the rat brain. Brain Research, 179, 93-101.

Bell, S. G., & Ellis, L. J. (1987). Use of fentanyl for sedation of mechanically ventilated neonates. Neonatal Network, 5(6), 27-31.

Benedetti, C. (1990). Acute pain therapy: A review of its effects and therapy with systematic opioids. In C. Benedetti, R. Chapman, & G. Giron, (Eds.), Advances in pain research and therapy, Vol. 14 (pp. 367-423). New York: Raven Press.

Berry, F. A., & Gregory, G. A. (1987). Do premature infants require anesthesia for surgery? Anesthesiology, 67, 291-293.

Bhat, R., Abu-Harb, M., Chari, G., & Gulati, A. (1992). Morphine metabolism in preterm neonates. Journal of Pediatrics, 120(5), 795-799.

Bhat, R., Chari, G., Gulati, A., Aldana, O., Velamati, R., & Bhargava, H. (1990). Pharmacokinetics of a single dose of morphine in preterm infants during the first week of life. Journal of Pediatrics, 117(3), 477-481.

Bhat, R., Chari, G., & Iver, R. (1994). Postconceptual age influences pharmacokinetics and metabolism of morphine in sick neonates. Pediatric Research, 35(4, pt. 2), 81A.

Bistoletti, P., Nylund, L., Lagercrantz, H., Hjemdahl, P., & Strom, H. (1983). Fetal scalp catecholamines during labor. American Journal of Obstetrics and Gynecology, 147(7), 785-788.

Blenow, M., Zeman, J., Dahlin, I., & Lagercrantz, H. (1994). Increased noradrenaline turnover and depletion of noradrenaline stores in the brain following perinatal asphyxia. Pediatric Research, 35(4, pt. 2), 82A.

Bohus, B., Benus, R. F., Fokkema, D. S., Koolhaas, J. M., Nyakas, C., van Oortmerssen, G. A., Prins, A. J. A., de Ruiter, A. J. H., Scheurink, A. J. W., & Steffens, A. B. (1987). Neuroendocrine states and behavioral and physiological stress responses. In E. R. de Kloet, V. M. Wiegant, & D. de Wied (Eds). Progress in brain research (pp. 57-70). New York: Elsevier.

Bovill, J. G., Sebel, P. S., & Stanley, T. H. (1984). Opioid analgesics in anesthesia: With special reference to their use in cardiovascular anesthesia. Anesthesiology, 61, 731-755.

Boyce, W. T., Barr, R. G., & Zeltzer, L. K. (1992). Temperament and the psychobiology of childhood stress. Pediatrics, 90(3), 483-486.

Brazelton, T. B. (1973). Neonatal behavioral assessment scale. Clinics in Developmental Medicine, 50, Philadelphia: J.B. Lippincott.

Bromm, B., & Seide, K. (1982). The influence of tilidine and prazepam on withdrawal reflex, skin reaction and pain rating in man. Pain, 12, 247-258.

Bromm, B., & Treede, R.D. (1980). Withdrawal reflex, skin resistance reaction and pain ratings due to electrical stimuli in man, Pain, 9, 339-354.

Brown, J. S., Gee, H., Olah, K. S., Docker, M. F., & Taylor, E. W. (1992). A new technique for identification of respiratory sinus arrhythmia in utero. Journal of Biomedical Engineering, 263-267.

Bryan-Brown, C. W. (1986). Development of pain management in critical care. In M. F. Cousins & G. D. Phillips (Eds.), Acute pain management (pp. 1-20). New York: Churchill Livingstone.

Cabal, L. A., Siassi, B., & Hodgman, J. E. (1992). Neonatal clinical cardiopulmonary monitoring. In A. A. Fanaroff & R. J. Martin (Eds.), Neonatal-perinatal medicine: Diseases of the fetus and infant (pp. 437-455). St. Louis: Mosby.

Cabal, L. A., Siassi, B., Artal, R., Gonzalez, F., Hodgman, J., & Plajstek, C. (1985). Cardiovascular and catecholamine changes after administration of pancuronium in distressed neonates. Pediatrics, 75(2), 284-287.

Cabal, L. A., Siassi, B., Zanini, B., Hodgman, J. E., & Hon, E. E. (1980). Factors affecting heart rate variability in preterm infants. Pediatrics, 65(1), 50-56.

Campbell, B. C., Parikh, R. K., Naismith, A., Sewnauth, D., & Reid, J. L. (1984). Comparison of fentanyl and halothane supplementation to general anesthesia on stress response to upper abdominal surgery. British Journal of Anaesthesia, 56, 257-261.

Canarelli, J. P., Poulain, H., Clamadieu, C., Ricard, J., Maingourd, Y., & Quintaro, J. M. (1994). Ligation of the patent ductus arteriosus in preterm infants-indications and procedures. Neonatal Intensive Care, May/June, 50-53.

Cannon, W. B. (1929). Organization for physiological homeostasis. Physiological Reviews, 9(3), 339-431.

Carr, D. B., & Murphy, M. T. (1988). Operation, anesthesia, and endorphin system. International Anesthesia Clinics, 26, 199-205.

Carruthers, M., & Taggart, P. (1973). Vagotonicity of violence: Biochemical and cardiac responses to violent films and television programmes. British Medical Journal, 3, 384-389.

Cepeda, M. S., & Carr, D. B. (1993). The neuroendocrine response to postoperative pain. In F. M. Ferante & J. R. VadeBoncouer (Eds.), Postoperative pain management (pp. 79-106). New York: Churchill Livingstone.

Chan, C. W. Y., & Dallaire, M. (1989). Subjective pain assessment is linearly correlated with flexion reflex in man. Brain Research, 479, 145-150.

Charnay, Y., Paulin, C., Dray, F., Dubois, P. M. (1984). Distribution of enkephalin in human fetus and infant spinal cord: An immunofluorescence study. Journal of Comparative Neurology, 233, 415-423.

Chernow, B, Alexander, H. R., Smallridge, H. C., Thompson, W. R., Cook, D., Beardsley, D., Fink, M. P., Lake, C. R., & Fletcher, R. (1987). Hormonal responses to graded surgical stress. Archives of Internal Medicine, 147, 1273-1278.

Choonara, I. A., McKay, P., Hain, R., & Rane, A. (1989). Morphine metabolism in children. British Journal of Clinical Pharmacology, 28, 599-604.

Christie, J., Santiero, M., Stromquist, C., & Torres, B. (1995). Pharmacokinetics of continuous infusion of fentanyl in very premature newborns.

Critical Care Medicine, 23(1, suppl.), A320.

Chrousos, G. P., & Gold, P. W. (1992). The concepts of stress and stress system disorders. Journal of the American Medical Association, 267(9), 1244-1252.

Clendeninn, N. J., Petraitis, M., & Simon, E. J. (1976). Ontological development of opiate receptors in rodent brain. Brain Research, 118, 157-160.

Coda, B. (1989). Reflex measures related to nociception in humans. In C.R. Chapman & J.D. Loesser (Eds.), Advances in pain research and therapy, Vol. 12: Issues in pain measurement (pp. 361-364). New York: Raven Press.

Cohen, M. R., Pickar, D., Dubois, M., & Bunney, W. E. (1982). Stress-induced plasma beta-endorphin immunoreactivity may predict postoperative morphine usage. Psychiatry Research, 6, 7-12.

Cohen, G., Xu, C., & Henderson-Smart, D. (1991). Ventilatory response of the sleeping newborn to CO_2 during normoxic rebreathing. Journal of Applied Physiology, 71(1), 168-174.

Coll, C. G. (1990). Behavioral responsivity in preterm infants. Clinics in Perinatology, 17(1),113-123.

Collins, C., Koren, G., Crean, P., Klein, J., Roy, W. L., & MacLeod, S. M. (1985). Fentanyl pharmacokinetics and hemodynamic effects in preterm infants during ligation of patent ductus arteriosus. Anesthesia and Analgesia, 64, 1078-1080.

Cordero, L., Gardner, D. K., & O'Shaughnessy, R. (1991). Analgesia versus sedation during broviac catheter placement. American Journal of Perinatology, 8(4), 284-287.

Cousins, M. (1989). Acute and postoperative pain. In P. D. Wall & R. Melzack (Eds.), Textbook of Pain (2nd. ed., pp. 284-305). New York: Churchill

Livingstone.

Davies, C. L., Newman, R. J., Molyneux, S. G., & Grahame-Smith, D. G. (1984). The relationship between plasma catecholamines and severity of injury in man. Journal of Trauma, 24(2), 99-105.

DeBrouker, T., Willer, J.C., & Bergeret, S. (1989). The nociceptive flexion reflex in humans. In C. R. Chapman & J. D. Loesser (Eds.), Advances in pain research and therapy, Vol. 12: Issues in pain measurement (pp. 337-35). New York: Raven Press.

Dellinger, J. A., Taylor, H. L., & Porges, S. W. (1987). Atropine sulfate effects on aviator performance and on respiratory-heart period interactions. Aviation Space Environmental Medicine, 58, 333-338.

DiPietro, J. A., Cusson, R. M., Caughty, M. O., Fox, N. A. (1994). Behavioral and physiologic effects of nonnutritive sucking during gavage feeding in preterm infants. Pediatric Research, 36(2), 207-214.

DiPietro, J. A., & Porges, S. W. (1991). Vagal responsiveness to gavage feedings an index of preterm status. Pediatric Research, 29(3), 231-236.

Doberczak, T. M., Kandall, S. R., & Willets, I. (1991). Neonatal opiate abstinence syndrome in term and preterm infants. Journal of Pediatrics, 118(6), 933-937.

Domek, N. S., Barlow, C. F., & Roth, L. J. (1960). An ontogenetic study of phenobarbital-C^{14} in cat brain. Journal of Pharmacology and Experimental Therapy, 130, 285-293.

Donchin, Y. Constantini, S., Szold, Byrne, E. A., Porges, S. W. (1992). Cardiac vagal tone predicts outcome in neurosurgical patients. Critical Care Medicine, 20(7), 942-949.

Dowman, R. (1992). Possible startle response contamination of the spinal

nociceptive withdrawal reflex. Pain, 49, 187-197.

Duncan, P., Gregory, G. A., & Wade, J. (1980). The effect of nitrous oxide on the baroreceptor response of newborn and adult rabbits. Canadian Anesthetists' Society Journal, 28, 339-341.

Eiselt, M., Curzi-Dascalova, L., Clairambault, J., Kauffman, F., Medigue, C., & Peirano, P. (1993). Heart-rate variability in low-risk prematurely born infants reaching normal term: a comparison with full-term newborns. Early Human Development, 32, 183-195.

Elias-Jones, A. C., Barrett, D.A., Rutter, N., Shaw, P. N., & Davis, S. S. (1991). Diamorphine infusion in the preterm neonate. Archives of Disease in Childhood, 66, 1155-1157.

Eliot, R. J., Lam, B. S., Leake, R. D., Hobel, C. J., & Fisher, D. A. (1980). Plasma catecholamine concentrations in infants at birth and during the first 48 hours of life. Journal of Pediatrics, 96(2), 311-315.

Fahmy, N. R., Sunder, N., & Soter, N. A. (11983). Role of histamine in the hemodynamic and plasma catecholamine response to morphine. Clinical Pharmacology and Therapeutics, 33(5), 615-620.

Falconer, A. D., Lake, D., & MacDonald, I. A. (1982). The measurement of plasma noradrenaline by high-performance liquid chromatography with electrochemical detection: An assessment of sample stability and assay reproducibility. Journal of Neuroscience Methods, 6, 261-271.

Farrington, E. A., McGuinnes, G. A., Johnson, G. K., Erenberg, A., & Leff, R. D. (1993). Continuous intravenous morphine infusion in postoperative newborn infants. American Journal of Perinatology, 10(1), 84-87.

Faxelius, G., Lagercrantz, H., & Yao, A. (1984). Sympathoadrenal activity and peripheral blood flow after birth: Comparison in infants delivered

vaginally and by cesarean section. Journal of Pediatrics, 105(1), 144-148.

Feldberg, W., & Wei, E. (1977). The central origin and mechanism of cardiovascular effects of morphine as revealed by naloxone in cats. Journal of Physiology, 272, 99P.

Feldberg, W., & Wei, E. (1978). Central sites at which morphine acts when producing cardiovascular effects. Journal of Physiology, 275, 57P.

Fisher, D. M., Castagnoli, K., & Miller, R. D. (1985). Vecuronium kinetics and dynamics in anesthetized infants and children. Clinical Pharmacology and Therapeutics, 37, 402-406.

Fitzgerald, M. (1987). Cutaneous primary afferent properties in the hind limb of the neonatal rat. Journal of Physiology, 383, 79-92.

Fitzgerald, M. (1988). The development of activity evoked by fine diameter cutaneous fibers in the spinal cord of the newborn rat. Neuroscience Letters, 86, 161-166.

Fitzgerald, M. (1991a). A physiological study of the prenatal development of cutaneous sensory inputs to dorsal horn cells in the rat. Journal of Physiology, 432, 473-482.

Fitzgerald, M. (1991b). The developmental neurobiology of pain. In M. R. Bond, J. E. Charlton, & C. J. Woolf (Eds.), Proceedings of the Vth World Congress on Pain (pp. 253-261). New York: Elsevier.

Fitzgerald, M., & Anand, K.J.S. (1993). Developmental neuroanatomy and neurophysiology of pain. In N. L. Schechter, C. B. Berde, & M. Yaster (Eds.), Pain in infants, children, and adolescents (pp. 11-31). Baltimore: Williams & Wilkins.

Fitzgerald, M., Millard, C., & McIntosh, N. (1988). Hyperalgesia in premature infants. Lancet, 6, 292.

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

Fitzgerald, M., Shaw, A., & McIntosh, N. (1988). Postnatal development of the cutaneous flexor reflex: Comparative study of preterm infants and newborn rat pups. Developmental Medicine and Child Neurology, 30, 520-526.

Fox, N. A. (1983). Maturation of autonomic control in preterm infants. Developmental Psychology, 16(6), 495-504.

Fox, N. A., & Porges, S. W. (1985). The relationship between neonatal heart period patterns and developmental outcome. Child Development, 56, 28-37.

Franck, L. S. (1986). A new method to quantitatively describe pain behavior in infants. Nursing Research, 35(1), 28-31.

Franck, L. S. (1992). The influence of sociopolitical, scientific, and technological forces on the study and treatment of neonatal pain. Advances in Nursing Science, 15(1), 11-20.

Franck, L. S. & Gregory, G. A. (1993). Clinical evaluation and treatment of infant pain in the neonatal intensive care unit. In N. L. Schechter, C. B. Berde, & M. Yaster (Eds.), Pain in infants, children, and adolescents (pp. 519-535). Baltimore: Williams & Wilkins.

Frayn, K. N., Little, R. A., Maycock, P. F., & Stoner, H. B. (1985). The relationship of plasma catecholamines to acute metabolic and hormonal responses to injury in man. Circulatory Shock, 16, 229-240.

Friedman, W. F. (1972). The intrinsic physiologic properties of the developing heart. Progress in Cardiovascular Diseases, 15(1), 87-111.

Friesen, R. H., & Henry, D.B. (1986). Cardiovascular changes in preterm neonates receiving isoflurane, halothane, fentanyl, and ketamine.

Anesthesiology, 64, 238-242.

Gauntlett, I. S., Fisher, D. M., Hertzka, R. E., Kuhls, E., Spellman, M. J., & Rudolph, C. (1988). Pharmacokinetics of fentanyl in neonatal humans and lambs: Effects of age. Anesthesiology, 69, 683-687.

Gelhorn, E. (1968). Central nervous system tuning and its implications for neuropsychiatry. Journal of Nervous and Mental Disease, 147(2), 148-162.

Gill, N. E., Behnke, M., Conlon, M., McNeely, J. B., & Anderson, G. C. (1988). Effect of non-nutritive sucking on behavioral state in preterm infants before feeding. Nursing Research, 37(6), 347-350.

Glantz, S. A., & Slinker, B. K. (1990). Primer of applied regression and analysis of variance. New York: McGraw-Hill.

Gold, P. W., Goodwin, F. K., & Chrousos, G. P. (1988). Clinical and biochemical manifestations of depression: Relation to the neurobiology of stress. New England Journal of Medicine, 319(7), 413-420.

Goldenthal, E. I. (1971). A compilation of LD50 values in newborn and adult animals. Toxicology and Applied Pharmacology, 18, 185-207.

Goldstein, B., DeKing, D., Delong, D. J., Kempski, M. H., Cox, C., Kelly, M. M., Nichols, D. D., & Woolf, P. D. (1993). Autonomic cardiovascular state after severe brain injury and brain death in children. Critical Care Medicine, 21(2), 228-233.

Goldstein, B., Woolf, P. D., DeKing, D., Delong, D. J., Cox, C., & Kempski, M. H. (1994). Heart rate power spectrum and plasma catecholamine levels after postural change and cold pressor test. Pediatric Research, 36(3), 358-363.

Goldstein, D. S. (1986). Sample handling and preparation for liquid chromatography and electrochemical assays for plasma catecholamines. In A. M

Krstulovic (Ed.), Quantitative analysis of catecholamines and related compounds. (pp. 126-135). New York: Halstead Press.

Goldstein, D. S., Feuerstein, G., Izzo, J. L., Kopin, I. J., & Keiser, H. R. (1981). Validity and reliability of liquid chromatography with electrochemical detection for measuring plasma levels of norepinephrine and epinephrine in man. Life Sciences, 28, 467-475.

Goldstein, D. S., McCarty, R., Polinsky, R.J., & Kopin, I. J. (1983). Relationship between plasma norepinephrine and sympathetic neural activity. Hypertension, 5, 552-559.

Goldstein, R. F. & Brazy, J. E. (1991). Narcotic sedation stabilizes arterial blood pressure fluctuations in sick premature infants. Journal of Perinatology, 11(4), 365-371.

Gordon, D., Herrera, V. L., McAlpine, L., Cohen, R. J., Akselrod, S., Lang, P., & Norwood, W. I. (1988). Heart-rate spectral analysis: A noninvasive probe of cardiovascular regulation in critically ill children with heart disease. Pediatric Cardiology, 9, 69-77.

Gorski, P. A., Hole, W. T., Leonard, C. H., & Martin, J. A. (1983). Direct computer recording of premature infants and nursery care: Distress following two interventions. Pediatrics, 72(2), 198-202.

Gottfried, A. W., & Gaiter, J. L. (1985). Infants under intensive care: Environmental neonatology. Baltimore: University Park Press.

Goudsouzian, N. G., Crone, R. K., & Todres, I. D. (1981). Recovery from pancuronium blockade in the neonatal intensive care unit. British Journal of Anaesthesia, 53, 1303-1309.

Grauer, T. T. (1989). Environmental lighting, behavioral state, and hormonal response in the newborn. Scholarly Inquiry for Nursing Practice, 3(1),

53-66.

Greenough, A., Lagercrantz, H., Pool, J., & Dahlin, I. (1987). Plasma catecholamine levels in preterm infants: Effects of birth asphyxia and apgar score. Acta Paediatrica Scandinavia, 76, 54-59.

Greenough, A., Nicolaides, K. H., & Lagercrantz, H. (1990). Human fetal sympathoadrenal responsiveness. Early Human Development, 23, 9-13.

Gregory, G. A. (1982). The baroreceptor responses of preterm infants during halothane anaesthesia. Canadian Anesthetists' Society Journal, 29(2), 105-106.

Gregory, G. A. (1992). Neonatal Anesthesia. In A. A. Fanaroff & R. J. Martin (Eds.), Neonatal-perinatal medicine: Diseases of the fetus and infant. (pp. 446-464). St. Louis: Mosby.

Gregory, G. A. (1994). Pharmacology. In G.A. Gregory (Ed.), Pediatric anesthesia (3rd. ed., pp. 13-45). New York: Churchill Livingstone.

Gregory, G. A. (1994). Anesthesia for premature infants. In G.A. Gregory (Ed.). Pediatric anesthesia (3rd. ed., pp. 351-373), New York: Churchill Livingstone.

Greisen, G., Frederiksen, P. S., Hertel, J., & Christensen, (1985). Catecholamine response to chest physiotherapy and endotracheal suctioning in preterm infants. Acta Paediatrica Scandinavia, 74, 525-529.

Groome, I. J., Mooney, D. M., Bentz, L. S., & Singh, K. P. (1994). Analysis of heart rate variability during quiet sleep in normal human fetuses between 36-40 weeks gestation. Early Human Development, 38, 1-10.

Grossman, P. (1992). Respiratory and cardiac rhythms as windows to central and autonomic biobehavioral regulation: Selection of window frames, keeping the panes clean and viewing the neural topography. Biological

Psychology, 34, 131-161.

Grossman, P., & Kollai, M. (1993). Respiratory sinus arrhythmia, cardiac vagal tone, and respiration: Within- and between-individual relations. Psychophysiology, 30, 486-495.

Gunnar, M. R., Connors, J., & Isensee, J. (1989). Lack of stability in neonatal adrenocortical reactivity because of rapid habituation of the adrenocortical response. Developmental Psychobiology, 22(3), 221-233.

Gunnar, M. R., Connors, J., Isensee, J. & Wall, L. (1988). Adrenocortical activity and behavioral distress in human newborns. Developmental Psychobiology, 21(4), 297-310.

Gunnar, M. R., Hertsgaard, L., Larson, M., & Rigatuso, J. (1992). Cortisol and behavioral responses to repeated stressors in the human newborn. Developmental Psychobiology, 24(7), 487-505.

Hallman, H., Farnebo, L., Hamberger, B., & Jonsson, G. (1978). A sensitive method for the determination of plasma catecholamines using liquid chromatography with electrochemical detection. Life Sciences, 23, 1049-1052.

Halter, J. B., Pflug, A. E., & Porte, D. (1977). Mechanisms of plasma catecholamine increase during surgical stress in man. Journal of Clinical Endocrinology, 45, 936-944.

Halliwill, J. R., & Billman, G. E. (1992). Effect of general anesthesia on cardiac vagal tone. American Journal of Physiology, 262, H1719-H1724.

Hansen-Flaischen, J. H., Brazinsky, S., Basile, C., & Lanken, P. N. (1991). Use of sedating drugs and neuromuscular blocking agents in patients requiring mechanical ventilation for respiratory failure. Journal of the American Medical Association, 266(20), 2870-2875.

Harpin, V. A., & Rutter, N. (1983). making heel pricks less painful.

Archives of Disease in Childhood, 58, 226-228.

Hartley, R., Green, M., Quinn, M., & Levene, M. I. (1993). Pharmacokinetics of morphine infusion in premature neonates. Archives of Disease in Childhood, 69, 55-58.

Hay, W. W., Brockway, J. M., & Eyzaguirre, M. (1989). Neonatal pulse oximetry: Accuracy and reliability. Pediatrics, 83(5), 717-722.

Hicks, H. C., Mobray, A. G., & Yhap, E. O. (1987). Cardiovascular effects of catecholamine response to high dose fentanyl-O_2 for induction of anesthesia in patients with ischemic coronary artery disease. Anesthesia and Analgesia, 60, 563-568.

Hill-Smith, I., & Purves, R. D. (1978). Synaptic delay in the heart: An iontophoretic study. Journal of Physiology, 279, 31-54.

Hoffman, B. B., & Lefkowitz, R. J. (1990). Catecholamines and sympathomimetic drugs. In A. Goodman Gilman, T. W. Rall, A. S. Nies, & P. Taylor (Eds.), The pharmacological basis of therapeutics (8th. ed., pp. 187-220), New York: Pergamon Press.

Hornchen, H., Betz, R., Kotlarek, F., & Roebruck, P. (1983). Microprocessor-based long term cardiorespirography II. Status evaluation in term and preterm newborns. Journal of Perinatal Medicine, 11, 32-42.

Huey, J. R., Paul, R. H., Hadjiev, R. A., Jilek, J., & Hon, E. H. (1979). Fetal heart rate variability: An approach to automated assessment. American Journal of Obstetrics, 134, 691-695.

Hug, C. C., & Murphy, M. R. (1979). Tissue redistribution of fentanyl and termination of its effects in rats. Anesthesiology, 55, 309-315.

Humphrey, T. (1964). Some correlations between the appearance of human fetal reflexes and the development of the nervous system. Progress in Brain

Research, 4, 93.

Hunt, C. E. (1991). Cardiorespiratory monitoring. Clinics in Perinatology, 18(3), 473-495.

Inoue, K., Samdelov, L. F., & Arndt, J. O. (1980). Fentanyl activates a particular population of vagal efferents which are cardioinhibitory. Archives of Pharmacology, 312, 57-63.

Irazuzta, J., Pascucci, R., Perlman, N., & Wessel, O. (1993). Effects of fentanyl administration on respiratory system compliance in infants. Critical Care Medicine, 21(7), 1001-1004.

Izard, C. E., Porges, S. W., Simmons, R. F., Haynes, D. M., & Cohen, B. (1991). Infant cardiac reactivity: Developmental changes and reactions with attachment. Developmental Psychobiology, 27(3), 432-439.

Jaattela, A., Alho, A., Avikainen, V., Karaharju, E., Kataja, J., Lahdensuu, M., Lepisto, P., Rokkanen, P., & Tervo, T. (1975). Plasma catecholamines in severely injured patients: A prospective study on 45 patients with multiple injuries. British Journal of Surgery, 62, 177-181.

Jaffe, J. H., & Martin, W. R. (1990). Opioid analgesics and antagonists. In A. Goodman Gilman, T. W. Rall, A. S. Nies, & P. Taylor (Eds.), The pharmacological basis of therapeutics (8th. ed., pp. 485-521), New York: Pergamon Press.

Jenkins, J. G., Reid, M., & McClure, B. G. (1980). Study of heart rate variability in sick newborn infants. Acta Paediatrica Scandinavia, 69, 393-396.

Johnson, M. M., & Sexton, D. L. (1990). Distress during mechanical ventilation: Patients' perceptions. Critical Care Nurse, 10(7), 48-57.

Karwande, S. V., & Rowles, J. R. (1992). Simplified muscle sparing thoracotomy for patent ductus arteriosus ligation in neonates. Annals of Thoracic

Surgery, 54, 161-163.

Karemaker, J. M. (1987). Neurophysiology of the baroreceptor reflex. In R. I. Kline & O. Rompelman (Eds.), The beat to beat investigation of cardiovascular function (pp. 27-49). Oxford: Clarendon Press.

Kato, M., Komatsu, T., Kimura, T., Sugiyama, F., Nakashima, K., & Shimada, Y. (1992). Spectral analysis of heart rate variability during isoflurane anesthesia. Anesthesiology, 77, 669-674.

Katona, P. G., & Jih, F. (1975). Respiratory sinus arrythmia: Non-invasive measure of parasympathetic cardiac control. Journal of Applied Physiology, 39, 801-805.

Katona, P. G., Frasz, A., & Egbert, J. (1980). Maturation of cardiac control in full term and preterm infants during sleep. Early Human Development, 4(2), 145-159.

Kehlet, H. (1984). The stress response to anaesthesia and surgery: release mechanisms and modifying factors. Clinical Anaesthesiology, 2, 315-339.

Kehlet, H. (1986). Pain relief and modification of the stress response. In M. F. Cousins & G. D. Phillips (Eds.), Acute pain management (pp. 49-76). New York: Churchill-Livingstone.

Kehlet, H. (1989). Surgical stress: The role of pain and analgesia. British Journal of Anesthesia, 63, 189-195.

Kehoe, P. (1988). Opioids, behavior, and learning in mammalian development. In E. M. Blass (Ed.), Handbook of behavioral neurobiology, Vol. 9, Developmental psychobiology and behavioral ecology (pp. 309-346). New York: Plenum Press.

Kehoe, P., & Blass, E. M. (1986). Behaviorally functional opioid systems in infant rats: II. Evidence for pharmacological, physiological, and psychological

mediation of pain and stress. Behavioral Neuroscience, 100(5), 624-630.

Kent, J., Pert, C., & Herkenham, M. (1982). Ontogeny of opioid receptors in rat forebrain: Visualization by in vitro autoradiography. Developmental Brain Research, 2, 487-504.

Kero, P. (1974). Heart rate variations in infants with the respiratory distress syndrome. Acta Paediatrica Scandinavia, (suppl. 250), 1-69.

Kinnman, E., & Levine, J. D. (1995). Involvement of the sympathetic postganglionic neuron in capsaicin-induced secondary hyperalgesia in the rat. Neuroscience, 65(1), 283-291.

Kirby, M. L. (1981). Development of opiate receptor binding in rat spinal cord. Brain Research, 205, 400-404.

Kirkin, J. K. (1990). Neonatal patent ductus surgery. In W. A. Long (Ed.), Fetal and neonatal cardiology (pp. 754-759). Philadelphia: W. B. Saunders.

Koehntop, D. E., Rodman, J. H., Brundage, D. M., Hegland, M. G., & Buckley, J. J. (1986). Pharmacokinetics of fentanyl in neonates. Anesthesia & Analgesia, 65, 227-232.

Koltry, F. S., Ebert, T. S., Vicuns, E. J., Roerig, D. L., & Kampine, J. P. (1984). Baroreceptor reflex control of heart rate during morphine, diazepam, N_2O/O_2 anesthesia in humans. Anesthesiology, 61, 538-563.

Kono, K., Philbin, D. M., Coggins, C. H., Moss, J., Roscow, C. E., Schneider, R. C., & Slater, E. E. (1981). Renal function and stress response during halothane or fentanyl anesthesia. Anesthesia and Analgesia, 60, 552-556.

Kopin, I. J. (1989). Plasma levels of catecholamines and dopamine-B-hydroxylase. In U. Trendelenberg & N. Weiner (Eds.), Catecholamines II. (pp. 211-256). Berlin: Springer-Verlag.

Kopin, I. J., Eisenhofer, G., & Goldstein, D. (1988). Sympathoadrenalmedullary system and stress. In G. P. Chrousos, D. L. Loriaux, & P. W. Gold (Eds.), Mechanisms of physical and emotional stress (pp. 11-23). New York: Plenum Press.

Koren, G., & Maurice, L. (1989). Pediatric uses of opioids. Pediatric Clinics of North America, 36(5), 1141-1156.

Koren, G., Butt, W., Chinyanga, H., Soldin, S., Tan, Y. K., & Pape, K. (1985). Post-operative morphine infusion in newborn infants: Assessment of disposition characteristics and safety. Journal of Pediatrics, 107(6), 963-967.

Kostovic, I., & Goldman-Rakic, P. S. (1984). Transient cholinesterase staining in the mediodorsal nucleus of the thalamus and its connections in the developing human and monkey brain. Journal of Comparative Neurology, 219, 431-447.

Kupferberg, H. J., & Way, E. L. (1963). Pharmacologic basis for the increased sensitivity of the newborn rat to morphine. Journal of Pharmacology and Experimental Therapy, 141, 105-112.

Lagercrantz, H., & Bistoletti, P. (1973). Catecholamine release in the newborn infant at birth. Pediatric Research, 11, 889-893.

Lagercrantz, H., Nilsson, E., Redham, I., & Hjemdahl, P. (1986). Plasma catecholamines following nursing procedures in a neonatal ward. Early Human Development, 14, 61-65.

Latson, T. W., & O'Flaherty, D. (1993). Effects of surgical stimulation on autonomic reflex function: Assessment by changes in heart rate variability. British Journal of Anaesthesia, 70, 301-305.

Laubie, M., & Schmitt, H. (1983). Origin of the hypotensive and sympathoinhibitory effect of morphinomimetic agents. European Journal of

Pharmacology, 91, 431-439.

Laubie, M., Schmitt, H., Canellas, J., Roquebert, J., & DeMichel, P. (1974). Centrally mediated bradycardia and hypotension induced by narcotic analgesia: Dextromoramide and fentanyl. European Journal of Pharmacology, 28, 66-75.

Leslie, F., Tso, S., & Hurlbut, D. (1982). Differential appearance of opiate receptor subtypes in neonatal rat brain. Life Sciences, 31, 1393-1396.

Levene, M. I., & Quinn, M. W. (1992). Use of sedatives and muscle relaxants in newborn babies receiving mechanical ventilation. Archives of Disease in Childhood, 67, 870-873.

Lewis, M. (1992). Individual differences in responses to stress. Pediatrics, 90(3), 487-490.

Lewis, M., Thomas, D. A., & Worobey, J. (1990). Developmental organization, stress, and illness. Psychological Science, 1(5), 316-318.

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

Lynn, A. M., Nespeca, M. K., Opheim, K.E., & Slattery, J. T. (1993). Respiratory effects of intravenous morphine infusions in neonates, infants, and children after cardiac surgery. Anesthesia and Analgesia, 77, 695-701.

Lynn, A. M., & Slattery, J. T. (1987). Morphine pharmacokinetics in early infancy. Anesthesiology, 66, 136-139.

Manuck, S. B., Kasprowicz, A. L., & Muldoon, M. F. (1990). Behaviorally-evoked cardiovascular reactivity and hypertension: Conceptual issues and potential associations. Annals of Behavioral Medicine, 12(1), 17-29.

Marin-Padilla, M. (1983). Structural organization of the human cerebral cortex prior to the appearance of the cortical plate. Anatomical Embryology, 168,

21-40.

Martin, W. R. (1983). Pharmacology of opioids. Pharmacology Review, 35, 283-323.

Martin, C. B., Siassi, B., & Hon, E. H. (1974). Fetal heart rate patterns and neonatal death in LBW infants. Obstetrics and Gynecology, 44(4), 503-510.

Maulik, P., Siani, V., & ZiGrossi, S. T. (1983). Clinical significance of short-term variability computed from heart rate waveforms. Journal of Perinatal Medicine, 4, 243-248.

McIntosh, N., Stephen, R., Smith, A., Cunningham, S. (1994). Are blood pressure waves due to hormone surges? Pediatric Research, 35(4, pt. 2), 241A.

McLaughlin, C. R., Lichtman, A. H., Fanselow, M. S., & Cramer, C. P. (1990). Tonic nociception in neonatal rats. Pharmacology, Biochemistry & Behavior, 36, 859-862.

Mehandru, P., Assel, B., Johnson, V., Fanaroff, A., & Kalhan, S. (1991). Catecholamine response to birth in preterm newborns. Pediatric Research, 229(4, pt. 2), 227A.

Meretoja, O. A. & Luosto, T. (1990). Dose-response characteristics of pancuronium in neonates, infants, and children. Anaesthesia and Intensive Care, 18, 455-459.

Miller, J., Law, A. B., Parker, R., A., Sundell, H., Silberberg, A. R., & Cotton, R. B. (1994). Journal of Pediatrics, 125, 97-103.

Mirochnick, M., Meier, J., Frank, D. A., Cabral, H., Needleman, R., & Zuckerman, B. (1994). Plasma catecholamine concentrations in cocaine exposed and unexposed neonates. Pediatric Research, 35(4, pt. 2), 25A.

Moller, I. W., Dinesen, K., Sondergards, D., Knigge, & Kehlet, H. (1988). Effect of patient controlled analgesia on plasma catecholamine, cortisol,

and glucose concentrations after cholecystectomy. British Journal of Anaesthesia, 61, 160-164.

Morse, C. A., & McIntosh, N. (1994). Assessing analgesia of morphine and hyperalgesia of withdrawal in neonates using the flexor withdrawal reflex. Children and Pain. Third International Symposium on Pediatric Pain. June 7-9, 197A.

Munck, A., Guyre, P. M., & Holbrook, N. J. (1984). Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocrine Reviews, 5(1), 25-44.

Neuman, M. R. (1992). Biomedical engineering aspects of neonatal monitoring. In A. A. Fanaroff & R. J. Martin (Eds.), Neonatal-perinatal medicine: Diseases of the fetus and infant (pp. 420-436). St. Louis: Mosby.

Newlin, D. B., Wong, C. J., & Cheskin, L. J. (1992). Cardiovascular responses to naloxone challenge in opiate dependent individuals. Pharmacology, Biochemistry and Behavior, 43, 357-360.

Newnham, J. P., Marshall, C. L., Padbury, J. F., Lam, R. W., Hobel, C. J., & Fisher, D. A. (1984). Fetal catecholamine release with preterm delivery. American Journal of Obstetrics & Gynecology, 149(8), 888-893.

Nistrup-Madsen, S., Fog-Muller, F., Christiansen, C., Vester-Andersen, T., & Engquist, A. (1978). Cyclic AMP, adrenaline and noradrenaline in plasma during surgery. British Journal of Surgery, 65, 191-193.

Nitsche, E. M., Richter, A., Raasch, W., Dominiak, P., Moller, J., Gortner, L. (1994). Catecholamines in VLBW infants. Pediatric Research, 35(4, pt. 2), 243A.

Norris, S., Campbell, L. A., & Brenkert, S. (1982). Nursing procedures and alterations in transcutaneous oxygen tension in premature infants. Nursing

Research, 31(6), 330-336.

Oberlander, T. F., Berde, C. B., Lam, K. H., Rappaport, L. A., & Saul, J. P. (1995). Infants tolerate spinal anesthesia with minimal overall autonomic changes: Analysis of heart rate variability in former premature infants undergoing hernia repair. Anesthesia and Analgesia, 80, 20-27.

Oberlander, T. F., Berde, C. B., Lam, K. H., & Saul, J. P. (1994). Halothane causes withdrawal of cardiac sympathetic modulation in infants: Transfer function analysis of respiratory sinus arrhythmia. Pediatric Research, 35(4, pt. 2), 87A.

O'Gara, P. T. (1988). The hemodynamic consequences of pain and its management. Journal of Intensive Care Medicine, 3, 3-5.

Panksepp, J., Meeker, R., & Bean, N. J. (1979). The neurochemical control of crying. Pharmacology, Biochemistry and Behavior, 12, 437-443.

Pasternak, G. W., Zhang, A. Z., Tecott, L. (1980). Developmental differences between high and low affinity opiate binding sites: Their relationship to analgesia and respiratory depression. Life Sciences, 27, 1185-1190.

Perry, E. H., Bada, H. S., Ray, J. D., Korones, S., Arheart, K., & Magill, H. L. (1990). Blood pressure increases, birth weight-dependent stability boundary, and intraventricular hemorrhage. Pediatrics, 85(5), 727-733.

Petrie, R. H. (1993). Dose/response effects of intravenous meperidine on fetal heart rate variability. Journal of Maternal-Fetal Medicine, 2, 215-222.

Petrie, R. H., Yeh, S., & Murata, Y. (1978). The effects of drugs on fetal heart rate variability. American Journal of Obstetrics and Gynecology, 130, 294-299.

Phillips, G. D., & Cousins, M. F. (1986). Neurological mechanisms of pain and the relationship to pain, anxiety, and sleep. In M. F. Cousins & G. D.

Phillips (Eds.), Acute pain management (pp. 21-48), New York: Churchill-Livingstone.

Phillips, B. L., McQuitty, J., & Durand, D. J. (1988). Blood gases: Technical aspects and interpretation. In J. P. Goldsmith & E. H. Karotkin (Eds.), Assisted ventilation of the newborn (2nd. ed., pp. 213-232). Philadelphia: W. B. Saunders.

Phillippe, M. (1983). Fetal catecholamines. American Journal of Obstetrics and Gynecology, 146(7), 840-855.

Pokela, M. (1994). Pain relief can reduce hypoxemia in distressed neonates during routine treatment procedures. Pediatrics, 93(3), 379-383.

Porges, S.W. (1983). Heart rate patterns in neonates. A potential diagnostic window to the brain. In T. M. Field & A. Sostek (Eds.), Infants born at risk: Physiological and perceptual processes (pp. 3-22). New York: Grune & Stratton.

Porges, S. W. (1985). Method and apparatus for evaluating rhythmic oscillations in aperiodic physiologic response systems. U. S. patent no. 4,510,944.

Porges, S. W. (1988) Neonatal vagal tone: Diagnostic and prognostic implications. In P. M. Vietze & H. G. Vaughan (Eds.), Early identification of infants with developmental disabilities (pp. 147-159). New York: Grune & Stratton.

Porges, S. W. (1991). Vagal mediation of respiratory sinus arrythmia: Implications for drug delivery. In W. J. M. Hrushesky, R. Langer, & F. Theeuwes (Eds.), Annals of the New York Academy of Sciences, Vol. 618, Temporal control of drug delivery (pp. 57-66), New York: New York Academy of Sciences.

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

Porges, S. W., & Borher, R. E. (1990). The analysis of periodic processes in psychosocial research. In J. T. Cacioppo & L. G. Tassinary (Eds.), Principles of psychophysiology (pp. 708-753). Cambridge: Cambridge University Press.

Porges, S. W., McCabe, P. M. & Yongue, B. G. (1982). Respiratory-heart rate interactions: Psychophysiological implications for pathophysiology and behavior. In J. T. Cacioppo & R. E. Petty (Eds.), Perspectives in cardiovascular psychophysiology (pp. 223-263), New York: Guilford Press.

Porter, F. L. (1989). Pain in the newborn. Clinics in Perinatology, 16(2), 549-564.

Porter, F. L., Miller, P., Cole, S., & Marshall, R. E. (1991). A controlled clinical trial of local anesthetic for lumbar punctures in newborns. Pediatrics, 88(4), 663-669.

Porter, F. L., Porges, S., & Marshall, R. E. (1988). Newborn pain cries and vagal tone: Parallel changes in response to circumcision. Child Development, 59, 495-505.

Porter, F. L., Ultman, M., Miller, J. P., Arfken, C., Cohlan, B., & Altman, D. (1989). Vagal tone in preterm infants at risk for chronic lung disease. Pediatric Research, 25, 227A.

Potter, E. K. (1981). Inspiratory inhibition of vagal responses to baroreceptor and chemoreceptor stimuli in the dog. Journal of Physiology, 316, 177-190.

Prechtl, H. F. R. (1974). The behavioral states of the newborn infant (A review). Brain Research, 76, 185-212.

Pretorius, M. B., Wong, C. J., & Newlin, D. B. (1990). Cardiovascular

components of the response to morphine. NIDA Research Monograph Series, 105, 417-418.

Price, D. D. (1989). Nociceptive reflexes in man. In C. R. Chapman & J. D. Loesser (Eds.), Advances in pain research and therapy, Vol. 12 (pp. 3533-359). New York: Raven Press.

Prietsch, V., Knoepke, U., & Obladen, M. (1994). Continuous monitoring of heart rate variability in preterm infants. Early Human, Development, 37, 117-131.

Purcell-Jones, G., Dormon, F., & Sumner, E. (1987). The use of opioids in neonates: A retrospective study of 933 cases. Anaesthesia, 42, 1316-1320.

Quinn, M. W., Otoo, F., Rushforth, J. A., Dean, H. G., Puntis, J. W. L., Wild, J., & Levene, M. I. (1992). Effect of morphine and pancuronium on the stress response in ventilated preterm infants. Early Human Development, 30, 241-248.

Quinn, M. W., Wild, J., Dean, H. G., Hartley, R., Rushforth, J. A., Puntis, J. W. L., & Levene, M. I. (1993). Randomised double-blind controlled trial of effect of morphine on catecholamine concentrations in ventilated preterm babies. Lancet, 342, 324-327.

Rakic, P., & Goldman-Rakic, P. S. (1982). Development and modifiability of the cerebral cortex. Neuroscience Research Progress Bulletin, 20, 433-451.

Ralston, H. J. (1984). Synaptic organization of spinothalamic projections to the thalamus, with special reference to pain. Advances in pain research and therapy, Vol. 6, (pp. 183-195). New York: Raven Press.

Randall, D. C. (1991). Plasticity of the unconditional response: Evidence linking pain and cardiovascular regulation. Journal of Cardiovascular Electrophysiology, (suppl. 2), 576-582.

Rawlings, D. J., Miller, P. A., & Engel, R. R. (1980). The effect of circumcision on transcutaneous PO_2 in term infants. American Journal of Diseases in Childhood, 134, 676-678.

Ready, L. D. (1990). Patient controlled analgesia - Does it provide more than comfort? (editorial). Canadian Journal of Anaesthesia, 37, 719-720.

Richardson, D. K., Gray, J. E., McCormick, M. C., Workman, K., & Goldmann, D. A. (1993). Score for neonatal acute physiology: A physiologic severity index for neonatal intensive care. Pediatrics, 91(3), 617-623.

Rizvi, T., Wadhas, S., Bijlani, V. (1987). Development of spinal substrate for nociception. Pain, (suppl. 4), 195-1919.

Robinson, S., & Gregory, G.A. (1981). Fentanyl-air-oxygen anesthesia for ligation of patent ductus arteriosus in preterm infants. Anesthesia and Analgesia, 60(5), 331-334.

Romijn, H. J., Hofman, M. A., & Gramsbergen, A. (1991). At what age is the developing cerebral cortex of the rat comparable to that of the full-term newborn human baby? Early Human Development, 26, 61-67.

Roscow, C. E., Moss, J., Philbin, D. M., & Savarese, J. S. (1982). Histamine release during morphine anesthesia. Anesthesiology, 56, 93-96.

Rossner, B. (1995). Fundamentals of biostatistics (4th ed.). Belmont, CA: Duxbury Press.

Rudolph, A. J., Vallbona, C., & Desmond, M. M. (1965). Cardiodynamic studies in the newborn III. Heart rate patterns in infants with idiopathic respiratory distress syndrome. Pediatrics, 36(4), 551-559.

Sanner, J. H., & Woods, L. A. (1965). Comparative distribution of tritium labelled dihydomorphine between maternal and fetal rats. Journal of Pharmacology and Experimental Therapy, 148, 176-184.

Scheinin, B., Scheinin, M., Asantila, R., Lindberg, R., Vinamaki, O., (1987). Sympatho-adrenal and pituitary hormone responses during and immediately after thoracic surgery - modulation of four different anesthetics. Acta Anaesthesiology Scandinavia, 31, 762-767.

Schmeling, D. J., & Coran, A. G. (1991). Hormonal and metabolic responses to operative stress in the neonate. Journal of Parenteral and Enteral Nutrition, 15(2), 215-238.

Schultz, S., Roikjaer, O., Hasselstrom, L., Jensen, N. H., Kehlet, H. (1988). Epidural bupivacaine and morphine plus systemic indomethacin eliminates pain but not systemic response and convalescence after cholecystectomy. Surgery, 103(3), 321-327.

Schwartz, M. E., & Jefferies, I. P. (1990). Neonatal pain assessment: Physiological responses to heel lancing. International Pediatrics, 5(4), 344-349.

Sebel, P. S., Bovill, J. G., Schellekens, A. P. M., & Hawker, C. D. (1981). Hormonal responses to high dose fentanyl anaesthesia. British Journal of Anaesthesia, 53, 941-948.

Selye, H. (1976a). Stress in health and disease. Boston: Butterworths.

Selye, H. (1976b). Forty years of stress research: Principal remaining problems and misconceptions. Canadian Medical Association Journal, 115, 53-56.

Severinghaus, J. W. & Kelleher, J. F. (1992). Recent developments in pulse oximetry. Anesthesiology, 76, 1018-1038.

Sherman, J. E., Strub, H., & Lewis, J. W. (1984). Morphine analgesia: Enhancement by shock-associated cues. Behavioral Neuroscience, 98(2), 293-309.

Shook, J. E., Watkins, W. D., Camporesi, E. M. (1990). Differential roles of opioid receptors in respiration, respiratory disease, and opiate-induced respiratory depression. American Review of Respiratory Diseases, 142, 895-909.

Slotkin, T. A., & Seidler, F. J. (1988). Stress in the fetus and newborn. In G. P. Chrousos, D. L. Loriaux, & P. W. Gold (Eds.), Mechanisms of physical and emotional stress (pp. 283-294). New York: Plenum Press.

Slotkin, T. A., & Seidler, F. J. (1989). Catecholamines and stress in the newborn. In S. Breznitz, & O. Zinder (Eds.), The molecular biology of stress (pp. 133-142). New York: Alan R. Liss.

Spyer, M. K. (1988). Central nervous system control of the cardiovascular system. In R. Bannister (Ed.), Autonomic failure (2nd. ed., pp. 56-79), Oxford: Oxford University press.

Stanley, T. H. (1981). Pharmacology of intravenous narcotic anesthetics.. In R. D. Miller (Ed.), Anesthesia, Vol. 1 (pp. 425-449), New York: Churchill Livingstone.

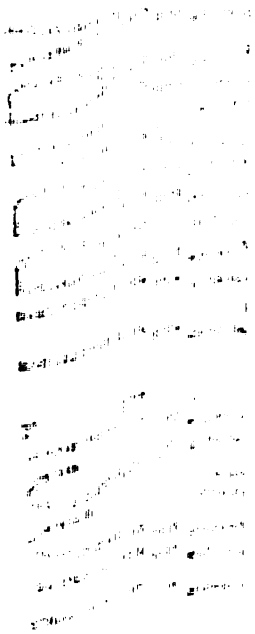
Stanely, T. H., Isern-Amaral, J., & Lathrop, G. D. (1975a). The effects of morphine anesthesia on urine norepinephrine after coronary artery surgery. Analgesia and Anesthesia, 54, 509-516.

Stanely, T. H., Isern-Amaral, J., & Lathrop, G. D. (1975b). Urine epinephrine excretion in patients undergoing mitral or aortic valve replacement. Canadian Anaesthesia Journal, 22, 478-485.

Stanley, T. T., Berman, L., Green, O., & Robertson, D. (1980). Plasma catecholamine and cortisol response to fentanyl-oxygen anesthesia for coronary-artery operations. Anesthesiology, 53(3), 250-253.

Sternbach, R. A. (1989). Acute versus chronic pain. In P. D. Wall & R. Melzack (Eds.), Textbook of pain (2nd. ed., pp. 242-246). New York: Churchill Livingstone.

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



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

Stulik, K., & Pacakova, V. (1986). Electrochemical detection of catecholamines and related compounds. In A. M. Krstulovic (Ed.), Quantitative analysis of catecholamines and related compounds (pp. 137-207). New York: Halstead Press.

Swartjes, J. M., van Geijn, H. P., Mantel, R., & Schoemaker, H. C. (1992). Quantitated fetal heart rhythm at 20, 32, and 38 weeks of gestation and dependence on rest-activity patterns. Early Human Development, 28, 27-36.

Teitel, D. F., Sidi, D., Chin, T., Brett, C., Heymann, M. A., & Rudolph, A. M. (1985). Developmental changes in myocardial contractile reserve in the lamb. Pediatric Research, 19(9), 948-955.

Tepperman, J., & Tepperman, H. M. (1991). Metabolic and endocrine physiology: An introductory text (pp. 229-246). Chicago: Yearbook Medical Publishers.

Thompson, C. R., Brown, J. S., Gee, H., & Taylor, E. W. (1993). Heart rate variability in healthy term newborns: The contribution of RSA. Early Human Development, 31, 217-228.

Tomancik, L. (1987). Directions for using Semmes-Weinstock monofilaments. Wood Dale, IL: Stoelting.

Torda, C. (1978). Effects of recurrent postnatal pain-related stressful events on opiate receptor-endogenous ligand systems. Psychoneuro-endocrinology, 3, 85-91.

Tronick, E., Als, H., & Brazelton, T. B. (1978). Early development of neonatal and infant behavior. In F. Falkner & J. M. Tanner (Eds.), Human growth, Vol. 3: Neurobiology and nutrition (pp. 305-328). New York: Plenum

Press.

Tronick, E. Z., Scanlon, K. B., & Scanlon, J. W. (1990). Protective apathy, a hypothesis about the behavioral organization and its regulation to clinical and physiological status of the preterm infant during the newborn period. Clinics in Perinatology, 17(1), 125-154.

Udelsman, R., & Chrousos, G. P. (1988). Hormonal responses to surgical stress. In G. P. Chrousos, D. L. Loriaux, & P. W. Gold (Eds.), Mechanisms of physical and emotional stress (pp. 265-272). New York: Plenum Press.

Vacanti, J. P., Crone, R. K., Murphy, J. D., Smith, S. D., & Black, P. R. (1984). The pulmonary hemodynamic response to perioperative anesthesia in the treatment of high-risk infants with congenital diaphragmatic hernia. Journal of Pediatric Surgery, 19(6), 672-679.

Van Praag, H., & Frenk, H. (1991). Evidence for opiate tolerance in newborn rats. Developmental Brain Research, 60, 99-102.

van Ravenswaaij-Arts, C. M. A., Hopman, J., Kollee, L., van Amen, J., Stoelinga, G., & van Geijn, H. (1991). The influence of respiratory distress syndrome on heart rate variability in very preterm infants. Early Human Development, 27, 207-221.

van Ravenswaaij-Arts, C. M. A., Hopman, J. C., Kollee, L. A., Stoelinga, G. B., & van Geijn, H. P. (1995). The influence of artificial ventilation on heart rate variability in very preterm infants. Pediatric Research, 37(1), 124-130.

van Ravenswaaij-Arts, C., Kollee, L., Hopman, J. C. W., Stoelinga, G., & van Geijn, H. (1993). Heart rate variability. Annals of Internal Medicine, 118(6), 436-447.

Walker, J. S., Chay, P. C. W., & Duffy, B. J. (1991). Pharmacokinetic and pharmacodynamic relationships of morphine in term and preterm neonates.

Clinical and Experimental Pharmacology and Physiology, (Suppl. 18), 65A.

Wallen, L. D. (1992). Developmental physiology of the fetus and newborn and physiological changes related to birth, cerebral adaptation, and apnea. Current Opinion in Pediatrics, 4, 200-205.

Walsh, E. S., Paterson, J. L., O'Riordan, J. B. A., & Hall, G. M. (1981). Effects of high-dose fentanyl anaesthesia on the metabolic and endocrine response to cardiac surgery. British Journal of Anaesthesia, 53, 1155-1165.

Way, W. L., Costley, E. C., & Way, E. L. (1965). Respiratory sensitivity of the newborn infant to meperidine and morphine. Clinical Pharmacology and Therapeutics, 6(4), 454-461.

Wear, R., Robinson, S., & Gregory, G. A. (1982). The effect of halothane on the baroreceptor response of adult and baby rabbits. Anesthesiology, 56, 188-191.

Weil, J. V., McCullough, R. E., Kline, J. S., & Sodal, I. E. (1975). Diminished ventilatory response to hypoxia and hypercapnia after morphine in normal man. New England Journal of Medicine, 292(21), 1103-1106.

Weissman, C. (1990). The metabolic response to stress: An overview and update. Anesthesiology, 73, 308-327.

Weissman, C., & Hollinger, I. Modifying systemic responses with anesthetic techniques. Anesthesiology Clinics of North America, 6(1), 221-235.

Weissman, C., & Jamdar, S. (1992). The effect of exogenous catecholamines on the measurement of plasma catecholamine concentrations. Anesthesia and Analgesia, 74, S345.

Wessel, D. L. (1993). Hemodynamic responses to perioperative pain and stress in infants. Critical Care Medicine, 21(9) Suppl., S361-S362.

Willer, J. C. (1977). Comparative study of perceived pain and nociceptive

flexion reflex in man. Pain, 3, 69-80.

Willer, J.C. (1985). Studies on pain: Effects of morphine on a spinal nociceptive flexion reflex and related sensation in man. Brain Research, 331, 105-114.

Willer, J. C., Bergeret, S., & Gaudy, J. H. (1985). Epidural morphine strongly depresses nociceptive flexion reflexes in patients with postoperative pain. Anesthesiology, 63, 675-680.

Willer, J. C., & Bussel, B. (1980). Evidence for a direct spinal mechanism in morphine induced inhibition of nociceptive reflexes in humans. Brain Research, 187, 212-215.

Willer, J. C., Dehen, H., & Cambier, J. (1981). Stress-induced analgesia in humans: Endogenous opioids and naloxone reversible depression of pain reflexes. Science, 212, 689-691.

Willer, J. C., Roby, A., & LeBar, D. (1984). Psychophysical and electrophysical approaches to pain relieving effects of heterotrophic nociceptive stimuli. Brain, 107, 1095-1112.

Willette, R. N., & Sapru, H. N. (1982). Peripheral versus central cardiorespiratory effects of morphine. Neuropharmacology, 21, 1019-1026.

Wilmore, D. W. (1976). Hormonal responses and their effects on metabolism. Symposium on response to infection and injury, II. Surgery Clinics of North America, 56(5), 999-1018.

Woo, M. A., Stevenson, W. G., Moser, D. K., & Middlekauf, H. R. (1994). Complex heart rate variability and serum norepinephrine levels in patients with advanced heart failure. Journal of the American College of Cardiology, 23(3), 565-569.

Wortsman, J., Frank, S., & Cryer, P. E. (1984). Adrenomedullary

response to maximal stress in humans. American Journal of Medicine, 77, 779-784.

Yaster, M. (1987). The dose response of fentanyl in neonatal anesthesia. Anesthesiology,66,433-435.

Yaster, M., & Deshpande, J.K. (1988). Management of pediatric pain with opioid analgesics. Journal of Pediatrics, 113(3), 421-429.

Yeager, M. P., Glass, D. D., Neff, R. K., & Brinck-Johnsen, T. (1987). Epidural anesthesia and analgesia in high-risk surgical patients. Anesthesiology, 66, 729-736.

Yli-Hankala, A., Porkkala, T., Kaukinen, S., & Hakkinen, V. (1991). Respiratory sinus arrythmia is reversed during positive pressure ventilation. Acta Physiologica Scandinavia, 141, 399-407.

Yongue, B. G., McCabe, P. M., Porges, S. W., Rivera, M., Kelley, S. L., & Ackles, P. K. (1982). The effects of pharmacological manipulations that influence vagal control of the heart on heart period, heart period-variability, and respirations in rats. Psychophysiology, 19, 426-432.

Yoshizato, T., Koyanagi, T., Takashima, T., Satoh, S., Akazawa, K., & Nakano, H. (1994). The relationship between age-related heart rate changes and developing brain function: A model of anencephalic human fetuses in utero. Early Human Development, 36, 101-112.

Zamir, N., & Maixner, W. (1986). The relationship between cardiovascular and pain regulatory systems. In D. D. Kelly (Ed.), Annals of the New York Academy of Sciences, Vol. 467, Stress-induced analgesia (pp. 371-384). New York: New York Academy of Sciences.

Zeltzer, L. K., Barr, R. G., McGrath, P. A., & Schechter, N. L. (1992). Pediatric pain: Interacting behavioral and physical factors. Pediatrics, 90 (5), 816-821.

Zhang, A. Z., & Pasternak, G. W. (1981). Ontogeny of opioid pharmacology and receptors: High and low affinity site differences. European Journal of Pharmacology, 73, 29-40.

Ziegler, M. G., Woodson, L. C., & Kennedy, B. (1986). Radioenzymatic assay of catecholamines, metabolites, and related enzymes. In A. M. Krstulovic (Ed.), Quantitative analysis of catecholamines and related compounds (pp. 263-280). New York: Halstead Press.

Appendix A

Data Collection Form

Part 1: Demographic Data

Patient ID:		SITE: 1 = CHO 2 = UCSF
MR#: SEX: M F	GA: DOB: PNA AGE AT SURGERY:	BW: AGA SGA LGA CW:
Exclusion Criteria: Y N		
Parental Consent: Y N		
Prenatal History and pre-study neonatal course: Delivery: V C-S Factors: Infection Asphyxia Twin Other: Apgars: Surfactant: Y N BUN: CR:		
Prenatal drug exposure: N Y Describe:		
Indomethacin: N Y Age at last dose and total dose:		
Opioids received prior to study (drug/total dose per day/days):		
PDA Ligation Duration of Surgery: Anesthesia (drug/dose): Postop MS Order: Chest tube:		
Comments:		

Data Collection Form Part II: Data Collection Sheet

Patient ID:			MR#
BASELINE MEASURES Date/Time:	PRE-MORPHINE Date/Time:	20 MIN POST-MORPHINE Date/Time:	1 HOUR POST-MORPHINE Date/Time:
Ventilator FiO2: Rate: Pres: MAP:	Ventilator FiO2: Rate: Pres: MAP:	Ventilator FiO2: Rate: Pres: MAP:	Ventilator FiO2: Rate: Pres: MAP:
Behavioral State: 1-DS 2-LS 3-D 4-A 5-C	Behavioral State: 1-DS 2-LS 3-D 4-A 5-C	Behavioral State: 1-DS 2-LS 3-D 4-A 5-C	Behavioral State: 1-DS 2-LS 3-D 4-A 5-C
O2 SAT:	O2 SAT:	O2 SAT:	O2 SAT:
HR:	HR:	HR:	HR:
RR:	RR:	RR:	RR:
BP:	BP:	BP:	BP:
VTI:	VTI:	VTI:	VTI:
E/NE:	E/NE:	E/NE	
FRT: Heel: Quality:	FRT: Heel: Quality:	FRT: Heel: Quality:	FRT: Heel: Quality:
ABG-Time: pH: pCO2: pO2: HCO3: BE:	ABG-Time: pH: pCO2: pO2: HCO3: BE:	ABG-Time: pH: pCO2: pO2: HCO3: BE:	ABG-Time: pH: pCO2: pO2: HCO3: BE:
Last procedure: Time:	Last procedure: Time:	Last procedure: Time:	Last procedure: Time:
	Indication of MS:		
Observations:	Observations:	Observations:	Observations:

Appendix B

Behavioral State Scale

State		Description
1	Deep Sleep	Deep sleep with regular breathing, eyes closed, no spontaneous activity except startles or jerky movements at quite regular intervals; external stimuli produce startles with some delay; suppression of startles is rapid, and state changes are less likely than from some other states. No eye movements.
2	Light Sleep	Light sleep with eyes closed; rapid eye movements can be observed under closed lids; low activity level, with random movements and startles or startle equivalents; movements are likely to be smoother and more monitored than in state 1; responds to internal and external stimuli with startle equivalents, often with a resulting change of state. Respirations are irregular, sucking movements occur off and on.
3	Drowsy	Drowsy or semi-dozing; eyes may be open or closed, eye lids fluttering; activity level variable, with interspersed mild startles from time to time; reactive to sensory stimuli, but response often delayed; state change after stimulation frequently noted. Movements usually smooth.
4	Alert	Alert with bright look; seems to focus attention on source of stimulation, such as an object to be sucked, or a visual or auditory stimulus; impinging stimuli may break through, but with some delay in response. Motor activity is at a minimum.

State		Description
5	Crying	Eyes open; considerable motor activity, with thrusting movements of the extremities, and even a few spontaneous startles; reactive to external stimulation with increase in startles or motor activity, but discrete reactions difficult to distinguish because of general high activity level.

From Brazelton, T. B. (1973). Neonatal behavioral assessment scale. Clinics in Developmental Medicine, 50, p. 5., Philadelphia: J. B. Lippincott Co.

Vectors of Behavioral States

State	Eyes Open	Resp. Regular	Gross Movement	Vocalization
State 1	-1	+1	-1	-1
State 2	-1	-1	-1	-1
State 3	+1	+1	-1	-1
State 4	+1	-1	+1	-1
State 5	0	-1	+1	+1

From Prechtl, H. F. R. (1974). The behavioral states of the newborn infant (A review). Brain Research, 76, p. 187.

Appendix C-1

PARENTAL CONSENT FOR CHILD TO BE A RESEARCH SUBJECT

NAME OF THIS STUDY
Effects of IV Morphine on the Stress Responses and Cardiorespiratory Stability of Premature Neonates Following Surgery.

PURPOSE OF THIS STUDY
Morphine is often given to babies after surgery to relieve their pain. However, we need more information about how well it works in relieving the pain of babies and how often babies experience side effects such as shallow breathing. Because your baby will be having an operation, you are asked to give your permission for your baby to participate in this study.

SPONSORSHIP
Linda Franck, RN, MS and Dr. Christine Miaskowski, Department of Physiological Nursing, University of California, San Francisco, are primarily responsible for conducting this study at Children's Hospital Oakland. Linda Franck can be reached at Children's Hospital, (510) 428-3435. Dr. Miaskowski can be reached at (415) 476-9407.

PROCEDURES
If you decide to permit your baby to participate in this study, your baby will undergo the following procedures:

1) Recording of information from the monitors, including the ECG pattern before, and after your baby receives morphine ordered by the doctor;
2) Leg withdrawal reflex (tested by touching plastic bristles to the heel) will be done before and after your baby receives morphine ordered by the doctor;
3) A maximum of 3 blood samples will be needed for measuring stress hormones before and after your baby receives morphine ordered by the doctor. A total of 1/2 teaspoon over a day will be taken from IV lines your baby already has and no venipunctures will be done for this procedure.

All the procedures will take place at your baby's bedside and take approximately 1.5 hours to obtain. The procedures will be done before your baby goes to surgery (10 minutes), and before and after your baby receives the morphine ordered by the physician during the immediate post-operative period (1 hour and 20 minutes total time).

RISKS/DISCOMFORTS
1) There are no risks to your baby from recording information from the monitors;
2) The plastic bristles may be perceived by your baby as uncomfortable and your baby will be able to move away from the bristle. The test will be very brief and is similar to what is done to test reflexes of all newborn babies.

3) Blood samples will be obtained from your baby solely for the purposes of this study. Whenever possible, the blood samples will be drawn at a time when blood is being obtained for other laboratory tests and will be taken from IV lines your baby already has in place. No additional venipunctures will be performed for this study.

BENEFITS
The information obtained from the study may help in the treatment of other babies recovering from surgery. There may be no direct benefit to your baby for participating in this study. However, the information about your baby's responses to morphine will be shared with your baby's physicians and may be used to alter pain therapy.

ALTERNATIVES
If you choose not to have your baby participate in this study, your baby's care will not be affected.

CONFIDENTIALITY
Your baby's medical records and the records of this study will be handled as confidentially as other medical records. Any published data will not identify participants by name.

TREATMENT AND COMPENSATION FOR INJURY
In the rare event your baby is injured as a result of being in this study, treatment will be available at Children's Hospital; however, there will be no compensation, and treatment will not be provided free of charge.

QUESTIONS
If you have any questions, either before deciding whether to have your baby participate or during the course of this study, please direct your questions to Linda Franck RN, MS at (510) 428-3435 or Dr. Miaskowski at (415) 476-9407. Additionally, if you wish to speak to a physician who is not involved with this research project, and is available for reference, you may contact:

Dr. Gerdsen, Director of Medical Affairs
Children's Hospital
747-52nd Street
Oakland, CA 94609
(510) 428-3331

PARTICIPATION IN RESEARCH IS VOLUNTARY
You have the right to refuse to have your baby take part in this study. You may withdraw your baby at any time without jeopardizing your baby's medical care at Children's Hospital.

CONSENT TO ALLOW MY BABY TO PARTICIPATE IN THE RESEARCH AND LIST OF RIGHTS
Your signature below indicates that you consent to your baby's participation in this study. You will be given a copy of this form and a copy of the "Lists of Rights of a Participant in a Medical Experiment" to keep.

_____ _____
Date Parent or Guardian Signature

_____ _____
Date Parent or Guardian Signature

_____
Witness

HOSPITAL DE NIÑOS-OAKLAND

(CHILDREN'S HOSPITAL OAKLAND)

CONSENTIMIENTO DE LOS PADRES PARA QUE SU NIÑO SEA SUJETO DE INVESTIGACION

TITULO DEL ESTUDIO:

Efectos de la Morfina (por vía intravenosa) sobre la Tolerancia al Agotamiento y la Estabilidad Cardiorespiratoria de un bebé prematuro, luego de haber tenido cirugía.

PROPOSITO DEL ESTUDIO:

Con frecuencia, luego de cirugía, se les suministra morfina a los bebés para calmarles el dolor. Sin embargo,necesitamos recopilar más información en cuanto a la eficacia de la droga para aliviar el dolor de los recién nacidos, así como para saber con qué frecuencia experimentan efectos secundarios como lo es: la respiración ligera.
Debido a que su bebé va a tener una operación, se le pide su permiso para que el(ella) pueda participar en este estudio.

PATROCINADORES:

Linda Franck RN,MS (enfermera certificada con Maestría en Ciencias) y la Dra. Christine Miaskowski (del Departamento de Asistencia Fisiológica de la Universidad de California, San Francisco) son las personas responsables de llevar a cabo este estudio en el Children's Hospital Oakland.

Linda Frank puede ser localizada en el Children's Hospital por el siguiente número de teléfono: (510) 428-3435 ; y la Dra. Christine Miaskowski puede ser localizada en el Hospital de la Universidad de California, San Francisco (UCSF) en el Departamento de Asistencia Fisiológica, Box 0610, Office 611Y, San Francisco, California 94143-0610, teléfono: (415) 476-9407.

PROCEDIMIENTO:

Si usted decide permitir que su bebé participe en este estudio, él(ella) será sometido(da) a los siguientes procedimientos:

1- Recopilación de datos generados por los monitores conectados a su bebé, inclusive una grabación del patrón de comportamiento de su electrocardiograma, antes y después de que su bebé reciba la dosis de morfina ordenada por el médico;

2- Prueba del reflejo retractable de las piernas (se examina rozando los talones con unas cerdas plásticas). Se llevará a cabo antes y después de que su bebé reciba la dosis de morfina ordenada por el médico;

3- Se necesitará extraer tres (3) muestras de sangre para medir las hormonas del agotamiento antes y después de que su bebé reciba la dosis de morfina ordenada por el médico; A lo largo del día se extraera total equivavalente a 1/2 cucharadita de sangre,la cual se tomara por la vía intravenosa puesta ya en su bebé, evitando hacerle otra punzada.

Todos los procedimientos mencionados tomarán apenas unos minutos y se realizarán a la cabecera de su bebé. Estos se llevarán acabo antes de la operación (período preoperatorio) e inmediatamente luego de la mísma (período posoperatorio); antes y después de que su bebé reciba la dosis de morfina ordenada por el médico.

RIESGOS Y MALESTARES:

1- No existe ningún tipo de riesgo para su bebé durante la recopilación de datos de los monitores;

2- Su bebé puede sentir molestia a causa de las cerdas plásticas. Debido a esto, él(ella) podria tratar de retirar sus piernas de las cerdas. Esta prueba dura poco tiempo y se asemeja al examen de reflejos que se les hace a todos los recién nacidos.

3- Las muestras de sangre que se obtendrán de su bebé solamente serán para el propósito de este estudio. Estas muestras se tomarán al mismo tiempo que se extrae sangre para otras pruebas de laboratorio; evitando hacerle otra punzada.

VENTAJAS:

La información que se obtiene de este estudio podrá aplicarse en el tratamiento de recuperación posoperatoria de otros bebés. Pueda ser que su bebé no se beneficie directamente al participar en este estudio; Sin embargo, los datos recopilados sobre la reacción de su bebé al suministro de morfina, se le harán llegar a su pediatra quien podrá emplearlos para modificarle el tratamiento de su bebé.

ALTERNATIVAS:

Si usted elige que su bebé no participe en este estudio, esto no alterará la asistencia médica que él(ella) recibe.

ACUERDO CONFIDENCIAL:

Tanto el expediente médico de su bebé como lo resultados de este estudio serán tratados confidencialmente al igual que otros casos. Si se publica alguna información de este estudio, no se identificarán a los nombres de los participantes.

TRATAMIENTO Y COMPENSACION DEBIDO A LESIONES:

En el caso extremo de que su bebé sea lastimado debido a su participación en este estudio, él(ella) sera tratado(da) en el Children's Hospital; Sin embargo, no habrá compensación por la lesión y el tratamiento no se podrá ofrecer exento(libre) de costos.

PREGUNTAS:

Si le surgen preguntas, ya sea antes de que usted decida aprobar la participación de su bebé en el estudio,

o en el transcurso de el mismo; favor comuníquese con Linda Franck (RN,MS) llamando a el teléfono: (510) 428-3435 o a la Dra.Christine Miaskowski por el teléfono: (415) 476-9407.

Además,si usted desea consultar con un médico que no esté involucrado en este proyecto de investigación, y que esté disponible para dar referencias, puede comunicarse con:

Dr. Jeffrey Gould, Director Médico
Children's Hospital
747-52nd Street
Oakland,Ca 94609
(510) 428-3331

LA PARTICIPACION EN ESTA INVESTIGACION ES VOLUNTARIA:

Usted está en el derecho de negarse a quesu bebé participe en este estudio. Podrá retirar a su bebé en cualquier instante del mismo sin poner en riesgo la asistencia médica que él(ella) recibe del Children's Hospital.

CONSENTIMIENTO PARA PERMITIR QUE MI BEBE PARTICIPE EN ESTA INVESTIGACION Y LISTA DE SUS DERECHOS

A continuación, su firma indica que usted dá consentimiento para que su bebé participe en este estudio.

Se le entregarán, para que usted las retenga, copias de este formulario y de "la lista de derechos del participante en un experimento médico".

Fecha	**Firma del Padre O Representante**
Fecha	**Firma del Padre O Representante**
	Testigo

Appendix C-3

Effects of IV Morphine on the Stress Responses and Cardiorespiratory Stability of Premature Neonates Following Surgery.

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
PARENTAL CONSENT FOR CHILD TO BE A RESEARCH SUBJECT

A. PURPOSE AND BACKGROUND

Dr. Christine Miaskowski and Linda Franck from the School of Nursing are conducting a study to learn how well morphine (a pain-relieving medication) reduces stress responses in babies after surgery and how often babies experience side effects such as shallow breathing. Because my baby will be having an operation, I am being asked to give permission for my baby to participate in this study.

B. PROCEDURES

If I agree to permit my baby to be in this study, the following procedures will be performed before surgery and again during the first day after my baby has surgery. The procedures will be done before and after the nurse gives my baby morphine ordered by the doctor for relief of postoperative pain:

1) Recording of information from the monitors, including the heart rate pattern;
2) Leg withdrawal reflex (tested by touching plastic bristles to the heel);
3) Blood samples will be taken for measuring stress hormones. A total of 1/2 teaspoon over a day will be taken from intravenous (IV) lines my baby already has and no needle punctures will be done for this procedure.

Participation in this study will take a total of 1 hour over a period of two days. All the procedures will take place at my baby's bedside at the Medical Center at UCSF. The procedures will be done before my baby goes to surgery (10 minutes), and before and after my baby receives the morphine ordered by the physician during the immediate postoperative period (40 minutes total time).

C. RISKS/DISCOMFORTS

1) Recording monitor data: There are no risks to my baby from recording information from the monitors;
2) Reflex testing: The plastic bristles will not harm my baby. The test is very brief and is similar to reflex tests done on all newborns.
3) Blood samples: Rarely, the IV line can be accidentally dislodged or infection can be introduced during the blood drawing procedure. Whenever possible, the blood samples will be drawn at a time when blood is being obtained for other laboratory tests. Blood will be only taken from IV lines my baby already has in place. No additional needle punctures will be performed for this study. The amount of blood taken for this study is similar to that needed for 2 to 3 blood tests and increases only slightly my baby's need for blood transfusion.
4) Confidentiality: Participation in research may involve loss of privacy. My baby's research records will be handled as confidentially as possible within the law. All records will be coded and kept in locked files so that only the study investigators have access to them. No individual identities will be used in any reports or publications resulting from this study.

8/25/93

Treatment and Compensation for Injury

If my baby is injured as a result of being in this study, treatment will be available. The costs of such treatment may be covered by the University of California, depending on a number of factors. The University does not normally provide any other form of compensation for injury. For further information about this, I may call the office of the Committee on Human Research at (415) 476-1814.

D. BENEFITS

There may be no direct benefit to my baby from participating in this study. However, the information about my baby's responses to morphine will help in the postoperative treatment of babies undergoing surgery in the future.

E. ALTERNATIVES

If I choose not to have my baby participate in this study, my baby's care will not be affected.

F. COSTS

I will not be charged for any of the study procedures. The costs of the laboratory tests associated with this study will be covered by the study.

G. REIMBURSEMENT

I will not be reimbursed for my baby's participation in this study.

H. QUESTIONS

This study has been explained to me by Dr. Miaskowski or Linda Franck and my questions were answered. If I have any other questions about the study, I may call Dr. Miaskowski at (415) 476-9407 or Linda Franck at (510) 428-3435.

I. CONSENT

I have been given copies of this consent form and the Experimental Subject's Bill of Rights to keep.

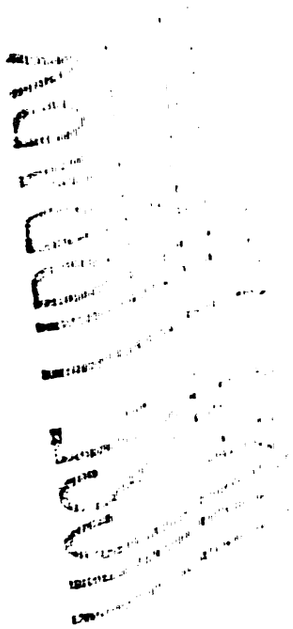
PARTICIPATION IN RESEARCH IS VOLUNTARY. I have the right to decline to have my baby participate or to withdraw your baby at any time without jeopardy to my baby's medical care.

If I wish to have my baby participate, I should sign below.

_____	_____
Date	Legally Authorized Representative (Parent or Guardian)
_____	_____
Date	Person Obtaining Consent

CHR Approval Number: H7025-09429-01

8/25/93



Appendix D

Measurement Sequence

Each measurement series was conducted in the same order, from least to most arousing. Behavioral state, VTI, and vital signs were obtained first, followed by blood samples for E and NE, and then FRT.

Preoperative (baseline) Measures:

1. Neonate must in a light or deep sleep state for 3 minutes.
2. Record ventilator settings and behavioral state.
3. Record 3 minutes of heart rate data from monitor. Document oxygen saturation, HR, RR, and BP from monitor.
4. Assist nurse in drawing 0.5 ml blood sample for E and NE assays, when blood for other laboratory test is to be obtained.
5. Apply von Frey hairs to heel, beginning with 0.4 g and increasing size (i.e., force), until FRT is observed.

Postoperative Measures:

When the nurse determined that the first postoperative dose of morphine was needed, the following measures were be obtained:

Pre-Morphine:

1. Record ventilator settings and behavioral state.
2. Record 3 minutes of heart rate data from monitor. Document oxygen saturation, HR, RR, and BP from monitor.
3. Assist nurse in drawing 0.5 ml blood sample for E and NE assays.
4. Apply von Frey hairs to the heel, beginning with 0.4 g and increasing size (i.e., force), until FRT is observed.

Post-Morphine, Time 1:

Twenty minutes after the nurse administered the dose:

5. Record ventilator settings and behavioral state.

6. Record 3 minutes of heart rate data from monitor. Document oxygen saturation, HR, RR, and BP from monitor.

7. Assist nurse in drawing 0.5 ml blood sample for E and NE assays.

8. Apply von Frey hairs to the same heel as used for pre-morphine measure, beginning with 0.4 g and increasing size (i.e., force), until FRT is observed.

Post-Morphine, Time 2:

One hour after the nurse administered the dose:

9. Record ventilator settings and behavioral state.

10. Record 3 minutes of heart rate data from monitor. Document oxygen saturation, HR, RR, and BP from monitor.

11. Apply von Frey hairs to the same heelas used for pre-morphine measure, beginning with 0.4 g and increasing size (i.e., force), until FRT is observed.

Appendix E

Preliminary Data

Phase 1

In phase 1, a 1 ml sample of whole blood was obtained from five neonates in order to determine plasma E and NE levels using HPLC-ED. The blood sample was obtained at the time of routine blood sampling by venipuncture or heelstick. The samples were placed in iced lithium-heparin microtainers and transported to the clinical laboratory where they were immediately centrifuged, yielding approximately 0.5 ml of plasma. The plasma samples were placed in a -20°C freezer for later E and NE assay using HPLC-ED.

The plasma samples were prepared for HPLC-ED assay using the extraction procedure specified by the manufacturer (ESA, Inc., Bedford, MA) with an internal standard dihydroxybenzylamine (DHBA) added to each sample.

Demographic data on the five neonates is shown in Table III-A. The mean plasma E and NE levels in the five neonates were 6.68 (range: 0.58-15.13) and 22.62 (range: 14.87-36.92), respectively (Table III-B). There was wide variability in the E and NE levels of the five neonates. Overall, the E and NE levels were elevated, but within the range of levels reported in other studies (see Table II for a summary of the results of these studies).

Phase 2

In phase 2, E, NE, VTI, and FRT were measured in nine neonates, before and after a single IV dose of morphine (0.1 mg/kg) was administered. As noted in Table III-C, not all of the measures were performed in all the neonates. Because a secondary aim of phase 2 was to determine if 0.5 ml of whole blood was sufficient for detection of plasma E and NE as compared to a 1 ml sample, a 1.5 ml sample of whole blood was obtained from one neonate. This neonate was receiving a continuous

infusion of morphine (0.02 mg/kg) at the time of blood sampling.

Demographic data and the specific measures performed on the neonates are shown in Table III-C. The mean gestational and postnatal ages of the ten neonates in the phase 2 sample were 31 weeks (range: 24-40 weeks) and 19 days (range: 1-90 days), respectively. The mean birth weight of the neonates was 1.8 kg (range: 0.7-3.5 kg).

E and NE Assays:

Blood samples were obtained from one neonate at 5 days and again at 11 days of age. The samples (0.5 ml) were drawn from the umbilical artery catheter immediately before and fifteen minutes after the administration of an IV dose of morphine. The pre- and post morphine samples each yielded 0.25 ml plasma. In the second neonate (i.e, receiving a continuous infusion of morphine), a 1.5 ml sample of whole blood was obtained from the ECMO circuit and split into two unequal samples (i.e., 1 ml and 0.5 ml), yielding 0.5 ml and 0.25 ml of plasma. All phase 2 plasma samples were prepared for HPLC-ED assay using the methods described in phase 1 except that the DHBA internal standard was not used for the phase 2 assays.

Epinephrine levels were below the limits of detection in all of the samples. Norepinephrine levels for the six samples (two neonates) are shown in Table VIII. The post-morphine levels were increased approximately one-fold the pre-morphine levels. There was a 9% difference between the levels in the two samples of differing volume from the same neonate. The NE levels were lower than those obtained during phase 1, but were within the range reported in the literature (Table II).

Vagal Tone Index:

ECG data were recorded onto computer disk for four neonates immediately before and fifteen minutes after the administration of an IV dose of morphine. The ECG data were analyzed using the MXedit program. No editing of the MXedit file was

necessary and VTI was calculated directly from the recorded R-R interval data (Table III-D).

The mean pre-morphine and post-morphine VTI values were 3.43 (range: 2.26-5.36) and 3.35 (range: 1.75-6.91), respectively. The VTI values for these ventilated neonates were within the range of VTI values reported for non-ventilated neonates (Porges, 1992). The VTI values changed following morphine administration in all neonates, increasing in two of the neonates and decreasing in two of the neonates.

Flexor Reflex Threshold:

FRT was measured in seven neonates before and after the administration of an IV dose of morphine (Table III-D). The mean pre-morphine FRT was 3.04 g (range: 0.5-6.9 g) and the mean post-morphine FRT was 2.45 (range: 0.8-6.9 g). The values were generally higher but within the range reported by Fitzgerald, et al. (1988; 1989) and Andrews and Fitzgerald (1994). The FRT increased in three of the neonates, decreased in two neonates, and demonstrated no change in two neonates following morphine administration.

Discussion

The data from this preliminary investigation demonstrated the technical feasibility of measuring plasma E and NE levels, VTI, and FRT in critically ill premature and full term neonates in the NICU. Adequate plasma could be extracted from 0.5 ml of whole blood to perform NE assays only. One ml whole blood samples may be required to detect both E and NE.

Electrocardiogram data can successfully be transferred to computer disk from the neonate's cardiorespiratory monitor for later calculation of VTI. Mechanical ventilation does not appear to interfere with VTI estimates in the ill neonate. Lastly, FRT can be measured in neonates following morphine administration.

Changes were demonstrated in all measures following morphine administration. The direction and magnitude of change varied among the neonates, as well as among the measures. Due to the variability demonstrated in the phase 1 and phase 2 pilot studies, it is evident that the measures of neonatal nociceptive stress responses will need to be performed in a larger sample of neonates in order to evaluate the effect of morphine on these measures.

Appendix E-1

Preliminary Data

Phase 1: Demographic Data

Neonate	GA/BW/PNA	Diagnosis/Clinical Status
#1	28 wks 1.3 kg 8 days	Mild RDS Nasal cannula
#2	41 wks 3.3 kg 15 days	Meconium Aspiration, PHTN, 10 days post-ECMO
#3	37 wks 3.4 kg 2 days	Hyperbilirubinemia No respiratory distress
#4	33 wks 2.2 kg 8 days	PDA, Arrythmias, last dose indomethacin given 48 hours prior
#5	28 wks 1.1 kg 15 days	RDS, Twin B, surfactant, ventilated (CPAP)

Appendix E-2

Preliminary Data

Phase 2: Demographic Data and Measures Performed

Neonate	GA BW PNA	Diagnosis Clinical Status	Reason for Morphine 0.1 mg/kg (Frequency)	Measure
#1 female	30 wks 1.5 kg 4 days	RDS; Oscillating ventilator; chest tube; Paralysis d/c'd X 12 hrs.	Sedation (Q 4 hours)	FRT
#2: male	37 wks 3.5 kg 5 days and 11 days	RDS/PHTN; Oscillator; then conventional vent.; Dopamine drip	Sedation (Q 4 hours)	E, NE, FRT at 5 days of age E, NE at 11 days of age
#3: male	38 wks 3.3 kg 5 days	Mec. Asp.; ventilated; chest tube	Analgesia, sedation (Q 6 hours)	FRT, VTI
#4: male;	24 wks 0.7 kg 2 months (1 kg)	RDS/NEC; ventilated; colostomy	Postop day 4, analgesia/ sedation (PRN)	FRT
#5: female	32 wks 2.3 kg 6 days	RDS; Ventilated; ileostomy; prenatal cocaine exposure	Postop day 6, analgesia/ sedation (PRN)	FRT
#6: male;	26 wks 0.75 kg 1 day	RDS; ventilated;	Analgesia/ sedation for perc. line insertion	FRT
#7: male	24 wks. 0.5 kg 3 months (1.5 kg)	RDS; ventilated; Reintubated X 1 wk.	Sedation (PRN)	VTI

Neonate	GA BW PNA	Diagnosis Clinical Status	Reason for Morphine 0.1 mg/kg (Frequency)	Measure
#8: male	25 wks 0.75 kg 16 days	RDS, PDA ligation; Ventilated; 10 days since last dose indocin	Postop 12 hrs.; first postop dose MS.	VTI
#9: male	34 wks 2.5 kg 5 days	NEC; Ventilated; Ileostomy	Post-op 24 hours; 2 dose MS	FRT, VTI
#10: male	40 wks 2.5 kg 2 days	Asphyxia; ECMO	Continuous MS drip 0.02 mg/kg/hr	E, NE

Appendix E-3

Preliminary Data

Phase 1: Plasma E and NE Levels (nmol/l)

NEONATE	LEVELS (nmol/l)
#1:	E: 13.34 NE: 21.83
#2:	E: 3.74 NE: 36.92
#3:	E: 0.59 NE: 14.87
#4:	E: 0.58 NE: 20.99
#5:	E: 15.13 NE: 18.49

Phase 2: Pre and Post Morphine Plasma NE Levels (nmol/l)
Note: E not detectable in samples

NEONATE	PRE-MORPHINE	POST-MORPHINE
#2: (5 days)	NE: 1.49	NE: 2.99
#2: (11 days)	NE: 1.56	NE: 2.91
#10: (Split sample)	NE: 2.32 (0.25 ml plasma) NE: 2.12 (0.5 ml plasma)	—

Appendix E-4

Preliminary Data

Vagal Tone Index

NEONATE	PRE-MORPHINE	POST-MORPHINE
#3:	5.36	6.91
#7:	2.26	2.73
#8:	3.46	1.75
#9:	2.63	1.99

Flexor Reflex Threshold

NEONATE	PRE-MORPHINE	POST-MORPHINE
#1:	2.0 g	0.8 g
#2:	6.9 g	2.0 g
#3:	0.5 g	2.1 g
#4:	2.1 g	2.3 g
#5:	2.1 g	2.3 g
#6:	0.8 g	0.8 g
#9:	6.9 g	6.9 g

Table 1

Stress Responses and Clinical Outcome

Response	Complication
Metabolic	
Hypermetabolism	Increased oxygen consumption
Hyperglycemia	Hyperosmolar state, diuresis
Hyperlactatemia	Metabolic acidosis
Protein catabolism	Impaired healing, growth, etc.
Lipolysis	Ketone production, acidosis
Cardiovascular	
Increased blood pressure	Hypertension, increased afterload
Increased heart rate	Tachyarrythmias
Increased cardiac output	Myocardial ischemia
Respiratory	
Increased oxygen consumption	Hypoxia
Decreased tidal volume	Atelectasis
Decreased functional residual capacity	Hypercarbia, increased work of breathing
Ventilation/perfusion mismatch	Hypoxemia
"Fighting the ventilator"	Pneumothorax
Decreased cough effort	Airway plugging, pneumonia
Diaphragmatic splinting	Atelectasis, hypercarbia
Gastrointestinal (GI)	
Gastric acid secretion	Stress ulcers, GI hemorrhage
Decreased gut motility	Paralytic ileus, biliary sludge syndrome
Splanchnic vasoconstriction	Mucosal ischemia, bacterial translocation
Renal	
Sodium retention	Congestive heart failure
Syndrome of inappropriate antidiuretic hormone release	Hyponatremia
Free water retention	Increased "third space"
Other Physiologic Responses	
Transcellular potassium, magnesium, and calcium shifts	Electrolyte disorders
Hypercoagulability	Thrombolytic complications
Increased fibrinolysis	Disseminated intravascular coagulation, hemorrhage
Altered immune function	Infectious complications
Cytokine production	Reperfusion injury, shock, hepatorenal syndrome, capillary leak syndrome

From: Anand, K.J.S. (1993). Relationship between stress responses and clinical outcome in newborns, infants, and children. Critical Care Medicine, 21(9, Suppl.), S358-S359.

Table 2

Plasma Epinephrine and Norepinephrine Levels in Neonates

AUTHOR/ YEAR	ASSAY	VOLUME OF BLOOD	FINDINGS: BASELINE (nmol/l)	FINDINGS: STRESS (nmol/l)
Anand, et al., 1985 N=8 Preterm N=10 Term	double isotope REA	----	Mean ± SEM Preterm E: 1.2±0.4 NE: 6.3±0.8 Term E: 0.4±0.1 NE: 5.9±1.7	Mean ± SEM at Endop; 6; 12; and 24 hours Preterm E:2.2±0.7;1.5±0.9 ; 1.3±0.8;0.8±0.4 NE:6.3±0.8;15.9± 3.0;8.3±2.7;8.2± 2.3;6.2±1.5 Term E:2.4±1.0;0.5±0.2 ; -;0.5±0.2 NE:8.7±2.2;7.0±2 .0; -;5.2±1.0
Anand, et al., 1985 Term and preterms* N=14 * No stat. significant difference in levels	double isotope REA	----	Mean ± SEM E: 0.44±0.11 NE:4.79±0.82	Mean ± SEM at Endop; 6; 12; and 24 hours E:2.40±0.93;0.44± 0.11;0.27±0.05;0. 44± 0.20 NE:9.57±2.42;7.2 6± 1.89;7.98±2.48;5. 14± 0.71
Anand & Aynsley-Green, 1988 Term N=71 minor stress N=12 mod. stress N=11 severe stress	double isotope REA	----	----	Greatest change from baseline: Mild stress E: +1 NE:+3 Mod. stress E: +5 NE:+10 Severe stress E: +20 NE:+27

AUTHOR/ YEAR	ASSAY	VOLUME OF BLOOD	FINDINGS: BASELINE (nmol/l)	FINDINGS: STRESS (nmol/l)
Anand & Aynsley-Green, 1988 Term N=18 w/ N2O2 only N=18 w/ halothane	double isotope REA	1-2ml	----	Greatest change from baseline: With N2O2 only E: +1.6 NE:+3.5 With halothane E: +0.8 NE:+1.0
Anand, et al., 1987 PDA ligation Preterm N=8 w/ N2O2 only N=8 w/ fentanyl (12.2±1.5 mcg/kg)	double isotope REA	1-1.5ml	----	Greatest change from baseline): With N2O2 only: E: +2 NE:+8 With fentanyl: E: -0.6 NE:-0.4
Anand & Hickey, 1992 Term N=15 light anesthesia N=30 deep anesthesia	double isotope REA	----	Mean ± SD Light E: 1.7±0.5 NE:6.2±1.3 Deep E: 2.1±0.6 NE:7.9±1.4	Greatest change from baseline: Light E: +6 NE:+32 Deep E: +2 NE:+12

AUTHOR/ YEAR	ASSAY	VOLUME OF BLOOD	FINDINGS: BASELINE (nmol/l)	FINDINGS: STRESS (nmol/l)
Baumgartner, et al., 1992 Heathy Term (n=27) NICU Term (n=13) NICU Preterms (n=5) 5 days old	REA, single isotope	300-500 ul arterial	Median (range) Healthy Terms: E: 0.59 (0.25-1.64) NE:1.58 (0.89-3.16) NICU Terms: E: 0.41 (0.098-1.25) NE:2.12 (1.05-3.13) NICU Preterms: E: 1.29 (0.66-1.37) NE:4.63 (3.61-10.23)	
Blenow, et al., 1994 Term Asphyxiated N=21 Non-asphyxiated N=11	HPLC	CSF	Mean NE asphyxiated with poor prognosis: 1.36 NE controls: 2.43 NE asphyxiated with good prognosis: 3.25 ($p < 0.05$)	----
Cabal, et al., 1985 Term and preterm N=6	modified REA	----	Mean $\pm$ SD E: 0.29+0.22 NE:5.66$\pm$3.27	Mean $\pm$ SD at 30 min. after pancuronium E: 1.09+0.82 NE:20.2$\pm$19.8

AUTHOR/ YEAR	ASSAY	VOLUME OF BLOOD	FINDINGS: BASELINE (nmol/l)	FINDINGS: STRESS (nmol/l)
Greenough et al., 1987 Preterm N=49	HPLC-ED	1ml plasma	----	E: 3.8 (0.1-18.9)* NE:20.2(1.2-118.5) *E below detection in 24 cases
Greisen, et al., 1985 Preterm N=13 suctioning N=8 phenobarb-itone	single isotope derivati ve	----	Median (Range) E: 0.16 (0.0-2.5) NE:2.1 (0.5-21.0)	After Suctioning E: 2.0-fold increase NE:1.5-fold increase With phenobarbitone E:0.34-fold increase NE: no change
Lagercrantz, et al., 1986 Term N=14	HPLC-ED sensitivi ty 0.1 nmole/l	1-1.5ml	Mean + SD Handling E: 0.42+0.55 NE:4.19+2.46 Heelstick E: 0.18+0.16 NE:5.23+3.58 Gavage E: 0.39+0.40 NE:5.42+3.97	Mean + SD Handling E: 0.40+0.45 NE:6.76+5.04 Heelstick E:0.24+0.25 NE:6.09+4.67 Gavage E: 0.30+0.34 NE:5.78+4.04
McIntosh, et al., 1994 Term/Preterm NICU N=7	HPLC	0.5-1.0ml	Median (MAD) NE during BP wave peaK: 7.05(3.27) NE during BP wave trough: 4.9 (2.1) (p=0.023)	----

AUTHOR/ YEAR	ASSAY	VOLUME OF BLOOD	FINDINGS: BASELINE (nmol/l)	FINDINGS: STRESS (nmol/l)
Mirochnick, et al., 1994 Term cocaine exposed (N=24) unexposed (N=22) 24-72 hours after birth	----	----	Mean±SD Cocaine Exposed NE: 6.06±3.00* E: 0.64±0.58 Unexposed NE: 4.73±4.65* E: 0.38±0.27 *(p=0.03)	----
Nitsche, et al., 1994 Preterms NICU N=11 no dopamine N=20 dopamine infusion	HPLC-ED	----	Mean (Range) No Dopamine Day 1 NE: 1.85 (0.08-6.85) E: 0.42 (0.08-0.81) Day 2 NE: 2.09 (0.54-3.03) E: 0.34 (0.14-10.42) Day 3 NE: 1.45 (0.37-3.54) E: 0.39 (0.04-2.50) Day 4 NE: 2.23 (1.18-7.27) E: 0.33 (0.05-16.18) Day 5 NE: 1.78 (1.45-4.44) E: 0.24 (0.02-1.16)	Dopamine Prior to infusion NE: 1.40 (0.37-14.18) E: 0.45 (0.12-38.69) Day 1 NE: 2.45 (0.64-11.99) E: 0.29 (0.15-2.58) Day 2 NE: 1.88 (0.53-21.39) E: 0.37 (0.04-34.80) Day 3 NE: 3.18 (1.52-32.24) E: 0.85 (0.13-8.97) Day 5 No dopamine NE: 4.27 (0.74-23.69) E: 0.44 (0.16-1.46)

AUTHOR/ YEAR	ASSAY	VOLUME OF BLOOD	FINDINGS: BASELINE (nmol/l)	FINDINGS: STRESS (nmol/l)
Quinn, et al., 1992 Preterms N=95	REA	1 ml	Mean (Range) Morphine (n=29) E: 1.6 (0.7-15.9) NE:4.2 (1.4-70.5) Pancuronium (n=28) E: 2.0 (0.8-8.3) NE:5.7 (1.9-14.1) MS + Pancuronium (n=38) E: 1.9 (0.7-8.2) NE:4.8(0.9-29.2)	After 6 hours MS E: 0.81 (0.4-31.6) NE:1.8 (0.7-23.3) Pancuronium E: 1.4 (0.7-28.5) NE:4.2 (2.6-35.1) MS + Pancuronium E: 1.0 (0.2-39.0 NE: 3.4 (1.2-25.0)
Quinn, et al., 1993 Preterms N=38	REA	1 ml	Mean (Range) Morphine (n=19) E: 0.94 (0.66-2.44) NE:3.00 (2.00-8.48) Placebo (n=19) E: 1.22 (0.99-1.90) NE:4.18 (2.43-8.08)	After 24 hours Morphine E: 0.69 (0.36-1.34) NE:2.89 (1.32-9.27) Placebo E: 1.34 (0.69-2.19) NE:4.87 (3.03-11.64)

Table 3

Characteristics of the Pregnancies

(N=25)

	%	(N)
Prenatal Care		
Complete	60%	(15)
Late, after 23 weeks	16%	(4)
None	24%	(6)
Twin Gestation		
No	76%	(19)
Yes	12%	(3)
Yes, with 1 fetal demise	12%	(3)
Maternal Drug Use		
None	80%	(20)
Opioids	4%	(1)
Alcohol/marijuana	4%	(1)
Alcohol/tobacco	4%	(1)
Marijuana	4%	(1)
Polydrug use	4%	(1)
Prenatal Complications		
Infection	36%	(9)
Premature Rupture of Membranes	32%	(8)
Placenta Previa/Abruption	8%	(2)
Hypertension/Pre-eclampsia	8%	(2)
Incompetent cervix	4%	(1)
Bleeding	4%	(1)
Liver disease	4%	(1)
None	4%	(1)
Steroids Administered Prior to Delivery		
Yes	40%	(10)
No	60%	(15)
Mode of Delivery		
Vaginal	52%	(13)
Cesarean Section	48%	(12)

Table 4

Neonatal Preoperative Characteristics

(N=25)

	Mean	SD	Range
Gestational Age (wks)	26.30	2.35	23.00-32.00
Birth Weight (kg)	0.87	0.35	0.52- 2.20

	%	(N)
Gender		
Female	64%	(16)
Male	36%	(9)
Ethnicity		
African American	36%	(9)
Hispanic	32%	(8)
Caucasian	24%	(6)
Asian	8%	(2)
Apgar Score at 1 Minute		
≤ 3	32%	(8)
4-6	48%	(12)
≥ 7	20%	(5)
Apgar Score at 5 Minute		
≤ 3	8%	(2)
4-6	12%	(3)
≥ 7	80%	(20)
Primary Diagnosis		
Premature/ Respiratory distress	96%	(24)
Premature/ Tracheoesophageal fistula	4%	(1)
Surfactant Administered		
Yes	84%	(21)
No	16%	(4)

Neonatal Preoperative Characteristics (cont.)

	%	(N)
Significant Treatments Prior to Thoracotomy		
Hypotension	56%	(14)
Infection	40%	(10)
Respiratory distress	40%	(10)
Steroids	28%	(7)
High frequency ventilation	20%	(5)
Dilantin	4%	(1)
Caffeine	4%	(1)
Comorbid Conditions Prior to Thoracotomy		
Bronchopulmonary dysplasia	12%	(3)
Poor renal function	12%	(3)
Intraventricular hemorrhage (less than grade 3)	8%	(2)
Anemia	8%	(2)
Hyperbilirubinemia	8%	(2)
Pulmonary interstitial emphysema	8%	(2)
Imperforate anus	4%	(1)
Previous Surgery		
Yes	12%	(3)
No	88%	(22)
Opioids Received Prior to Surgery		
Yes	64%	(16)
No	36%	(9)
Hours Since Last Opioid Dose Prior to Surgery		
Less than 4 hours	4%	(1)
4-12 hours	8%	(2)
13-24 hours	4%	(1)
24-48 hours	12%	(3)
Greater than 48 hours	36%	(9)
No opioids given preoperatively	36%	(9)
Indomethacin Received Prior to Surgery		
Yes	74%	(19)
No	24%	(6)

Table 5

Neonatal Discharge Characteristics

	%	(N)
Alive at Discharge		
Yes	80%	(20)
No	20%	(5)
Medical Diagnoses at Discharge (N=20)		
Bronchopulmonary dysplasia	76%	(19)
Retinopathy of prematurity	24%	(6)
Bowel resection due to necrotizing enterocolitis	20%	(5)
Intraventricular hemorrhage (grade 3 or 4)	8%	(2)
Chylothorax	8%	(2)
Inguinal hernia repair	8%	(2)
Hydrocephalus	8%	(2)
Hepatomegaly	4%	(1)
Hearing loss	4%	(1)
None	12%	(3)

Table 6

Surgical Characteristics
(N=25)

	Mean	SD	Range
Postnatal Age at Surgery (days)	13.56	8.01	2.00 - 30.00
Weight at Surgery (kg)	0.93	0.39	0.53 - 2.50
Days Since Last Indomethacin Dose (n=19)	5.68	4.68	1.00 - 18.00
Days Opioids Received Prior to Surgery (n=17)	3.94	3.25	0.00 - 10.00
Preop BUN (mg/dl)	29.47	19.24	13.00 - 92.00
Preop Creatinine (mg/dl)	1.36	0.44	0.70 - 1.99
Fentanyl Anesthesia Dose (mcg/kg)	55.12	37.97	16.39 - 149.25
Paralysis Dose (mg/kg)	0.25	0.20	0.09 - 0.86
Duration of Surgery (minutes)	76.76	31.62	32.00 - 150.00

Surgical Characteristics (cont.)

	%	(N)
Thoracotomy for		
PDA ligation	96%	(24)
TEF repair	4%	(1)
Type of anesthesia		
IV fentanyl	84%	(21)
IV fentanyl and isoflurane	12%	(3)
IV fentanyl and local anesthesia	4%	(1)
Paralytic agent		
Pancuronium	84%	(21)
Vecuronium	16%	(4)
Chest Tube Placed Intraoperatively		
No	52%	(13)
Yes	48%	(12)

Table 7

Study Characteristics

	Baseline %(N)	Pre-MS %(N)	20 Minutes Post-MS %(N)	1 Hour Post-MS %(N)
Ventilator Mode				
IMV	52% (13)	52% (13)	52% (13)	54.2% (13)
SIMV	24% (6)	32% (8)	32% (8)	33.3 (8)
AC	24% (6)	16% (4)	16% (4)	12.5% (3)
Ventilator Settings (M±SD)				
FiO_2	0.37± 0.20	0.36± 0.13	0.36± 0.14	0.38± 0.19*
PIP	18.60± 3.97	20.12± 3.87	19.84± 3.98	19.96± 3.81*
PEEP	4.52± 0.71	4.44± 0.82	4.40± 0.87	4.12± 0.78*
Rate	33.48±10.08	37.04± 8.09	36.96± 8.10	36.68± 7.99*
MAP	7.82± 1.51**	7.88± 1.56*	7.92± 1.58*	7.98± 1.62**
Behavioral State				
Deep Sleep	8% (2)	76% (19)	76% (19)	83.3% (20)
Light Sleep	76% (19)	16% (4)	16% (4)	16.7% (4)
Drowsy	16% (4)	4% (1)	4% (1)	0 (0)
Alert	0 (0)	4% (1)	0 (0)	0 (0)
Crying	0 (0)	0 (0)	4% (1)	0 (0)
Most Recent Procedure				
Suctioning	72% (18)	80% (20)	4% (1)	16.7% (4)
Handling	12% (3)	16% (4)	4% (1)	8.3% (2)
Physical Exam	8% (2)	0 (0)	0 (0)	0 (0)
Echocardiogram	4% (1)	0 (0)	0 (0)	0 (0)
None	4% (1)	4% (1)	88% (22)	75.0% (18)
IV insertion	0 (0)	0 (0)	4% (1)	0 (0)
Time Since Last Procedure (minutes; M±SD)	124.13±99.29	126.04±82.65	15.00± 7.07	20.00± 19.15

	Baseline %(N)	Pre-MS %(N)	20 Minutes Post-MS %(N)	1 Hour Post-MS %(N)
Most Recent Arterial Blood Gas (M±SD)				
pH	7.35± 0.61	7.33± 0.96	7.29± 0.92#	7.34± 0.02&
PCO_2	43.28±10.18	44.32±10.50	45.63±10.93#	45.50±12.02&
PO_2	64.32±23.37	76.08±62.01	64.13±32.34#	67.00±31.11&
HCO_3	22.88± 3.90	22.52± 3.36	21.13± 2.64#	23.50± 4.95&
BE	-2.21± 3.13**	-3.47± 3.96*	-5.73± 3.00##	-2.00± 2.83&
Concurrent Therapies				
None	48% (12)	36% (9)	44% (11)	62.5% (15)
Dopamine	32% (8)	32% (8)	32% (8)	25.0% (6)
Handling	16% (4)	4% (1)	8% (2)	4.2% (1)
Transfusion	8% (2)	40% (10)	12% (3)	8.3% (2)
Insulin Infusion	8% (2)	12% (3)	12% (3)	16.7% (4)
Steroids	4% (1)	0 (0)	0 (0)	4.2% (1)
Fluid Bolus	0 (0)	8% (2)	4% (1)	4.2% (1)
Phototherapy	0 (0)	4% (1)	4% (1)	4.2% (1)
Dopamine Levels# (nmol/l; M±SD)	139.79±344.08 ***	256.09± 461.64.16****	229.34±444.31	-----
Quality of FRT Responses				
Brisk-Entire Leg	63.6% (14)	8.3% (2)	4.2% (1)	13.0% (3)
Brisk-Foot Only	18.2% (4)	16.7% (4)	25.0% (6)	26.1% (6)
Delayed-Leg	13.6% (3)	0	4.2% (1)	0
Delayed-Foot Only	0	25.0% (6)	12.5% (3)	13.0% (3)
Toes Only	4.6% (1)	8.3% (2)	12.5% (3)	4.4% (1)
No Response	0	41.7% (10)	41.7% (10)	43.5% (10)

* N = 24
** N = 23
*** N = 22
**** N = 21
# N=8
N=7
& N=2

Table 8

Stress Response Measures

Variable	Baseline	Pre-MS	20 min Post-MS	1 hr Post-MS	
	M ± SD	M ± SD	M ± SD	M ± SD	F-Statistic, p-value
Norepinephrine (N=18)	4.14±3.75	6.50±6.60	6.93±6.68	-----	F(2,34)=2.73, p=0.076
Vagal Tone Index[1] (N=22)	1.14±1.21	0.48±0.72	0.31±0.52	0.20±0.40	F(3,63)=7.18, p=0.0001
Flexor Reflex Threshold Present (N=11)	0.45±0.28	1.76±3.68	2.26±4.87	1.06±1.24	F(3,30)=0.75, p=0.53
Flexor Reflex Threshold Absent (N=10)	0.43±0.15	----	----	----	----

[1]Post hoc (Scheffe): Baseline > Pre-MS (p = .04), Baseline > 20 minutes Post-MS (p = .056), Baseline > 1 hour Post-MS (p = .001).

Table 9

Cardiorespiratory Measures

Variable	Baseline	Pre-MS	20 min Post-MS	1 hr Post-MS	
	M ± SD	M ± SD	M ± SD	M ± SD	F-Statistic, p-value
Heart Rate[1] (N=24)	152.16±12.13	160.45±19.58	159.82±17.86	158.36±19.74	F(3,69)=4.27, p=0.008
Respiratory Rate[2] (N=23)	52.96±16.42	39.87±12.65	40.04±12.59	40.60±14.50	F(3,66)=8.47, p=0.0001
Systolic Blood Pressure (N=24)	51.33± 8.12	56.29±18.58	52.95±13.76	53.33±13.45	F(3,69)=1.46, p=0.231
Diastolic Blood Pressure (N=24)	25.17± 6.83	30.25±12.82	27.96± 9.25	28.63±10.72	F(3,69)=2.28, p=0.086
Mean Blood Pressure (N=24)	36.04± 7.52	40.96±15.38	37.58±10.07	37.75±12.31	F(3,69)=1.53, p=0.214
Oxygen Saturation[3] (N=24)	97.08± 3.30	95.17± 3.21	95.00± 2.96	95.21± 3.13	F(3,69)=3.13, p=0.031

[1] Post hoc (Scheffe): Baseline < Pre-MS (p = .02), Baseline < 20 minutes Post-MS (p = .04).

[2] Post hoc (Scheffe): Baseline > Pre-MS (p = .001), Baseline > 20 minutes Post-MS (p = .002), Baseline > 1 hour Post-MS (p = 0.003).

[3] Post hoc (Scheffe): Baseline < Pre-MS (p = 0.02), Baseline < 20 minutes Post-MS (p = 0.04).

Table 10

Correlation Matrix of Stress Response Measures

Pearson Product-Moment Correlations (r-value/p-value/N)

	Baseline			Pre-MS			Post-MS 20 Minutes			Post-MS 1 Hour	
	VTI	FRT	HR	VTI	FRT[1]	HR	VTI	FRT[1]	HR	FRT[1]	HR
NE	-0.156 0.512 20	0.319 0.183 19		0.224 0.484 12	-0.275 0.387 12		-0.002 0.992 14	-0.299 0.299 14			
VTI		-0.410 0.058 22	-0.332 0.122 23		-0.189 0.519 14	-0.223 0.444 14		0.251 0.387 14	-0.439 0.117 14	0.153 0.618 13	-0.142 0.643 13
FRT											

[1] Includes only neonates who demonstrated flexor reflex in the postoperative period.

Table 11

Flexor Reflex Present or Absent

Variable	Baseline	Pre-MS	20 min Post-MS	1 hr Post-MS
	M ± SD	M ± SD	M ± SD	M ± SD
Vagal Tone Index/FRT Present[1] (N=12)	1.60±1.34	0.66±0.87	0.39±0.61	0.32±0.50
Vagal Tone Index/FRT Absent[1] (N=10)	0.59±0.78	0.26±0.43	0.22±0.41	0.05±0.11
Norepinephrine/FRT Present[2] (N=10)	4.51±4.30	7.58±7.91	7.94±8.25	-----
Norepinephrine/FRT Absent[2] (N=8)	3.68±3.15	5.15±4.66	5.66±4.20	-----

[1] Two-way ANOVA: Group effect ($F(1, 20) = 7.95$, $p = .01$); Time effect ($F(3, 60) = 6.71$, $p = .0006$); No interaction ($F(3, 60) = 1.47$, $p = .23$).

[2] Two-way ANOVA: No group effect ($F(1, 16) = 0.61$, $p = .45$); No time effect ($F(2, 32) = 2.45$, $p = .10$); No interaction ($F(2, 32) = 0.23$, $p = .80$).

Table 12

Neonatal Mortality

Variable	Status at Discharge: Alive (N=20)	Status at Discharge: Expired (N=5)
	M ± SD	**M ± SD**
Gestational Age[1]	26.73±2.35	24.60±1.56
Birth Weight	0.91±0.37	0.70±0.15
Baseline NE	6.35±6.47 (N=17)	6.41±4.71
Baseline VTI	0.42±0.69	0.71±0.85
Baseline FRT	0.41±0.23 (N=18)	0.48±0.25 (N=4)
Baseline Heart Rate[2]	149.04±10.65	164.58± 8.08

[1]Independent Student's t-test: t = 1.91; p = .07.

[2]Independent Student's t-test: t = -3.03; p= .006.

Figure 1 - The mean plasma norepinephrine (NE) levels in nmol/L (Y-axis) of the neonates (N = 18) at each of the study time points (X-axis). Each point in the figure represents the $\underline{M} \pm \underline{SD}$.

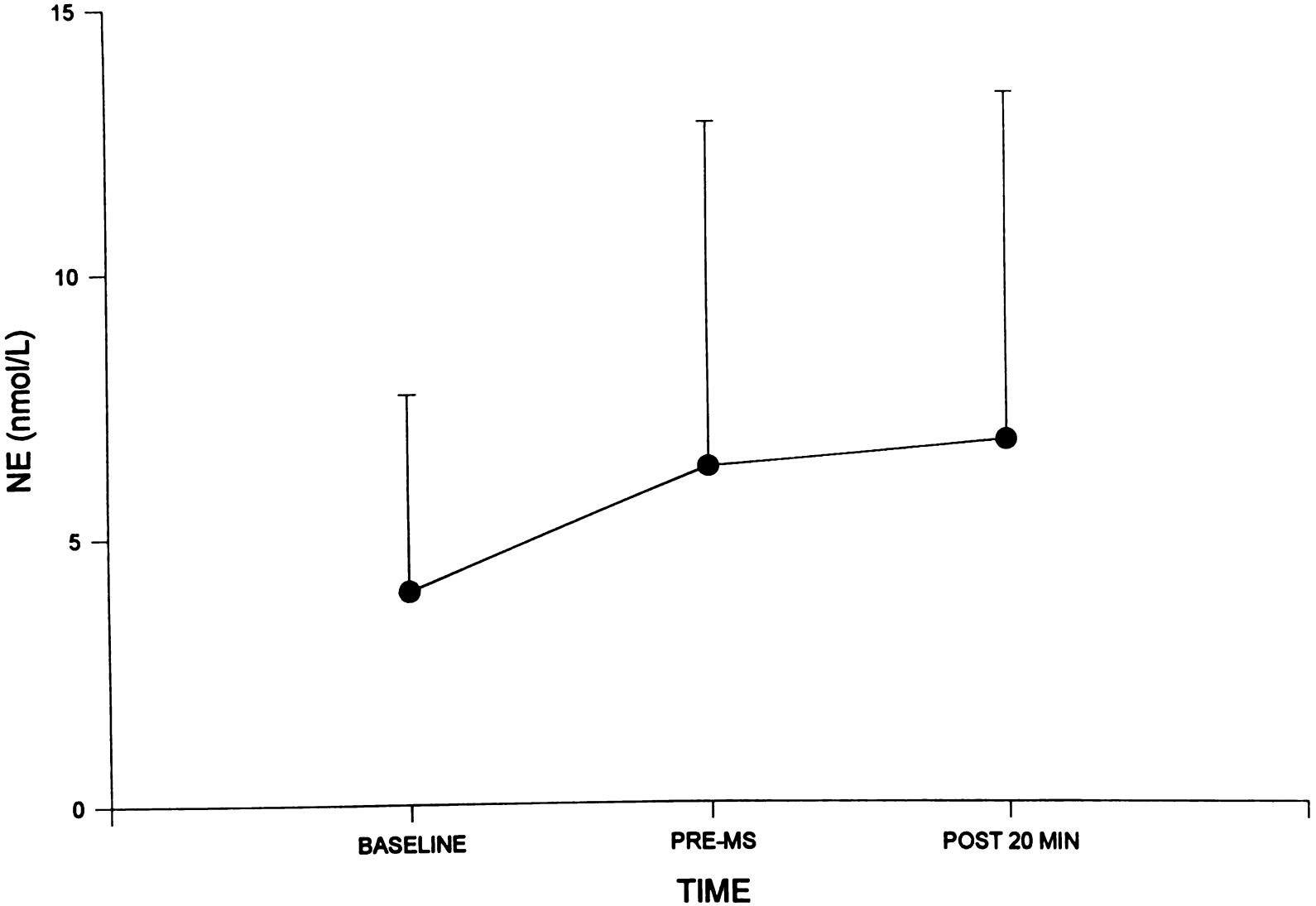

15
10
5
0
NE (nmol/L)
BASELINE
PRE-MS
POST 20 MIN
TIME

Figure 2 - The mean vagal tone index (VTI) levels in $ln/msec^2$ (Y-axis) of the neonates (N = 22) at each of the study time points (X-axis). Each point in the figure represents the $\underline{M} \pm \underline{SD}$. The asterisk above the pre-morphine point denotes a statistically significant difference from baseline ($p < .0001$).

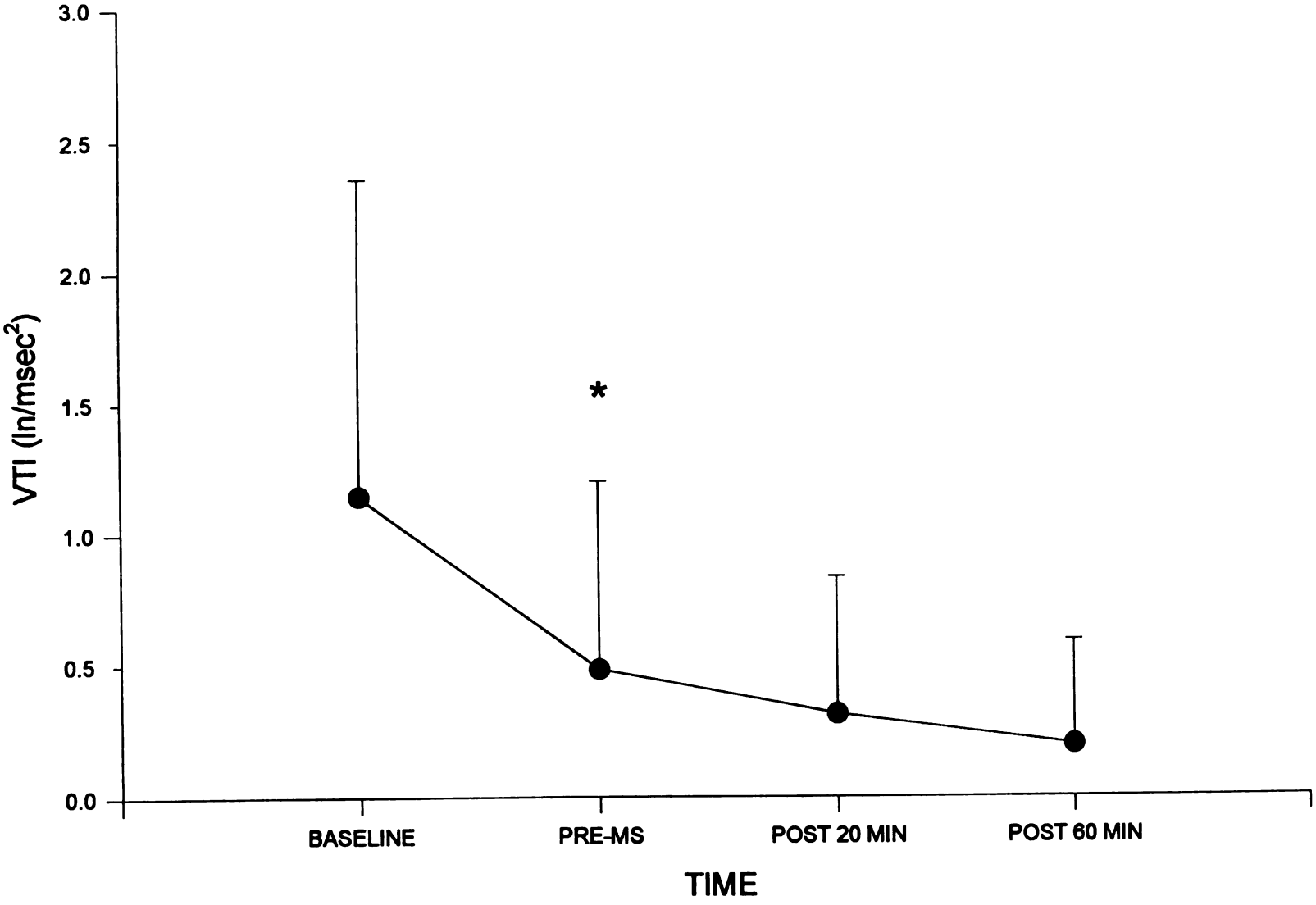

3.0
2.5
2.0
1.5
1.0
0.5
0.0
VTI (ln/msec²)
*
BASELINE
PRE-MS
POST 20 MIN
POST 60 MIN
TIME

Figure 3 - The mean flexor reflex threshold (FRT) in grams force (Y-axis) at each of the study time points (X-axis) for neonates who demonstrated a flexor reflex in the postoperative period (N = 11) and for neonates who did not demonstrate a flexor reflex in the postoperative period (N = 10). Each point in the figure represents the $\underline{M} \pm \underline{SD}$. The baseline time point represents neonates in both groups.

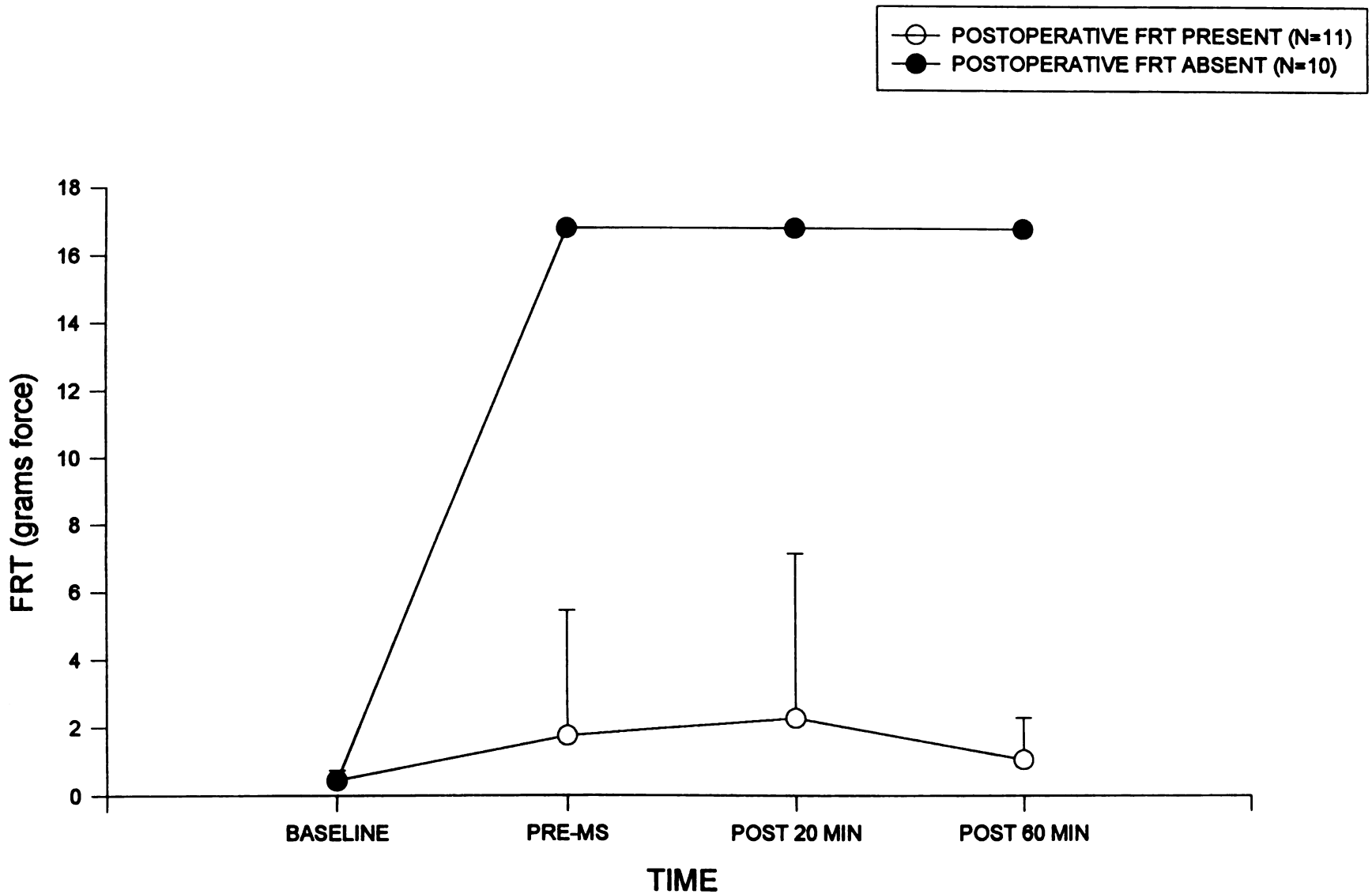

POSTOPERATIVE FRT PRESENT (N=11)
POSTOPERATIVE FRT ABSENT (N=10)
FRT (grams force)
18
16
14
12
10
8
6
4
2
0
BASELINE
PRE-MS
POST 20 MIN
POST 60 MIN
TIME

Figure 4 - The mean heart rate (HR) in beats per minute and systolic, mean, and diastolic blood pressure (BP) in mmHg (Y-axis) of the neonates (N = 24) at each of the study time points (X-axis). Each point in the figure represents the $\underline{M} \pm \underline{SD}$. Some error bars are contained within the symbols. The asterisk above the pre-morphine point denotes a statistically significant difference from baseline ($p < .001$).

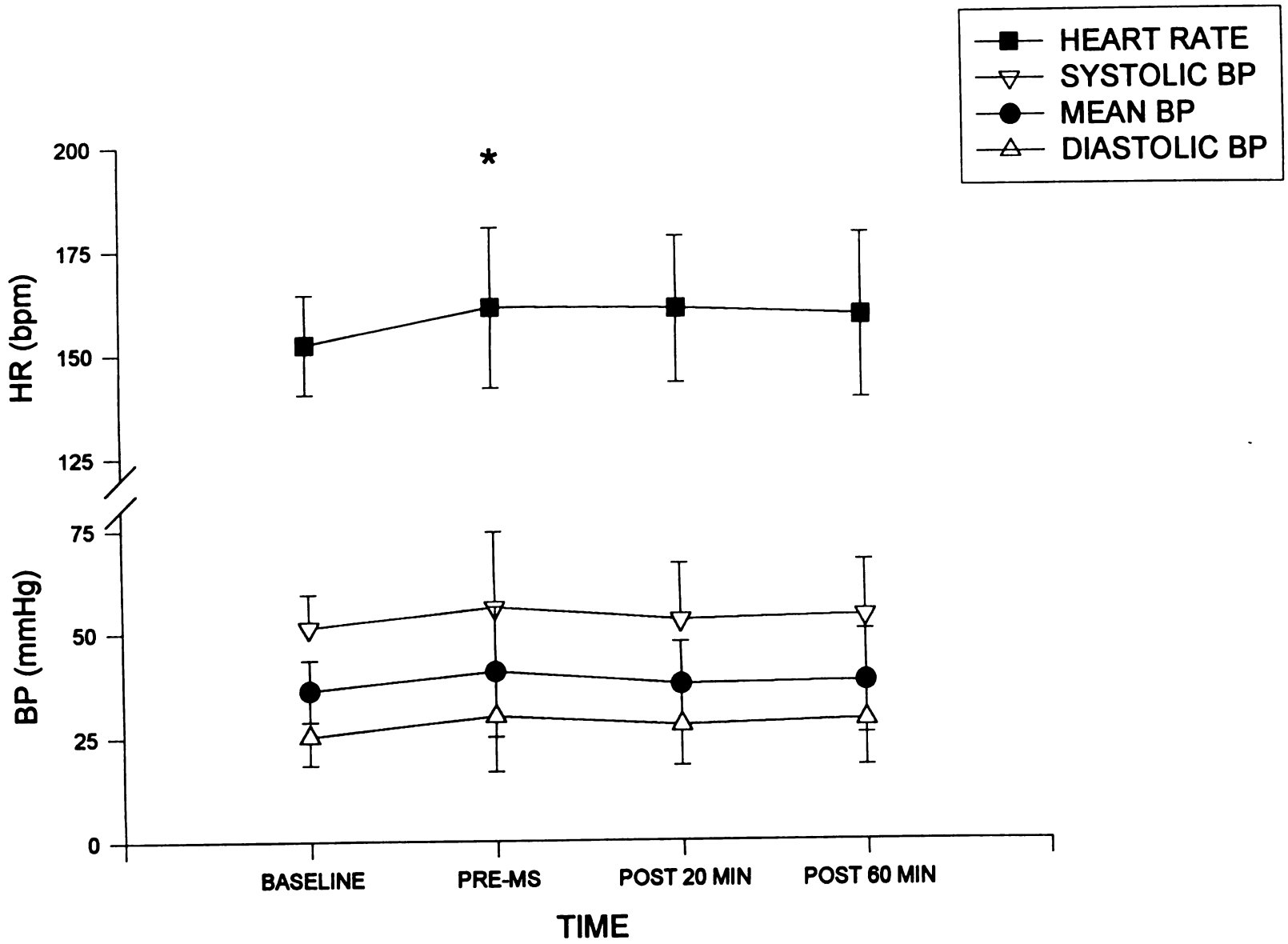

HEART RATE
SYSTOLIC BP
MEAN BP
DIASTOLIC BP
*
200
175
150
125
HR (bpm)
75
50
25
0
BP (mmHg)
BASELINE
PRE-MS
POST 20 MIN
POST 60 MIN
TIME

Figure 5 - The mean vagal tone index (VTI) in $ln/msec^2$ (Y-axis) at each of the study time points (X-axis) for the neonates who demonstrated a flexor reflex in the postoperative period (N = 12) and the neonates who did not demonstrate a flexor reflex in the postoperative period (N = 10). Each point in the figure represents the $\underline{M} \pm \underline{SD}$. The asterisk above the baseline and the 60 minutes post-morphine time points denotes statistically significant differences between the 2 groups ($p < .01$).

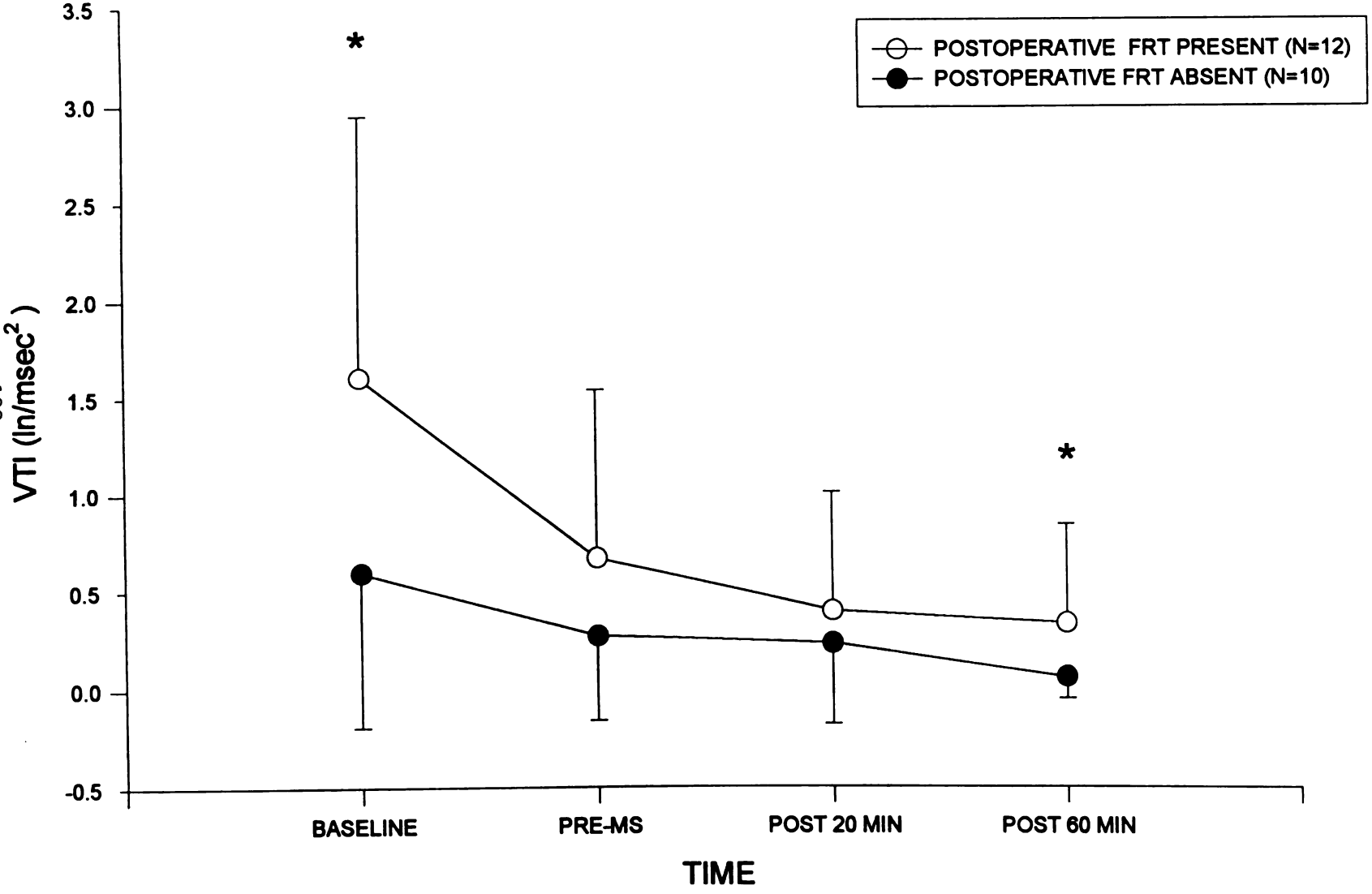

POSTOPERATIVE FRT PRESENT (N=12)
POSTOPERATIVE FRT ABSENT (N=10)
VTI (ln/msec²)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
*
*
BASELINE
PRE-MS
POST 20 MIN
POST 60 MIN
TIME

Figure 6 - The heart rates of the neonates who expired prior to discharge from the NICU (N = 5) and of the neonates who were alive at the time of discharge from the NICU (N = 20). Each bar in the figure represents the $\underline{M} \pm \underline{SD}$. Some error bars are contained within the bars. The asterisk above the bar denotes a statistical significant difference between the 2 groups ($p = .006$)

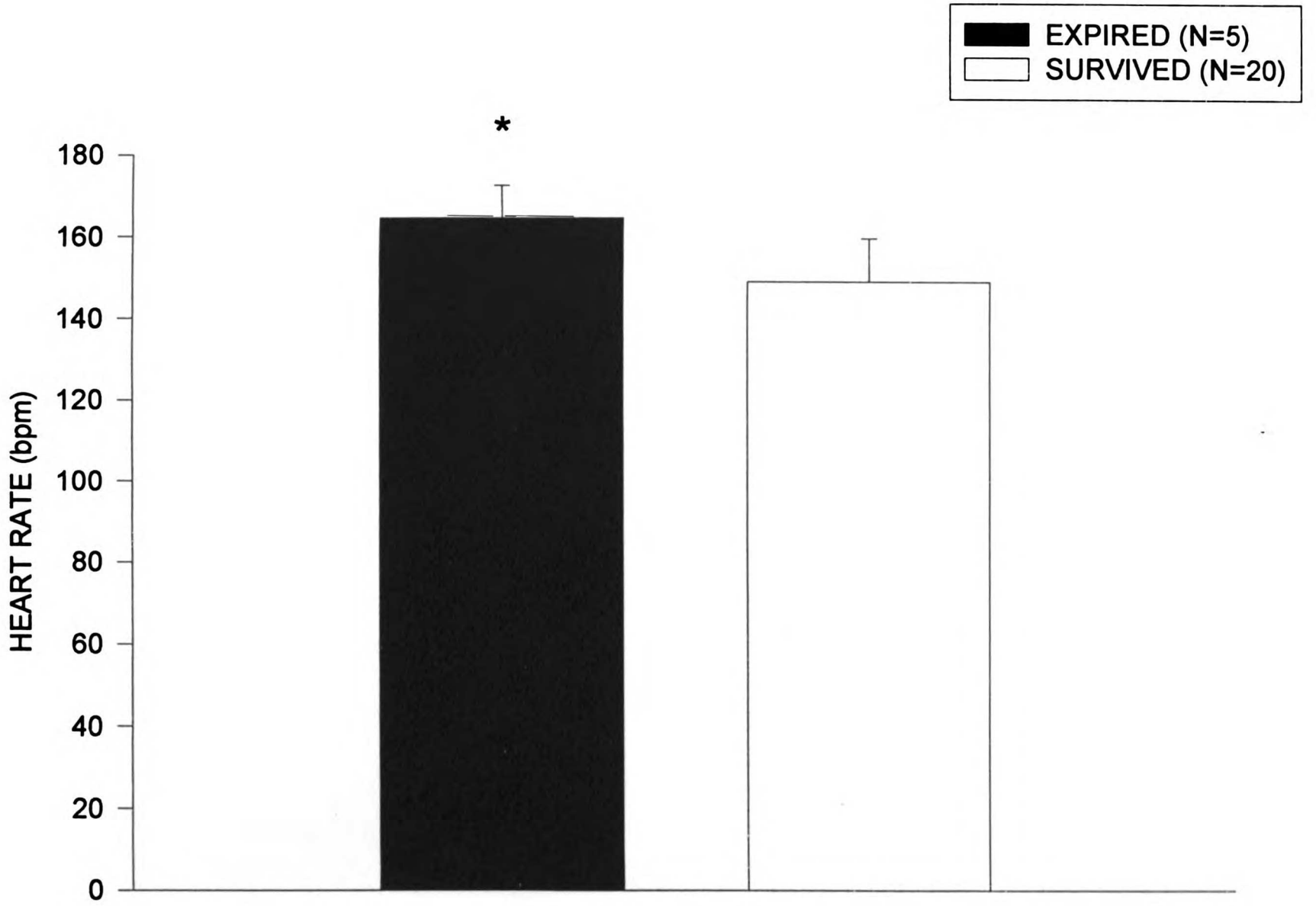
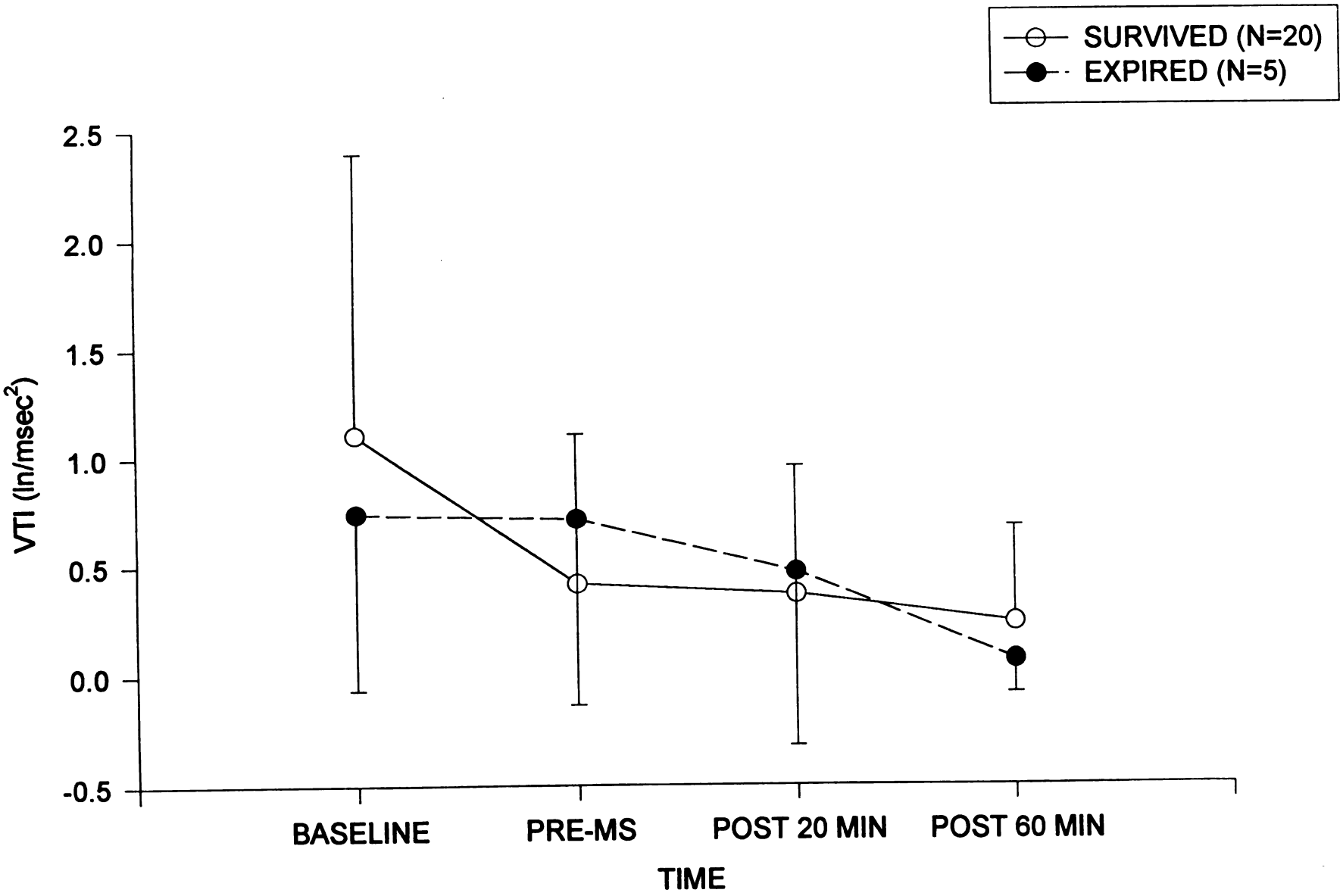

EXPIRED (N=5)
SURVIVED (N=20)
*
HEART RATE (bpm)
180
160
140
120
100
80
60
40
20
0

Figure 7 - The mean vagal tone index (VTI) in $ln/msec^2$ (Y-axis) of the neonates who were alive at the time of discharge from the NICU (N = 20) and of the neonates who expired prior to discharge from the NICU (N = 5) at each of the study time points (X-axis). Each point in the figure represents the mean $\pm$ SD Some error bars are contained within the symbols.


SURVIVED (N=20)
EXPIRED (N=5)
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
VTI (ln/msec²)
BASELINE
PRE-MS
POST 20 MIN
POST 60 MIN
TIME