A STUDY OF THE EFFECTS OF ANALGESIA IN ACUTE AND CHRONIC PAIN IN PRETERM INFANTS

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For Kate and Jennie.

Without their love, support and encouragement,

this work would not have been possible.

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ABSTRACT

Advances in obstetric and neonatal care during recent years have led to the survival of increasing numbers of extremely premature and low birth weight infants. These infants are, of necessity, subjected to a wide range of stressors and painful interventions during the course of intensive care. Invasive procedures such as heelstick and venepuncture are common in the care of very sick infants and responses to acute pain of this kind have been extensively investigated.

Many other procedures and interventions are performed which, although not causing direct tissue damage, are likely to be perceived as painful or distressing for neonates. These may be prolonged such as mechanical ventilation or repeated such as endotracheal suction. Many disease states will induce an inflammatory response likely to cause pain that may be persistent or chronic; for example, necrotising enterocolitis or meningitis. There has been only limited investigation of the physiological and behavioural responses to chronic or sub acute pain in the newborn population.

Pain assessment is the key to pain management. Self-report of pain is the gold standard for pain assessment in older age groups, but in the preverbal infant this is obviously not available. A simple and accurate means of identifying and measuring pain would enable appropriate initiation of analgesia and evaluation of its effects. There are no recognised physiological or behavioural indicators that can be used to

assist with the detection and assessment of chronic pain in infants whether or not they are undergoing neonatal intensive care, but there are situations in the neonatal intensive care unit in which one could anticipate that infants would experience chronic pain.

This study investigates mean heart rate, heart rate variability and the cutaneous flexor withdrawal reflex as potential indicators of chronic or acute pain in preterm infants. The relationship between mean heart rate, heart rate variability, pain and analgesia is explored in two different situations:

1. In preterm infants undergoing chronic pain or distress associated with mechanical ventilation, randomised to receive morphine or placebo. The groups are compared to assess whether mean heart rate, heart rate variability or the cutaneous flexor withdrawal reflex threshold are useful in the assessment of pain or withdrawal of opiate analgesia in the clinical situation.

2. In infants undergoing the acute pain and stress of ophthalmological examination for screening for retinopathy of prematurity. These infants were randomised to receive sucrose solution or placebo. These groups are similarly compared to assess whether mean heart rate or heart rate variability are useful for pain assessment.

Declaration

This thesis has been composed by myself and the results presented are a product of my own work.

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CHAPTER 1

INTRODUCTION

Historically, the newborn infant was believed to be incapable of experiencing pain. More recently, as increasing research has been carried out, it has become clear that even the preterm neonate is sufficiently developed anatomically, hormonally and neurophysiologically to perceive and respond to painful stimuli[1;2]. Furthermore, there is a growing body of evidence from both animal and human studies to suggest that prolonged or repetitive pain during the neonatal period may increase morbidity and alter behavioural responses both in the short and long term[3-7].

Yet all newborn infants are exposed to potentially painful or distressing events. Healthy term neonates undergo a number of unavoidable procedures such as injection of Vitamin K at birth, heel prick for the Guthrie test in the first week of life and immunisations during the first year. Sick term infants or the smaller and more vulnerable preterm infants are subjected to many more frequent and invasive procedures[8]. This pain or distress may be repeated as in the case of venepuncture or heel prick, or be prolonged as in babies who are mechanically ventilated.

Clinicians now accept that newborn infants can and do experience pain and there is a large amount of literature examining the problem of acute procedural pain in the newborn. There are, however, ongoing difficulties in the detection of pain in the neonatal period. In the care of older children and adults, doctors rely on self-report indicating that a person is in pain. This is not applicable in preverbal infants. The only means of identifying pain in the newborn is by careful observation of behavioural and physiological parameters. In the preterm infant, the limited repertoire of behavioural responses makes this challenging though facial responses appear to be good indicators of acute pain. In addition, there is always likely to be a degree of subjectivity in assessment of behaviour.

In an attempt to facilitate and standardise pain recognition and assessment, a number of scoring systems and scales for acute pain have been developed and validated for use in term and preterm infants[3;9;10]. Some deal with procedural pain, others are designed to be used to assess postoperative pain. Most rely on facial responses, though some also include physiological parameters such as changes in heart rate and oxygen saturation[11;12]. Although physiological changes are objective and may be useful when accompanied by changes in facial expression, they are in themselves non-specific. For example, the increased heart rate or decreased oxygen saturation observed when a baby is in pain may equally be due to pyrexia or respiratory disease respectively and similar changes not infrequently accompany non-painful interventions.

There has been only limited investigation of the physiological and behavioural effects of chronic or subacute pain in the newborn population. There is at present no simple clinical scoring system for chronic pain or distress and no recognised physiological indicator that can be used to assist with the identification and management of chronic pain in infants undergoing neonatal intensive care.

Heart rate variability is known to be reduced in sick preterm infants compared with well infants[13-15]. This may, in part, be due to the effects of chronic pain and stress associated with mechanical ventilation and other necessary interventions during intensive care. Increased heart rate and reduced heart rate variability have also been demonstrated in infants undergoing acutely painful procedures such as venepuncture[16;17]. The effects of analgesia and withdrawal of analgesia on mean heart rate and heart rate variability in preterm newborn infants have not been investigated.

The cutaneous flexor withdrawal reflex has been used by investigators in the field of neonatal pain as a research tool[18]. Previous work has shown that it correlates well with the perception of pain. It has been used to examine effects of topical anaesthesia in infants but its use as a clinical measure of the effects or adequacy of analgesia in ventilated neonates has not been investigated[19].

This study aims to explore the relationships between acute/procedural and sub acute/chronic pain, heart rate variability and analgesia in premature infants in the

intensive care situation. It also aims to assess whether mean heart rate, heart rate variability or the cutaneous flexor withdrawal reflex are clinically useful indicators of pain or opiate withdrawal in preterm infants.

CHAPTER 2

AIMS OF THE RESEARCH

The broad objectives of this study were:

To investigate the effects of analgesia in preterm newborn infants in two different situations

1. In ventilated infants – subacute/chronic pain/distress (morphine analgesia)
2. In infants undergoing ophthalmological examination as screening for retinopathy of prematurity - acute pain/distress (sucrose analgesia)

The specific objectives of this study were:

1. to examine differences in mean heart rate, heart rate variability and the cutaneous flexor withdrawal reflex between infants randomised to receive morphine or placebo for intensive care
2. to compare two different methods of heart rate monitoring in preterm infants

3. to examine differences in mean heart rate and heart rate variability between infants randomised to receive sucrose or placebo for ophthalmological examination
4. to assess whether mean heart rate, heart rate variability or the cutaneous flexor withdrawal reflex are clinically useful indicators of pain or opiate withdrawal in preterm infants

CHAPTER 3

PAIN IN THE NEWBORN

3.1 DEFINITIONS

The International Association for the Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage"[20]. According to this definition, the perception of pain is subjective and is dependent both on previous experience and self-report.

The term 'nociception' describes the behavioural and physiological effects induced by a noxious stimulus, without reference to the associated psychological and emotional responses. Since newborn infants neither have prior experience of pain nor the ability to communicate, nociception more accurately describes the phenomenon of acute pain in the neonate. However, the terms are frequently used interchangeably in literature on the subject of neonatal pain.

'Stress' refers to adaptive responses generated by external occurrences or internal factors. Such adaptive responses are normal responses. Stress will produce 'distress' only if it persists and leads to maladaptive behaviour.

The issue of chronic pain and distress in infants presents difficulties when considering the newborn. Previously, chronic pain has been defined as pain lasting for six months or recurring periodically over a six-month period. The IASP now recognises chronic pain as that which persists for longer than one month beyond the normal healing time for an injury or disease. Even this shorter period of time cannot be used in the neonatal period, which by definition is the first 28 days of life. Whilst such classifications are useful in the study of pain generally, much of the terminology is more suited to adults who are able to articulate their feelings. There is no definition entirely appropriate to describe ongoing pain or distress in the neonate, which may be present for hours or days rather than the weeks and months. For the sick, preverbal infant, it may be more appropriate to consider pain according to both the cause and likely duration of the insult. Pain resulting from an invasive procedure is immediate, often lasting only seconds or minutes, whereas the pain potentially caused by ongoing inflammation is likely to persist for hours or days. Detection and management of these contrasting phenomena will differ, so a distinction must be made.

For the purposes of this discussion, the pain induced by a defined invasive procedure will be referred to as 'acute' or 'procedural' pain. Pain which results from a

condition such as inflammatory disease or an intervention likely to lead to prolonged discomfort lasting for hours or longer, will be referred to as 'chronic' pain.

3.2 AN HISTORICAL PERSPECTIVE

Attitudes of medical professionals towards the issue of pain in newborn infants have changed markedly with the passage of time. Until the early 20th century, it was believed that children and infants experienced more pain than adults, with several medical texts of the time making reference to hyperalgesia in infants and the recognition of pain by facial expression.

However, a change in thinking occurred as a result of research into the developing nervous system. In 1872, Paul Emil Flechsig noted that although both myelinated and unmyelinated fibres were present, only myelinated fibres appeared to be fully functional. He suggested that complete myelination is required for perception of pain and this led to the widespread belief that the immature nervous system of newborn infants was not sufficiently developed to render them capable of experiencing pain. More recently, L.I. Swafford and D. Allen stated that "pediatric patients seldom need medication for the relief of pain after general surgery. They tolerate discomfort well"[21]. At this time, it was common for babies and young children to undergo surgical procedures without anaesthesia or pain relief. Additional 'proof' that babies

did not feel pain was the fact that they had no memory of painful experiences in early childhood. Concerns about appropriate opiate dosing and the possibility of encouraging opiate addiction in paediatric patients together with the belief that infants react adversely when given opiates have also played a part in the reluctance of physicians to prescribe adequate analgesia[22].

The anatomy of the developing fetal and neonatal nervous system has been clarified by extensive research in animals and humans. It is now clear that cutaneous sensory receptors begin to appear early in foetal life and continue to spread throughout all skin and mucous membranes until the 20th week[23;24]. Skin in the newborn period has a greater density of nociceptive nerve endings than is found in adults[25]. At around 8 to 10 gestational weeks, development of neural connections between the periphery and the dorsal horn of the spinal cord begins to occur[26]. Formation of synaptic connections and neurotransmitter vesicles continues to complete the development of the dorsal horn by around 30 weeks of gestation. Lack of complete myelination of nerve fibres has been suggested as an indicator of nervous system immaturity and the lack of ability of neonates to perceive pain[21;27]. In adult peripheral nerves, some transmission of nociceptive impulses occurs via unmyelinated and thinly myelinated fibres[25]. Incomplete myelination of nerve fibres leads to slower conduction of impulses, rather than absence of conduction. In infants, this slower conduction is offset by the relatively short distance travelled by impulses in babies as compared with adults. Myelination is completed during the second and third trimesters. The cerebral cortex starts to develop at 10 gestational weeks. This continues until term

and beyond with growth, differentiation and branching and migration of neurones. Nociceptive pathways from the periphery to the cortex pass through the thalamus, therefore maturity of neural connections in the thalamus is necessary for pain perception. Thalamocortical connections are completed by 20 – 24 weeks of gestation[28]. Electroencephalograms (EEGs) have been studied in fetuses and extremely preterm infants in an attempt to define cerebral function in physiological terms. A primitive EEG characterised by intermittent bursts is seen from 20 weeks of gestation. The EEG patterns mature over the next few weeks of gestation. By 28 weeks, EEG patterns are sufficiently defined as to make observation of identifiable sleep-wake patterns in fetuses possible[28]. There is also evidence to suggest that fetuses can mount physiological stress responses in utero. Interventions that would be expected to be painful for the fetus such as those which involve piercing of the abdomen cause a significant rise in production of cortisol and noradrenaline, even in early gestation. Procedures that would be unlikely to be painful do not induce such a response[29;30]. This evidence all points to the fact that newborn babies, even those born prematurely, do have the anatomical and functional requirements to allow perception of pain, although further development occurs during the postnatal period[25].

Considerable research in rats and human infants has been carried out to explore the cutaneous flexor withdrawal reflex. This is present in preterm infants from 26 weeks gestation[26;31]. It has been shown to correlate well with pain threshold and has been used as a measure of sensation in newborn infants. Investigation has shown that the

threshold for the cutaneous flexor reflex is very low in newborns and increases with increasing gestational age, rather than postnatal age[18]. Repeated stimulation in preterm babies results in sensitisation characterised by increase in the force of withdrawal with repeated stimuli[18]. This occurs in infants of below about 30 weeks gestation, but is seen less often with increasing gestational age. In contrast, in infants of more than 34 weeks gestation, repeated stimulation leads to habituation and decrease in the response, rather than sensitisation. The concept of wind-up has been well described in animal studies[32] and may also be important in human neonates. This describes the phenomenon where repeated painful stimuli are followed by exaggerated responses to mild, non-noxious stimuli that would not usually be perceived as painful.

In spite of increasing evidence that newborn babies experienced pain, perhaps even more acutely than older children and adults, routine analgesia for painful procedures was rarely used. Although it had been recognised by some that pain in infants and children frequently went untreated or, at best, under-treated[33;34] it was not until the mid to late 1980s that important work by Anand et al brought the subject into the public arena. Controversy and outcry arose because babies undergoing surgery as control subjects received no analgesia; this was standard practice at that time. The study showed that preterm infants were able to mount a significant hormonal and metabolic stress response to surgery and that this response could be attenuated by the use of opioid analgesia (fentanyl) during surgery[1;35]. It was also noted that infants receiving analgesia had fewer complications following surgery.

Research in the field of neonatal pain assessment has advanced considerably in recent years, with work concentrated mainly on the area of acute pain. Behavioural and physiological responses of infants undergoing painful procedures have been documented and a number of tools for the detection of pain have been developed and validated[10;12;36-42]. Knowledge of the pharmacokinetics of opioid and other analgesics in infants has been greatly increased.

The problem of chronic pain in the neonatal period has been less well investigated[43]. There is, at present, no recognised physiological indicator that can be used to assist with the identification and management of chronic pain in infants undergoing intensive care.

3.3 PAIN IN THE NEONATAL INTENSIVE CARE UNIT

Advances in obstetric and neonatal care during recent years have led to the survival of increasing numbers of extremely premature and low birth weight infants with gestational ages as low as 23 weeks. These tiny patients, undergoing life-saving and essential therapy during neonatal intensive care are, of necessity, subjected to a wide range of stresses and interventions. Older children or adults would certainly perceive many of these as painful or distressing. We cannot and will never know precisely

how the newborn baby perceives these interventions, but it is reasonable to suppose that those procedures reported as painful or distressing by articulate adults, would be similarly perceived by the preverbal infant.

Procedures causing direct tissue damage are common during the first hours and days of life for a preterm or seriously ill term baby. Such procedures include venepuncture, heel stick, arterial line insertion, chest drain insertion, lumbar puncture and suprapubic aspiration of urine. There can be little doubt that these invasive events cause acute pain and warrant consideration of specific analgesics appropriate to the degree of invasiveness of the procedure.

A number of other less invasive procedures are commonly performed such as endotracheal intubation and suction, nasogastric tube insertion and umbilical venous and arterial catheterisation and ophthalmological examination. Whilst these do not usually involve tissue damage, they are likely to produce varying degrees of procedural pain or discomfort.

Essential nursing interventions such as placement, repositioning and removal of monitoring electrodes or probes and handling associated with nappy or linen changes are regular occurrences. Such interventions are often undertaken in 'clusters' following more invasive procedures thus allowing longer rest periods for the infant. However, bearing in mind the phenomena of sensitisation and 'wind-up' previously described, it is possible that these relatively benign procedures might be perceived as

painful if approached in this way. Studies reporting changes in transcutaneous oxygen and pain behaviours in infants associated with "nonpainful" procedures support this view[44-46].

There are a number of conditions that commonly affect newborn babies and which may cause chronic pain. Preterm infants are at particular risk of necrotising enterocolitis, whilst infants born at any gestation may develop illnesses such as septicaemia, meningitis, pneumonia or osteomyelitis. All of these conditions will induce an inflammatory response within tissue, likely to cause pain that may persist for hours, days or weeks until the disease has run its acute course. Intraventricular haemorrhage affects a significant number of extremely premature infants and it is possible that this condition may cause pain if bleeding affects the meninges.

The sickest and most premature infants usually require mechanical ventilation. Adults recall that having endotracheal or gastric tubes in the mouth or nose is a major source of stress during their stay in an intensive care unit[47-49]. Although the sensation may not be specifically described as 'pain', it is likely that the experience of prolonged mechanical ventilation is also unpleasant and a source of ongoing distress for the newborn baby. Therapeutic paralysis makes detection of pain even more challenging and may in itself cause distress, as may tissue oedema that is often seen in this group of infants, particularly those receiving muscle relaxant drugs.

3.4 PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES TO PAIN

Invasive or acutely painful interventions in neonates are accompanied by a number of measurable physiological and biochemical responses many of which are undesirable. Significant changes have been demonstrated by investigators in association with many procedures commonly carried out during intensive care of the newborn. These include heel prick[50;51], venepuncture[52], circumcision[52-55], endotracheal intubation[56;57] and endotracheal suction[58;59]. Physiological changes described include increase in mean heart rate, change in respiratory patterns, increase in blood pressure and decrease in oxygen saturation or transcutaneous oxygen levels. Increase in skin conductance[60] and increased 'emotional' palmar sweating[61] have also been shown in term newborn infants exposed to heel prick, but do not occur in those born before 36 weeks gestation.

Many of these physiological parameters are routinely monitored in premature infants undergoing intensive care and as such, might be useful in the day-to-day evaluation of pain. However, most physiological responses are not specific to pain and change in response to other interventions that are not judged to be painful. By themselves, therefore, these measures are of limited value in pain assessment.

Newborn infants mount a significant hormonal and metabolic stress response to painful stimuli. Much of the work on this subject was carried out on babies

undergoing circumcision or surgery. Gunnar showed a marked increase in cortisol levels in infants after circumcision without anaesthesia[62]. In neonates having surgery with minimal anaesthesia, Anand demonstrated a marked catecholamine response at the end of surgery[1], increases in lactate and pyruvate and suppression of insulin secretion. Release of adrenaline at the time of surgery is thought to play an important part in initiation of other changes seen post-operatively in babies such as hyperglycaemia[1]. These metabolic and hormonal responses are attenuated by deep anaesthesia and analgesia[35;63;64]. There is little literature addressing more minor painful procedures such as endotracheal or oral suction[65] and heel prick[66].

3.5 BEHAVIOURAL RESPONSES TO PAIN

Behavioural indicators of pain in newborn infants have been extensively studied. Crying, changes in facial expression, gross motor movements and changes in behavioural state and sleep-wake cycles have all been evaluated.

Crying is the newborn baby's most effective method of communication with adults. Studies using spectrographic analysis of pre-recorded cries show that cries can be classified and those reflecting different types of distress can reliably be recognised by experienced observers[67;68]. Patterns of crying have also been shown to correlate

with intensity of pain in infants at circumcision[69]. Cry duration is less good as an indicator of pain intensity[70].

Facial expressions associated with exposure to pain are well characterised. Facial responses include brow bulge, eye squeeze, deepening of the naso-labial furrow, open mouth, vertically and horizontally stretched mouth and taut, cupped tongue. These facial features are demonstrated in premature infants[71;72] and term infants[73] although the younger the gestational age in preterm infants, the less the response[71]. Guinsburg et al demonstrated that there may also be a difference in facial indication of pain between male and female infants, with females showing greater facial expression than males[74].

Body activity caused by the pain of heel prick has been examined. Infants born at term show more whole body activity in response to pain than do premature infants[71].

Behavioural states in term and near term infants occur in cycles[75]. In premature infants, states are less well regulated. Pain in term infants has been shown to disrupt behavioural state and sleep-wake cycles. Emde et al reported that a prolonged period of non rapid-eye-movement sleep followed circumcision[76]. Other studies have shown increased wakefulness following circumcision[77]. Minor painful procedures have been shown to increase the behavioural state in neonates[78]. The behavioural state of an infant prior to a painful procedure has also been shown to affect facial activity in response to the painful stimulus[68;73]. Behavioural states are difficult to accurately

assess and are also altered by other conditions such as severe illness and neurological disorders or injury. The usefulness of such assessment in pain measurement is therefore limited.

3.6 ASSESSMENT OF PAIN

Assessment of pain is the key to appropriate management of pain. Without accurate and reliable means of assessing pain, it is impossible to evaluate the need for or the effectiveness of intervention. In the absence of self-report, the difficulties of identification and measurement of pain in babies are many. It is further limited by the narrow behavioural repertoire, particularly of premature infants and the lack of specificity of the associated physiological responses. This has led to the development of a number of clinical tools in the form of scoring systems for the assessment of pain and distress in neonates. For such a tool to be useful, it must enable the observer to detect the presence of pain and describe or grade the intensity of the pain. The measures included in any pain assessment score must be well defined and validated such that observations are comparable between observers and reliable between infants. The feasibility of its use in the clinical setting is dependent on its simplicity and ease of execution.

Most of the work, to date, has centred upon the area of acute pain. Both behavioural and physiological indicators of pain have been incorporated into pain assessment scores. Some have chosen to use a unidimensional approach, using a single type of pain indicator. Behavioural indicators, including facial activity, which appears to be the most specific of pain indicators in infants, form the basis of most unidimensional scoring systems. Although unidimensional measures have been useful in pain research, no single measure is reliable, valid, sensitive and specific and most unidimensional scores are not clinically useful for the recognition and assessment of pain in the newborn. Other investigators have combined behavioural and physiological indicators to produce multidimensional scores.

3.6.1 Scores for Assessment of Acute Pain

3.6.1.1 Unidimensional Measures

The *Neonatal Facial Coding System* (NFCS)[67;73] uses the characteristic facial expressions associated with pain (brow bulge, eye squeeze, nasolabial furrow, open lips, stretch mouth, lip purse, taut tongue, chin quiver and tongue protrusion) to assess the presence of pain. This score has been validated and its feasibility as a clinically useful tool has been tested[9].

The *Infant Body Coding System* (IBCS)[79] is a behavioural tool using gross motor movements in infants exposed to acute painful stimuli. Movements of the hand, foot, arm, leg, head and torso are observed and recorded as being present or absent.

The *Children's Hospital of Eastern Ontario Pain Scale* (CHEOPS)[80] was designed for use postoperatively in children over 1 year. It was adapted to form the *Modified Behavioural Pain Scale* (MBPS)[81] for use in infants from 4 – 6 months, but has not been validated in neonates. This scale uses 3 indicators including facial expression, cry and gross body movements.

The *Liverpool Infant Distress Scale* (LIDS)[39;82] was developed to assess the degree of distress due to postoperative pain in infants. It uses eight behavioural categories, including facial expression, quality and quantity of crying, scoring each on a scale of 0 to 5.

In France, the *Douleur Aiguë du Nouveau-né* (DAN)[41] has been validated as a discriminator of pain, but has not been proven to grade intensity of pain. The DAN scores pain from 0 – 10, evaluating facial expression, limb movements and vocal expression. It has been used in research to assess the effectiveness of analgesia[83].

3.6.1.2 *Multidimensional Measures*

The *Neonatal Infant Pain Scale* (NIPS)[36] was also adapted from the CHEOPS[80]. It utilises five behavioural and one physiological (breathing patterns) measure, each indicator scored on a 2 or 3-point scale. The scale has been validated and has been compared with other pain scales[42]. It is used mainly in research.

The *CRIES* (Crying, Requires oxygen for saturation above 95%, Increased vital signs, Expression and Sleeplessness)[37] is a postoperative score using five physiological and behavioural indicators giving a score of 0 – 10. The score was validated in a group of 24 babies.

The *Distress Scale for Ventilated Newborn Infants* (DSVNI)[84] assesses the response to pain in ventilated neonates. This scale was developed using elements from five other previously developed scoring systems (Neonatal Behavioural Assessment Scale(NBAS)[85], the Assessment of Preterm Infants' Behaviour(APIB)[86], the NFCS[67;73] the IBCS[79] and the Gustave-Roussy Child Pain Scale[87;88]. It includes measures of both physiological and behavioural responses, but is only useful in this small group of infants and has not been validated.

The *Scale for Use in Newborns* (SUN)[42] consists of three behavioural (movement, tone, face) and four physiological measures (neurological state, breathing, heart rate, blood pressure). Each category is scored from 0 – 4 with 2 being normal. The SUN's

validity and clinical utility were established during comparison with two other scales[42].

The *Premature Infant Pain Profile* (PIPP)[12] is perhaps the most extensively validated pain score for use in both term and preterm infants. It takes into account seven indicators, of which three are behavioural (brow bulge, eye squeeze, nasolabial furrow), two physiological (heart rate and oxygen saturation) and two contextual (gestational age and behavioural state). Each indicator is evaluated using a four-point scale. The total possible score for infants of less than 28 weeks gestation is 21 and for those of greater gestational age is 18. For both groups, a total score of more than 12 indicates moderate to severe pain, while a score of less than 6 indicates little or no pain. The validity and inter- and intra-rater reliability of the PIPP have been established in the clinical situation[89] and it has been used in research studies to assess the efficacy of pain relief interventions[90].

The *Comfort Scale*[91] was designed specifically for use in infants and children up to the age of 2 years in the intensive care situation. It has been shown to measure reliably the adequacy of sedation in these patients[92]. It was compared with the NIPS and SUN to establish its validity and clinical use, but was found to be the most complex of the scores and difficult to use[42].

3.6.2 Scores for Assessment of Prolonged Pain

The EDIN (Echelle Douleur Inconfort Nouveau-né)[10] represents the first scoring system to be developed for the assessment of prolonged pain in preterm newborn infants. It is a unidimensional tool, using only behavioural indicators. Facial activity, body movements, quality of sleep, and quality of contact with nurses and consolability are evaluated as measures of pain. Each measure is scored on a four-point scale of 0 – 3. The study to validate the EDIN involved 76 preterm infants. Scores in extreme situations, before and after analgesic therapy were compared to establish validity of the scoring system. Inter-rater reliability was found to be acceptable.

The N-PASS (Neonatal Pain, Agitation and Sedation Scale)[93] is a scale that is currently being developed and is in the early stages of evaluation in the clinical setting, but as yet there are no published data relating to N-PASS. The score uses crying and irritability, behavioural state, facial expression, extremities tone and vital signs (heart rate, respiratory rate, blood pressure and oxygen saturation) as indicators. Each indicator is evaluated on a score of –2 - +2.

3.7 MORPHINE ANALGESIA IN NEONATAL INTENSIVE CARE

A variety of systemic analgesic and sedative drugs are available for use in the newborn. Opiate analgesics are probably the most widely used in neonatal intensive care[94;95]. A number of opioids including morphine, diamorphine, fentanyl, alfentanil and sufentanil are used in treatment of neonates undergoing surgery or intensive care. Morphine is the oldest and most extensively studied in all ages and, as such, is also the most frequently used clinically. Since more recognition has been given to the problem of neonatal pain, its use in newborn intensive care has dramatically increased.

Morphine is an extract of opium and analgesia is mediated principally by μ-opioid receptors. It acts at the receptors to hyperpolarize cell membranes and reduce neuronal activity.

3.7.1 Morphine Pharmacokinetics and Pharmacodynamics

Information on pharmacokinetics of morphine is limited. This is perhaps due to the need for repeated blood sampling in such studies that presents a problem in the

neonatal population. In general studies have included small numbers of infants and results show great variation.

Plasma protein binding of morphine varies with age. In the premature infant, less than 20% is protein bound[96].

Studies to examine the volume of distribution of morphine vary in the ages of infants studied. Their results are conflicting. Some report similar steady state concentrations in preterm and term infants[97] while others report great variation depending on the postnatal age in preterm infants[96].

Morphine is metabolised by the liver via glucuronidation. Its metabolites are morphine-6-glucuronide and morphine-3-glucuronide, which are excreted by the kidneys. The rate of metabolism depends on the age of the patient and glucuronidation is less efficient in the newborn[98-101]. Morphine-6-glucuronide is a potent analgesic and has slow clearance compared with morphine. Adults and older children excrete less than 10% morphine in unchanged form, whereas sick neonates excrete much larger amounts.

Clearance of morphine depends on age, but there is also large inter-individual variation. The elimination half-life in preterm infants is 10-20 hours, decreasing to 1-2 hours in preschool children and settling at 2-4 hours in adulthood. Renal failure will lead to accumulation of morphine and its metabolites.

Plasma concentrations of morphine that are appropriate for analgesia are difficult to determine, because of the problems of differentiating between analgesia and sedation. Study results vary, reporting values of between 12 and 65ng/ml as effective concentrations[102]. This is indicative of the difficulties in performing such studies.

3.7.2 Tolerance

Tolerance refers to a decrease in the pharmacological effects of a drug with repeated administration or the requirement for an increase in dose to maintain the same clinical effect[103]. Opioid tolerance is due to adaptation of neuronal cells within the central nervous system. Research suggests that tolerance may develop more rapidly with continuous opiate infusions than with intermittent doses[104;105]. There is little information available to indicate the length of treatment required before development of tolerance in the preterm neonate.

3.8 OPIATE WITHDRAWAL IN PRETERM INFANTS

Little literature is available relating to the effects of discontinuation of therapeutic doses of opiates in preterm neonates. Opiate withdrawal effects are well documented in adults and children[106;107]. Term infants whose mothers have used opiate drugs during pregnancy are commonly known to display an identifiable constellation of signs and symptoms known as the neonatal abstinence syndrome[108;109]. Validated scores are used to grade the features of neonatal withdrawal in such circumstances[109].

Withdrawal behaviour has been studied in the past in infants exposed to opiate medication during extracorporeal membrane oxygenation[104]. This study used a modification of the Neonatal Abstinence Score to evaluate withdrawal and, in these term infants morphine was shown to be associated with fewer withdrawal effects than fentanyl. Since the behavioural repertoire of sick preterm neonates often differs from that of term babies, it is possible that they might also exhibit different types of response to opiate withdrawal or that the signs might differ in quantitative or qualitative terms.

The question of whether cessation of therapeutic doses of opiates in sick premature infants causes a recognisable withdrawal syndrome remains to be answered. Preterm infants are a difficult group to assess due to their limited behavioural repertoire and the relative inexperience of clinicians, particularly where infants of extremely early gestation are concerned. These babies are frequently very sick and receive multiple

therapies, usually including mechanical ventilation. A number of the elements of accepted scoring systems are therefore more difficult to apply, or lack relevance for the premature neonate in the intensive care setting; for example, sweating, sneezing, regurgitation, loose stools, fist sucking and excoriation of skin. Most of these features are unlikely to be observed in babies who are ventilated and in whom enteral feeds have not yet been introduced. It is likely, therefore, that attempts to assess withdrawal effects in this vulnerable population are often inappropriate and inadequate.

In order to prevent the onset of potential, but poorly defined withdrawal phenomena, opiate drugs are weaned over a variable period of time in some neonatal units. However, this is not a universal practice, with some clinicians having anxieties about continuing even low-dose opiate infusion in spontaneously breathing babies. There is no published evidence to suggest that any weaning regimen is optimal. The identification of a reliable physiological indicator of opiate withdrawal in this group of infants would allow evaluation of weaning protocols.

The effects of opiate withdrawal on mean heart rate and heart rate variability have not been explored. It would be expected that patterns associated with opiate withdrawal would become evident shortly after discontinuing therapy or during the weaning phase and then resolve gradually over time as the drug is excreted. Identification of a clear difference in heart rate or heart rate variability between the two study groups might be indicative of withdrawal phenomena.

CHAPTER 4

THE N.E.O.P.A.I.N. STUDY

(NEUROLOGICAL OUTCOMES AND PRE-EMPTIVE ANALGESIA IN NEONATES)

4.1 STUDY DESIGN AND METHODS

The N.E.O.P.A.I.N. Multicentre Trial was a randomised, double blind, placebo controlled trial investigating the use of routine morphine infusions in preterm ventilated infants. Primary outcomes to be investigated were mortality and poor neurological outcome. A total of 900 infants were recruited to the study in 16 neonatal intensive care units in North America, Scandinavia, France and Scotland.

Babies born at 23-32 weeks gestation were randomised to receive either continuous low-dose morphine infusion or placebo (dextrose 5%) from the time of intubation for the duration of the time spent on a ventilator (minimum 24 hours) up to a maximum of 14 days. Morphine infusion rates were stratified by gestational age. A loading dose of 100mcg/kg was used. Babies of 23-26 completed weeks of gestation received

maintenance doses of 10mcg/kg/hour, those of 27-29 weeks received 20mcg/kg/hour and babies of 30-32 weeks of gestation, received 30mcg/kg/hour.

4.2 N.E.O.P.A.I.N. STUDY POPULATION (SCOTTISH CENTRE)

The Scottish arm of the N.E.O.P.A.I.N. study was conducted on the Neonatal Unit of the Simpson Memorial Maternity Pavilion, Edinburgh, United Kingdom, between 1 August 2000 and 31 December 2001.

Of around 7000 babies delivered each year within the area served by the Neonatal Unit, approximately 550 infants are admitted to the Neonatal Unit, of whom 180 will require ventilation. During the study period, 210 infants between 23 and 32 completed weeks of gestation were admitted to the neonatal unit and were eligible to enter the N.E.O.P.A.I.N. study on the basis of gestational age. Of these, 95 (45%) did not require mechanical ventilation and a further 25 (12%) babies were ventilated for less than 24 hours. 28 babies were excluded for other reasons including congenital abnormalities, severe birth asphyxia or maternal opiate abuse. Parental consent was refused in 18 ventilated babies who would otherwise have been eligible to enter the study. A total of 44 infants were recruited to the N.E.O.P.A.I.N. Multicentre Study (Scottish Centre). 52% of the babies were male. The median gestational age of the

babies included was 28 weeks (range 23-32) and the median birth weight was 1022 g (range 500-1935). 22 babies were entered into each treatment group.

4.3 ADDITIONAL INVESTIGATION OF INFANTS

Babies enrolled into the N.E.O.P.A.I.N. study form a unique cohort from a randomised blinded study comparing the effects of treatment with morphine versus placebo. Research into pain in newborn babies is an emotive area. Some believe that all babies undergoing intensive care with ventilation should receive analgesia despite the lack, to date, of published data regarding short and long-term benefits of analgesia in this group. Given the difficulties and controversy surrounding pain and analgesia in the newborn, it may be inappropriate to conduct a similar study again. This group of preterm babies, therefore, provides an ideal opportunity to investigate other aspects and the effects of pain and analgesia.

We used the opportunity of participation in the N.E.O.P.A.I.N. trial to investigate heart rate variability and the cutaneous flexor withdrawal reflex in babies randomised to receive continuous low-dose morphine infusion or placebo. 38 of the 44 babies recruited to the N.E.O.P.A.I.N. were involved in this additional investigation.

4.4 ETHICS AND CONSENT

The Lothian Research Ethics Committee granted approval for this research. Verbal and written information about the multicentre study and the local additional research interventions was given to all parents. Parents were given the option of enrolling their baby in the N.E.O.P.A.I.N. Study without taking part in the local research, but no parent declined the extra measures. Informed, written consent for additional monitoring of heart rate using a Holter monitor and testing of the cutaneous flexor withdrawal reflex using von Frey hairs was obtained from parents of all infants enrolled in the N.E.O.P.A.I.N. Study.

CHAPTER 5

EFFECTS OF ANALGESIA ON MEAN HEART RATE AND HEART RATE VARIABILITY IN PRETERM VENTILATED INFANTS

5.1 BACKGROUND

A number of physiological variables have been used in term infants to assess pain. The changes in heart rate and transcutaneous oxygen partial pressures or oxygen saturation have been used as measures of pain in conjunction with behavioural measures. A simultaneous rise in heart rate and fall in partial pressure of oxygen is seen with procedures that involve tissue damage[11;110]. These measures have been used to evaluate the use of topical anaesthetic[111] and sucrose[112] analgesia for heel prick. In preterm infants and in ventilated infants of any gestation, the range of useful and relevant pain indicators is more limited. Emotional sweating has been used as an indicator of pain in infants exposed to heel prick[61] but this does not occur in babies of less than 36-37 weeks gestation. Cry, which has frequently been used as an indicator of pain, is difficult to assess in infants who have an endotracheal tube in place. An additional difficulty with all physiological measures is that absolute values

and change from baseline in these values are not specific for pain. In the case of heel prick, an increase in heart rate is observed with skin preparation, although the increase is greater with the invasive part of the procedure[71]. In preterm babies, assessment is further complicated by the variation in physiological parameters associated with increasing gestational age.

The variability of physiological parameters such as heart rate, respiratory rate, and oxygen and carbon dioxide tensions of the blood has been investigated as a measure of pain. McIntosh et al showed that variability of these parameters seemed to be more discriminating for distress caused by heel prick than the absolute heart rate[113;114]. Of the four parameters, variability of heart rate appeared to provide the most useful information about pain.

Heart rate variability is a measurement of beat-to-beat changes in heart rate. The normal variability in heart rate is due to the synergistic action of the two branches of the autonomic nervous system on the sinoatrial node. These act in parallel through mechanical, neural, humoral and other physiological mechanisms to maintain cardiovascular parameters in optimal ranges and to react appropriately to external or internal stimuli. In normal healthy individuals the heart rate varies constantly and an estimate of heart rate at any one point in time will represent the net effect of slowing by the parasympathetic nerves and acceleration by the sympathetic system. It is thought that greater increases and decreases in the heart rate of an individual represent a "healthier" state and better capacity to respond to demands presented by

stressors and the external environment. It might be anticipated therefore, that measurement of heart rate responses to painful events could provide a useful additional window into the complexity of pain reactivity in the infant.

The simplest measure of heart rate variability in the time domain is the standard deviation about the mean heart rate. This will reflect all components of variability during the time span over which measurements are made, but will be dependent on this length of time. This method was used by McIntosh et al[113] [114], but more complex methods have been used by other researchers. In the frequency domain, spectral analysis identifies changes in the power spectra of heart rate fluctuations in the low and high frequency ranges and is a more precise means of quantitatively assessing the function of the autonomic nervous system[15;115].

Heart rate variability has been extensively studied in the fetus, newborn infant, child and adult. Fetal heart rate variability has been used for many years as a measure of fetal well-being and reduction in this variability is recognised as a reliable indicator of fetal distress. Postnatally, spectral analysis has been used in healthy term [116-118] and preterm [14;119;120] newborn infants to identify normal patterns of heart rate variability and factors that influence these patterns. Cabal et al showed that, in preterm infants, heart rate variability is inversely related to heart rate and related to postnatal age[14].

Variability has been shown to increase with increasing gestational age, discriminating in particular between groups of preterm and term infants[117].The effects on heart rate variability of a number of disease states and therapeutic interventions have been investigated. Studies have shown decreased heart rate variability in infants with respiratory distress syndrome and that persistent reduction in variability is associated with an increased incidence of mortality[13]. Heart rate variability has been shown to increase as respiratory distress improves [15]. Mechanical ventilation in itself does not appear to exert an influence on variability[121]. Investigation of heart rate variability in infected infants has shown that these babies display abnormal heart rate characteristics when compared with non-septic infants[122]. Most studies of preterm infants have involved babies of gestational ages greater than 27 weeks. There is little information regarding heart rate variability in extremely premature infants of 23 – 26 weeks' gestational age. Heart rate variability decreases with increasing age, starting during childhood. The study of heart rate variability in older children has included attempts at defining normal patterns at rest and with exercise, highlighting the difficulties in obtaining accurate measurements in this age group[123;124]. Heart rate variability in children has also been studied in respiratory and cardiovascular abnormalities or disease processes[125;126]. The relationship between heart rate and emotional or psychological states has also been investigated[127;128] suggesting that heart rate variability decreases in the face of psychological stress. Patterns of variability in adults also alter with disease states and changing emotions. Much of the work in adults has been centred on the area of cardiovascular function in ischaemic heart disease and diabetes. Myocardial infarction has been shown to

produce a decrease in heart rate variability and reduction in variability in patients with heart failure is predictive of an increased risk of sudden death[129]. Decreased heart rate variability occurs in diabetic autonomic neuropathy[130] and is associated with higher mortality[131]. These studies are in keeping with the view that increased physiological variability reflects a state of greater well-being.

There are no data regarding the influence of pain on heart rate variability in adults and in the studies looking at painful conditions such as angina pectoris, the specific role of pain has not been addressed. Likewise, in children, there has been little work on this subject although a recent study has examined heart rate variability in children undergoing propofol anaesthesia for painful procedures. This study used spectral analysis and the authors concluded that it is possible to obtain useful information about the sympathetic response to pain from the low frequency power spectral data[132]. For the most part, however, the investigation of pain and its effect on heart rate variability has been confined to the neonate. In early studies the distress associated with heel prick was shown to produce an increase in heart rate variability, using the standard deviation of the mean heart rate as a measure but did not produce an increase in heart rate[113]. Variability of other physiological parameters also increased with the painful stimulus in this study as compared to a sham procedure. Further work again used this method to highlight this response and to demonstrate that the increase in variability could be attenuated by the use of an automatic spring-loaded device rather than a conventional lancet for heel prick[114]. Johnston et al found different results using similar methodology. They saw both increased heart

rate with heel prick and a differential response between sham and real procedures and suggested that the response may be more related to the gestational age of the infant than the nature of the stimulus[133]. Other studies have utilised frequency domain analysis of heart rate variability to assess pain responses in preterm infants. Using this method of assessment, increased heart rate and decreased variability were seen with heel prick[16]. These changes were thought to reflect activation of the sympathetic nervous system during the painful experience. When the procedure of heel prick was analysed more closely, the differential changes in heart rate variability indicated that the squeezing of the heel was the most stressful component of the procedure[134]. Heart rate variability has also been used to evaluate the effectiveness of treatment strategies for pain management. The response to venepuncture has been studied using spectral analysis[17]. This study revealed a difference between groups subjected to the procedure with and without the use of topical anaesthetic cream. The placebo group showed a greater response in terms of increased heart rate and decreased variability than the treatment group. Heart rate variability may be affected by factors other than pain such as gestational age, behavioural state and previous experience of pain, but has nevertheless been generally regarded as a useful measure of the response of both term and preterm infants to noxious stimuli. Behavioural indicators of pain probably remain the most sensitive, but do not always correlate well with intensity of physiological responses[135]. It is possible that combination of behavioural assessments with detailed measurement of variability of physiological parameters may add to the understanding of the complex relationships between different types of pain response.

The most appropriate method for assessment of heart rate variability will depend on the situation in which it is to be used and the resources available. Although spectral analysis is a more sophisticated method of measuring heart rate variability than analysis of the standard deviation of the mean heart rate and is valuable as a research tool, it is not, at present, suitable for routine use in the clinical setting. It is however, relatively simple to incorporate displays of variability based on mean and standard deviation of physiological parameters into computerised monitoring systems such as the Badger System, used on the Neonatal Unit of the Royal Infirmary of Edinburgh. If heart rate variability can provide a specific measure of pain, then this method is much more likely to be effective as a clinical tool to provide a real time evaluation of pain, distress and adequacy of analgesia. For these reasons, in the following work described in this chapter and in the study relating to acute pain described in Chapter 7 the chosen measure of heart rate variability is the standard deviation about the mean heart rate.

5.2 METHODS

5.2.1 Monitoring systems

5.2.1.1 The Badger System

Continuous monitoring using the Badger System is routine in all babies admitted to the Neonatal Intensive Care Unit in Edinburgh. The Badger System is a real time physiological intensive care monitoring system used at the cot side. Heart rate, blood pressure, oxygen saturation and other physiological parameters are sampled and recorded from the neonatal monitors at a rate of once per second [www.clevermed.com]. Monitoring of the babies is commenced as soon as possible after admission to the neonatal unit and is continued until the babies no longer require intensive care. All clinical data collected using this system is available for viewing at the cot side in the form of a trend graph as an aid to clinical management. The data is also stored and can be accessed retrospectively for research purposes. Data can be extracted and analysed using an additional computer program designed for this purpose [Badger viewer, Alan Maxwell]. All infants included in this study were monitored using the Badger System.

5.2.1.2 The Braemar DL700 Holter Monitor

A subset of infants was also monitored intermittently, using the Braemar DL700 Holter monitor [Braemar, Burnsville, MN, USA]. This is a device designed for continuous ambulatory ECG monitoring using 3 channels. The sampling rate of the monitor is 128 per second. The data is recorded onto a flash memory card and can then be downloaded onto a computer. Initially, the waveform data is stored in binary format. This is then converted into text format using a computer program specifically written for this function [Heartrate, Mansour Ahmadian]. A voltage threshold detector is used to identify the peak voltages that represent the R waves of the ECG and from this the beat-to-beat heart rate can be calculated.

5.2.2 Protocol for Monitoring of Infants

5.2.2.1 Badger System

36 of the 44 babies recruited to the NEOPAIN Trial were monitored as part of their routine clinical management using the Badger System. Monitoring gel electrodes (Blue Sensor electrodes, Medicotest) were placed on the baby's chest as soon after arrival on the Neonatal Unit as was considered appropriate by nursing staff caring for the baby. The time of commencement of recording depends upon clinical staff entering basic demographic patient data into the system and activating the recording

system. This is usually done within the first few hours of a baby's admission to the Neonatal Unit. Recording of data continued until a clinical decision was made to discontinue.

5.2.2.2 Braemar DL700 monitor

Additional monitoring using the Braemar DL700 Holter monitor was performed in a subset of 29 babies. This involved connection of five additional gel electrodes to the chest or back of the baby. Monitoring was carried out at the following times:

I. Prior to administration of the study drug
II. Daily for the duration of the maintenance dose of the study drug
III. Every 4 hours during the period of weaning of the study drug for up to 48 hours
IV. Every 4 hours after discontinuation of the study drug

Monitoring of the infants was performed on each occasion for a minimum of 15 minutes. Where possible, this followed a period of at least 15 minutes undisturbed rest and the infant remained undisturbed for the duration of the recording. In the less stable babies, this was not always possible as very frequent nursing or medical interventions were sometimes necessary. However, no period of recording was included in the analysis if a painful or significantly distressing intervention was required and performed during the time of recording. Where essential nursing or

medical procedures were needed at the times specified for monitoring, the monitoring was delayed until the procedure was completed and there had been time for physiological parameters to return to normal. All monitoring was carried out by one of three researchers (two research fellows and one research nurse).

The monitoring electrodes were positioned on the infant's chest or back, depending on whether the baby was being nursed supine or prone. The position of the baby was noted but was not altered for the purpose of the study since heart rate, rather than ECG waveform data was required. Where possible, monitoring electrodes were connected 5-10 minutes before monitoring was started, to avoid any changes in heart rate induced by this process.

5.2.3 Extraction of Heart Rate Data

Data from the Holter monitor were collected prospectively, but the stored data from the Badger system were viewed retrospectively. Wherever possible, data recorded on Channel 1 was used. In some cases data on this channel was poor due usually to movement artefact. In these circumstances, data from either Channel 2 or 3 was used, after first examining to ensure that heart rate and heart rate variability from all three channels were similar. For every period of monitoring using the DL700 Holter monitor, the start and finish times were noted using the time displayed on the Badger

system cot side computer corresponding to that baby. This was necessary to allow later comparison of the data collected by the two different methods to be as accurate as possible.

5.2.3.1 Calculation of mean heart rate and heart rate variability using Holter monitor data

ECG data from each 15-minute period of monitoring was converted into text format using the computer program referred to above. This returned a large amount of numerical data (128 data points per second), corresponding to the recorded ECG voltages for each sample in the format of a Microsoft Excel spreadsheet. Since it was impractical to work with such a large amount of data, a 10 second period of data was chosen and isolated. A random number generator [www.random.org] was used to determine the start point between 300 and 600 seconds. By using the middle 5 minutes of the recording time, it was hoped that the data would represent a period at which the infant was most likely to be at rest and there would be less likelihood of interference due to vigorous movements of the baby. Each peak in voltage representing the R wave of the ECG was identified and marked. A 20 beat period was selected from this 10-second time span. The mean heart rate was calculated across the 20 beats and the standard deviation about the mean heart rate was calculated as a measure of heart rate variability over that time. A set number of beats

were chosen rather than a given time period, because of the potential variation of heart rate variability with changes in heart rate.

5.2.3.2 Calculation of mean heart rate and heart rate variability using Badger System data

Types of patient monitors in use in the Neonatal Unit include Siemens and Hewlett Packard monitors. All babies needing intensive care are monitored from the time of admission to the Neonatal Unit using one of these. The type of monitor for any individual baby depends only on the equipment available at the time of admission.

The Badger System extracts data from the patient monitor at a rate of once per second. This one-second data produces a trend graph, which is constantly updated. A computer program allows isolation of physiological data over any specified time period during the baby's stay and calculation of the mean heart rate and heart rate variability over that time. In this study, where babies had been monitored at regular intervals for 15-minute periods using the Holter monitor, the same time period was recalled on the Badger System (to an accuracy of within 1 minute). The mean heart rate and standard deviation about the mean were calculated for the whole of that 15-minute time span.

5.2.4 Missing Data and Limitations of Data Collection Methods

5.2.4.1 Researcher availability

Data collection using the DL700 Holter monitor was limited to three researchers only, in order to minimise the likelihood of inaccuracies. It was impossible for a researcher to be present at all times when monitoring was required. This was due mainly to the unpredictable nature of work involving newborn infants, unanticipated clinical decisions affecting study drug administration or discontinuation and the high intensity of work involved in obtaining 4 hourly data over a period of several days. On most occasions, when monitoring took place between the hours of 06.00hrs and 02.00hrs, the required 15 minutes of data only was recorded. However, during the night, and at other times when a research fellow was unable to be present, Holter monitor recording was continuous for a number of hours to include the required 15-minute time span. Start and finish times for continuous overnight monitoring were sometimes less meticulously noted than were those for 15-minute periods. It is therefore less easy to be certain, when extracting data collected over several 4-hour periods, that times of data chosen from the two different methods will coincide exactly and this may be a source of error when comparisons are made. Sometimes, during prolonged periods of recording, Holter monitor battery failure meant that data was not always complete. Another explanation for missing data in these circumstances was removal of and failure to replace monitoring leads by medical or nursing staff for clinical or other reasons.

5.2.4.2 *Clinical reasons*

Although monitoring of babies begins on admission to the Neonatal Unit, data recording by the Badger System depends on activation of the cot side computer, following entry of the baby into the data management system. In very sick babies, with many other clinical pressures, this may be delayed until clinical stability has been achieved. In the extremely preterm who have particularly thin and fragile skin, it is occasionally felt inappropriate to attach ECG leads. This means that in 6 infants, initial data from the first few hours of life is unavailable, including baseline data before administration of the study drug and during the loading dose.

During the course of intensive care, there will be other unavoidable gaps in monitoring. ECG electrodes must always be removed for chest x-rays to be carried out, shorter periods of data are lost when electrodes are routinely replaced and in some instances the condition of a baby's skin may make invasive arterial monitoring preferable.

As the condition of an infant improves, less monitoring is clinically required and so Badger System monitoring is discontinued. For this reason, more data are available from intermittent Holter monitoring towards the end of the study period for all but the sickest babies who require a very prolonged period of intensive care. Data collection is also discontinued early in babies transferred to other hospitals for their ongoing care. Similarly, in babies for whom survival is unlikely, data is limited due

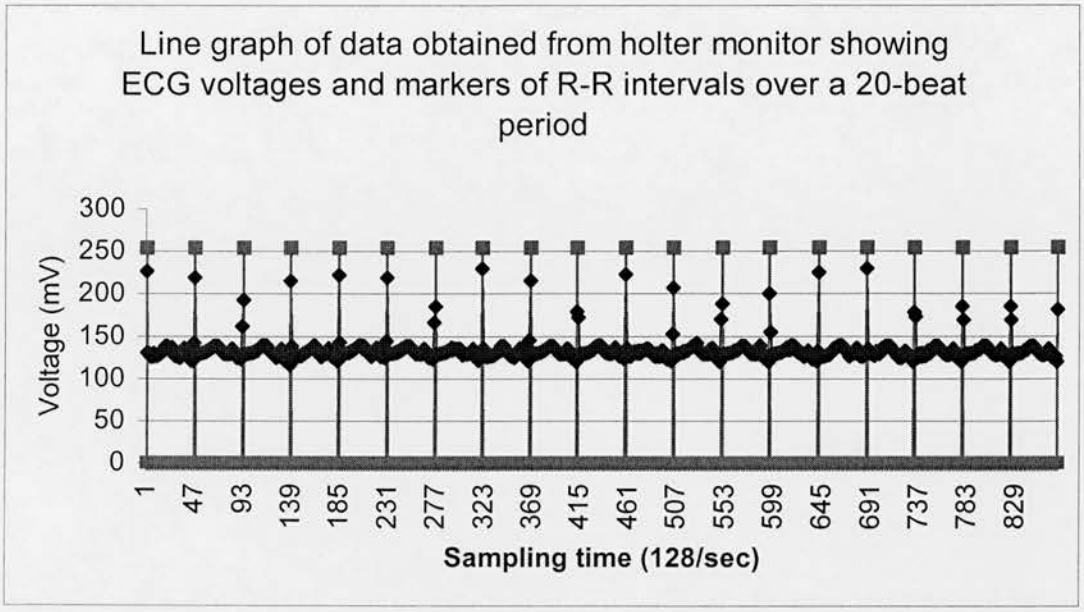
to elective discontinuation of routine clinical monitoring during compassionate care and the inappropriateness of continuing research in such babies.

5.2.4.3 Artefact

Corruption of data by artefact is seen when there is failure of, or interference with, detection or transmission of physiological data signals, adversely affecting the quality of the recording obtained. A common cause of artefact in ECG recording is poor contact between the electrode and skin, seen in babies who are very active. Accidental movement of the electrodes during routine care is also common, and accounts for brief periods of data that are difficult or impossible to interpret meaningfully. The problem of data artefact applies to both methods of monitoring. It is usually possible to identify artefact by carefully examining the ECG trace. Fallacious physiological changes due to artefact are usually abrupt, immediate and of large magnitude. The values may return to normal equally rapidly. This is in contrast to true physiological change in heart rate that tends to be more gradual both in onset and recovery. However, genuine profound changes may occur that bear some resemblance to artefact such as bradycardia accompanying endotracheal suction in unstable infants.

No systematic method for artefact detection or removal was available for data obtained in this study. However, since the study aimed to examine mean heart rate and heart rate variability in a small block of data representative of a longer time span, it was necessary to avoid periods of recording where artefact was obvious or suspected. When viewing the randomly chosen data from the Holter monitor recording, a line graph of the data including the R wave markers was plotted using Microsoft Excel and examined to ensure that the 20-beat trace appeared regular with no artefact included (Figure 5.1). If this was unsatisfactory, a further randomly selected 20-beat period was examined and utilised. All Badger data was examined for recognisable patterns indicating artefact or sudden change in physiological parameters. If either was seen, an alternative 15-minute period within 45 minutes of the chosen time was used. Extremely brief interruptions in monitoring, where there was clearly no deviation of the trace from baseline, were ignored. With both methods of monitoring, if no satisfactory portion of ECG trace could be identified around the ideal time required for the study, data for that monitoring period was omitted from the analysis.

Figure 5.1



5.3 RESULTS

5.3.1 Comparison of methods of ECG monitoring

36 infants were monitored using the Badger System. 29 infants had some monitoring using the Braemar DL700 holter monitor. Parallel data from both the Holter monitor and Badger system are available for 29 infants.

Figure 5.2

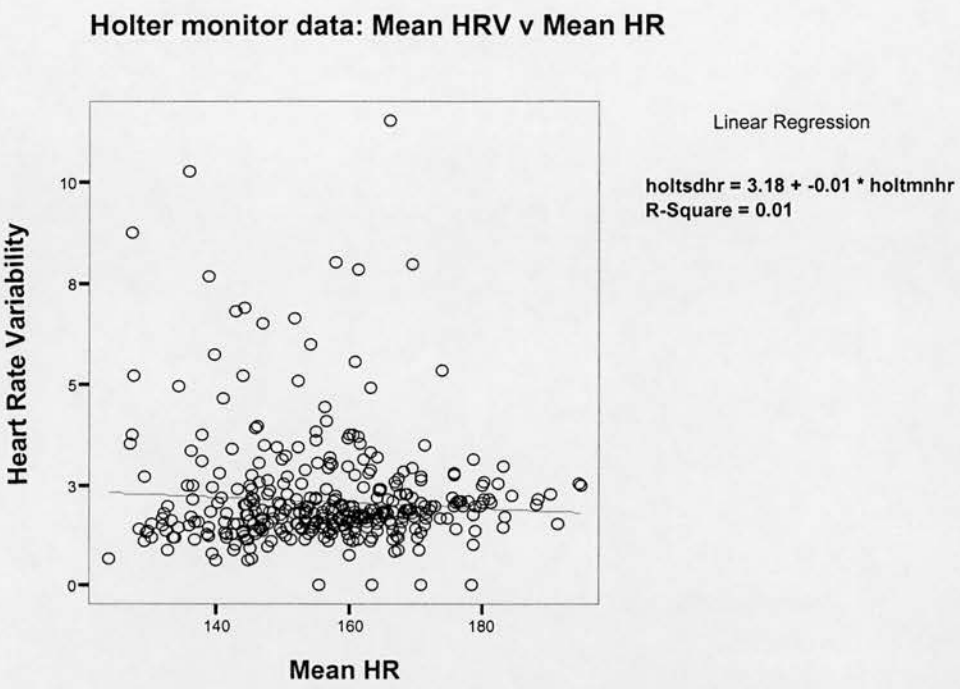
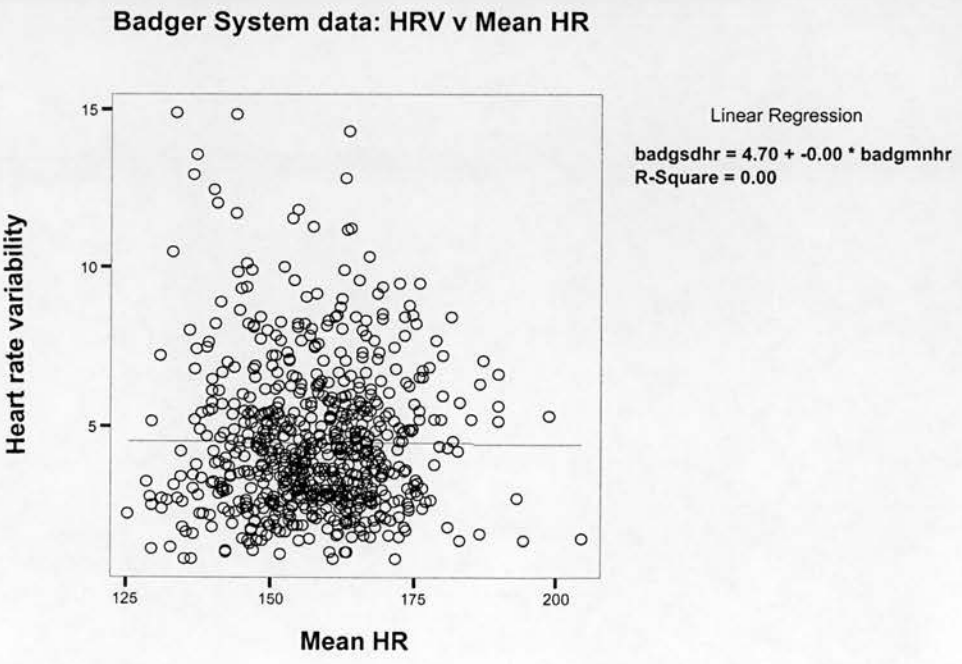


Figure 5.3



Plots of all heart rate data showing mean heart rate against heart rate variability for both groups show that in the case of Holter monitor recordings, increasing mean heart rate is accompanied by a small decrease in heart rate variability (Figure 5.2), in keeping with findings of other researchers.[14] This is not seen in the Badger System group (Figure 5.3) where there are a larger number of measurements available and calculations are made over a greater period of time. The spread of results is greater for both mean heart rate and heart rate variability when measured using the Badger System. The variability is generally higher in this group than the Holter monitor group. This is not an unexpected finding; it would be expected that measurements using the Holter monitor would be more precise, since variability in rate from one beat to another is measured over an extremely brief time period. In contrast, the Badger System uses averaged data, collected over a longer time. This small difference in absolute figures is unlikely to present a problem clinically if it is consistent.

In order further to compare the two different means of data collection and to estimate the level of agreement between them, Bland-Altman plots were used (Figures 5.4 and 5.5). In these plots, the differences between the two results for mean heart rate and the heart rate variability are plotted against the means of the measures. The parallel horizontal lines represent the 95% confidence limits for agreement. Figure 5.5 demonstrates that the difference between the two methods in the measurement of variability increases as the heart rate variability itself rises. However, for both mean heart rate and heart rate variability, most points (93% and 94% respectively) fall

within the 95% confidence limits, indicating that agreement between the two methods is acceptable.

Since there was a good level of agreement between the two methods of heart rate monitoring, further analyses of mean heart rate and heart rate variability data were carried out on data obtained from the Badger System.

Figure 5.4

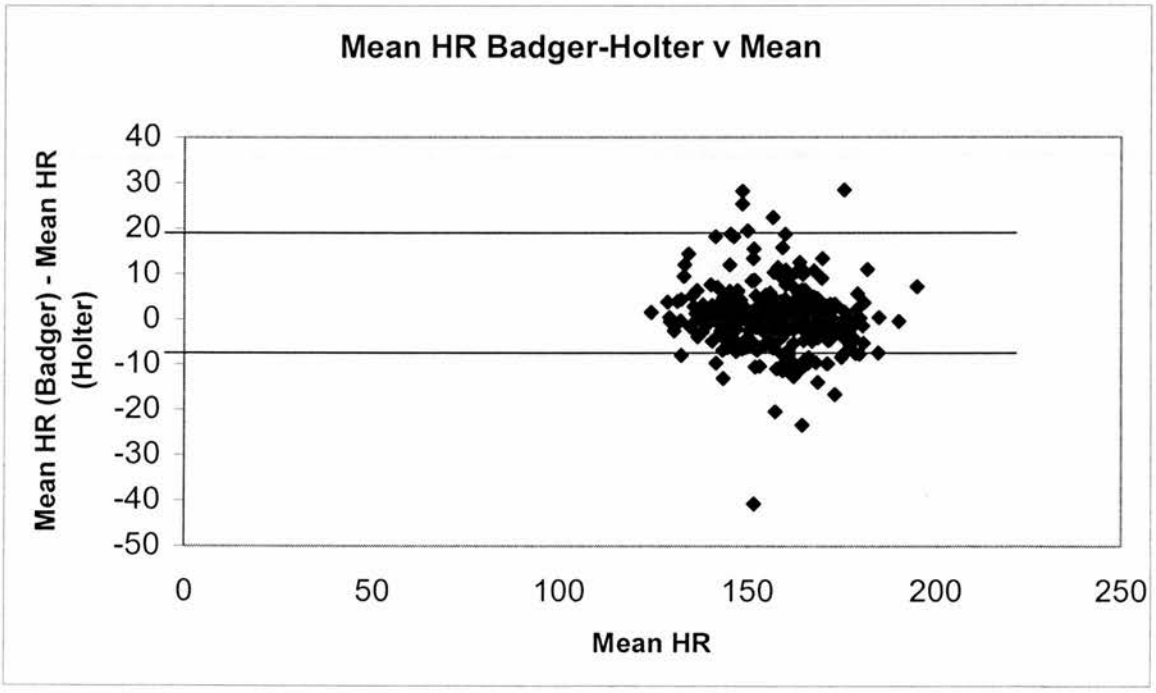
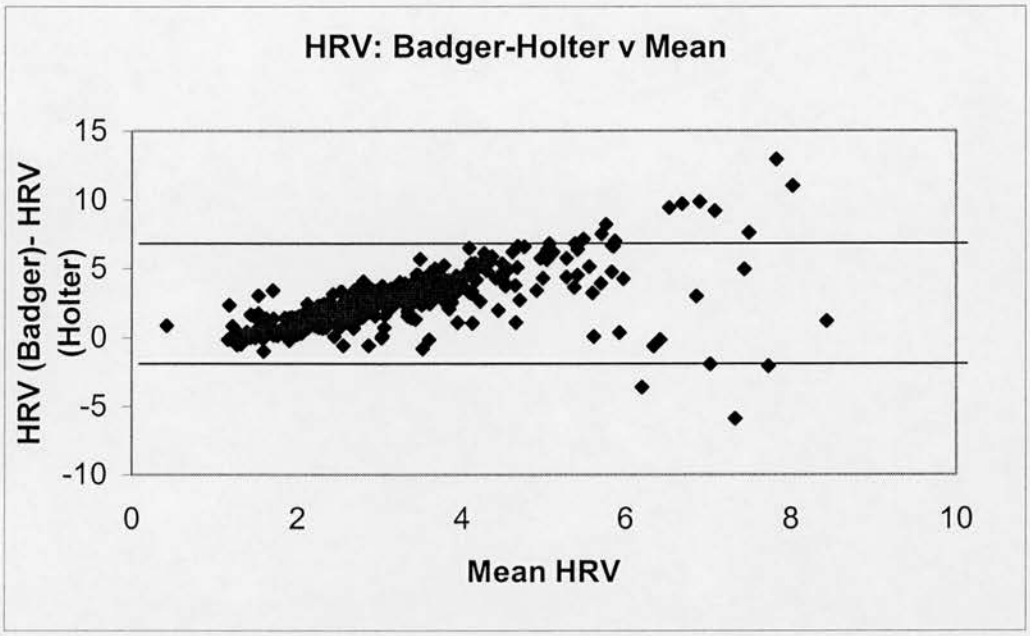


Figure 5.5



36 infants enrolled in the N.E.O.P.A.I.N. Study were monitored using the Badger System. Of these, 18 infants were randomly allocated to receive morphine infusion and 18 were allocated to receive placebo. Table 5.1 shows that there were no significant differences between the two groups in gender, gestational age, birth weight, severity of illness measured by the CRIB Score (Clinical Risk Index for Babies) [136] and number of days on study infusion. Table 5.2 show the numbers of infants remaining in the study and able to be studied at each point in time. Part A shows the numbers of babies receiving maintenance infusion from the time of starting the study drug until 14 days. Part B includes only those babies who received the study drug for more than 48 hours and therefore required a period of up to 48 hours' weaning. Part C shows the number of babies in each group who were studied

following discontinuation of the study infusion. This includes infants whose infusion had been weaned and those who had not required weaning.

To ascertain whether the commencement of morphine infusion led to a change in mean heart rate or heart rate variability, it was necessary to compare the changes from baseline in every baby. Baseline data for 6 babies were unavailable. In view of the already small numbers in the study, imputation of data would be difficult and could lead to erroneous results. These 6 infants were therefore excluded from the analysis of both mean heart rate and heart rate variability

Table 5.1: Characteristics of infants studied

	Morphine	Placebo	Significance*
No. of babies	18	18	
Male / female	10 / 8	8 / 10	
Birth weight (g)	1080 (590 – 1935)	840 (500 – 1500)	p = 0.217
Gestational age (weeks)	28 (24 – 31)	26.5 (23 – 31)	p = 0.223
No days on study drug	4 (2 – 9)	2 (1 – 14)	p = 0.747
CRIB Score	4 (0 – 13)	5 (1 – 18)	p = 0.309
Baseline mean HR (bpm)	155.32 (129.35–204.27)	153.66 (133.44–173.20)	p = 0.874
Baseline HRV (bpm)	2.47 (1.45 – 5.46)	2.24 (0.85 – 5.75)	p = 0.704

*Mann Whitney U; Values are expressed as median (range)

Table 5.2: Days of treatment with study drug

A

Sample	Morphine (no. infants)	Placebo (no. infants)
Baseline	18	18
Day 1	13	18
Day 2	16	16
Day 3	14	8
Day 4	11	8
Day 5	3	8
Day 6	2	7
Day 7	1	6
Day 8	1	6
Day 9	1	6
Day 10	0	6
Day 11	0	6
Day 12	0	5
Day 13	0	5
Day 14	0	3

B

Wean 4 hours	8	6
Wean 8 hours	8	6
Wean 12 hours	7	6
Wean 16 hours	6	6
Wean 20 hours	5	6
Wean 24 hours	5	6
Wean 28 hours	3	5
Wean 32 hours	3	5
Wean 36 hours	3	5
Wean 40 hours	3	5
Wean 44 hours	1	5
Wean 48 hours	1	5

C

Stop 4 hours	14	11
Stop 8 hours	14	11
Stop 12 hours	14	11
Stop 16 hours	13	10
Stop 20 hours	13	10
Stop 24 hours	14	12
Stop 28 hours	14	12
Stop 32 hours	14	12
Stop 36 hours	14	12
Stop 40 hours	14	12
Stop 44 hours	14	12
Stop 48 hours	13	12

Figure 5.6

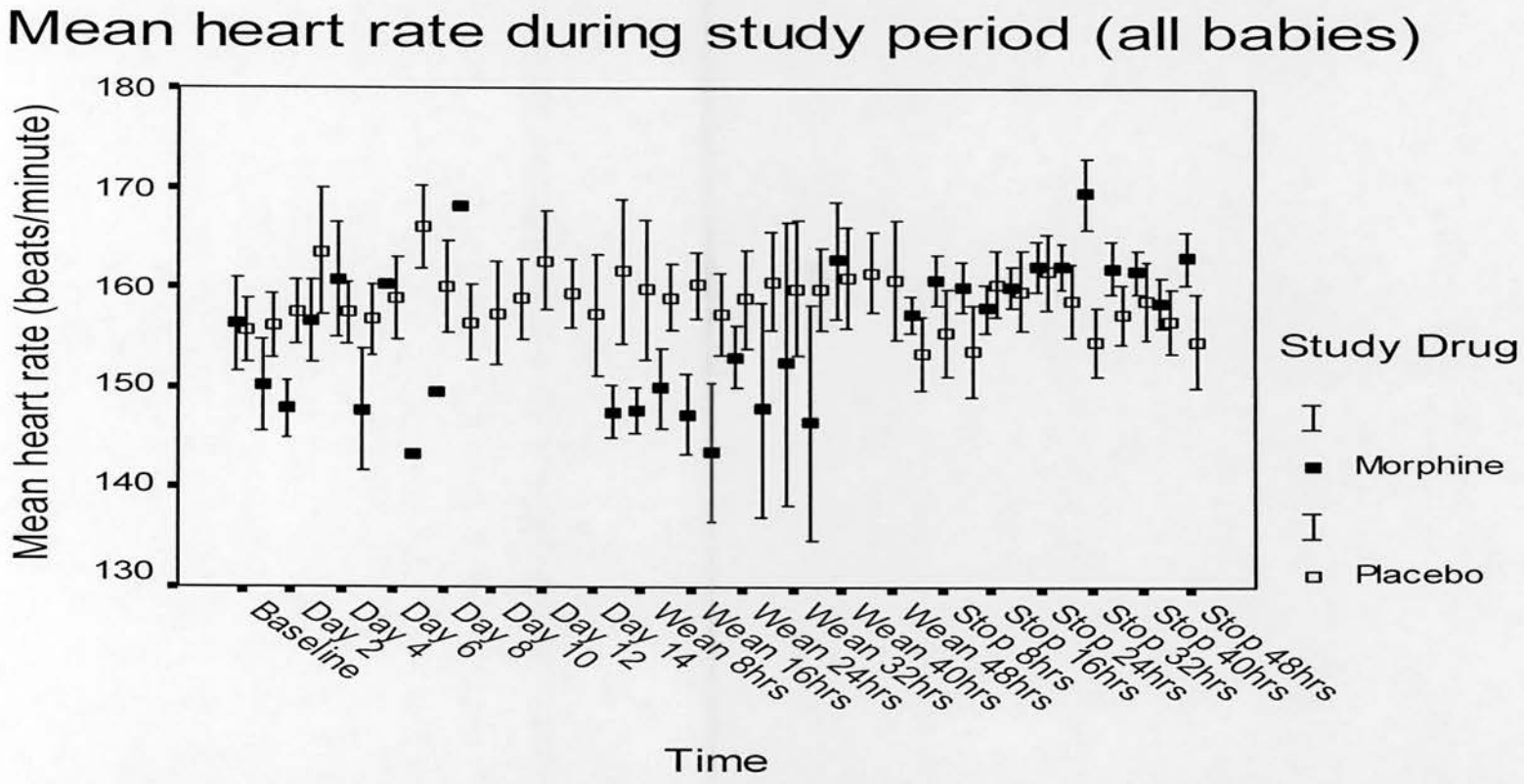
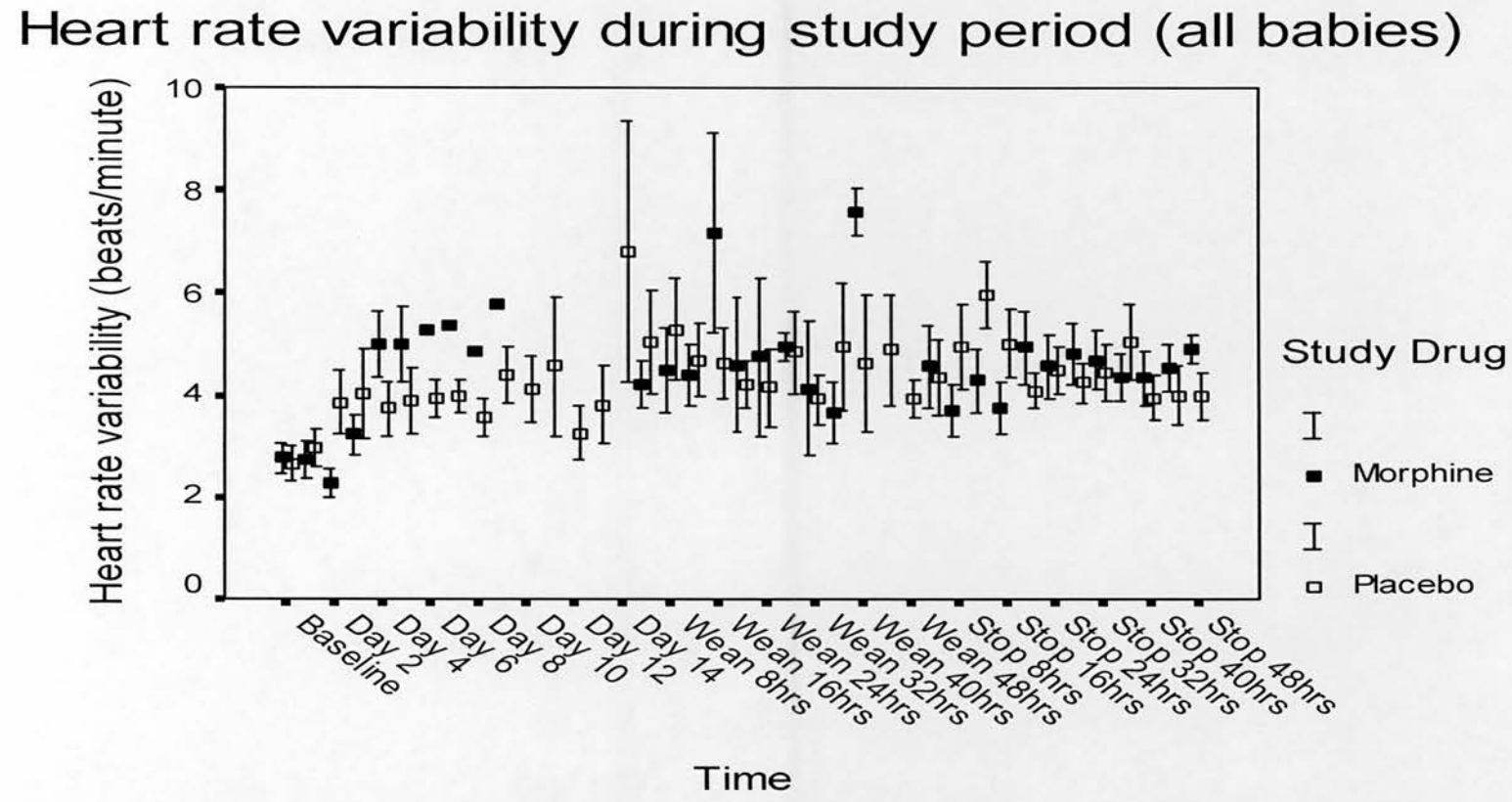

Mean heart rate during study period (all babies)
Mean heart rate (beats/minute)
180
170
160
150
140
130
Baseline
Day 2
Day 4
Day 6
Day 8
Day 10
Day 12
Day 14
Wean 8hrs
Wean 16hrs
Wean 24hrs
Wean 32hrs
Wean 40hrs
Wean 48hrs
Stop 8hrs
Stop 16hrs
Stop 24hrs
Stop 32hrs
Stop 40hrs
Stop 48hrs
Time
Study Drug
Morphine
Placebo
Error bars represent mean =/- SE

Figure 5.7


Heart rate variability during study period (all babies)
Heart rate variability (beats/minute)
10
8
6
4
2
0
Baseline
Day 2
Day 4
Day 6
Day 8
Day 10
Day 12
Day 14
Wean 8hrs
Wean 16hrs
Wean 24hrs
Wean 32hrs
Wean 40hrs
Wean 48hrs
Stop 8hrs
Stop 16hrs
Stop 24hrs
Stop 32hrs
Stop 40hrs
Stop 48hrs
Time
Study Drug
Morphine
Placebo
Error bars represent mean =/- SE

5.3.2 Comparison of mean heart rate and heart rate variability in babies receiving morphine and placebo

Figures 5.8 and 5.9 show the values for mean heart rate and heart rate variability over the first 4 days of administration of the study infusion. To avoid problems associated with multiple T testing [137] and unequal numbers of subjects within different groups, comparisons of change in mean heart rate and heart rate variability were made using analysis of covariance.

Figure 5.8

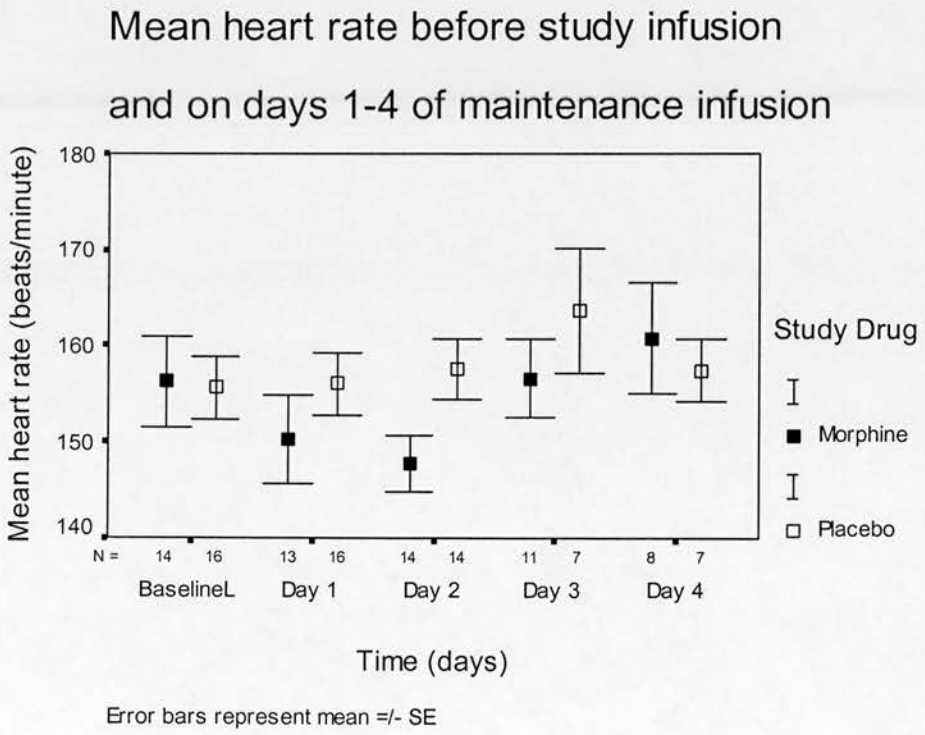


Figure 5.9

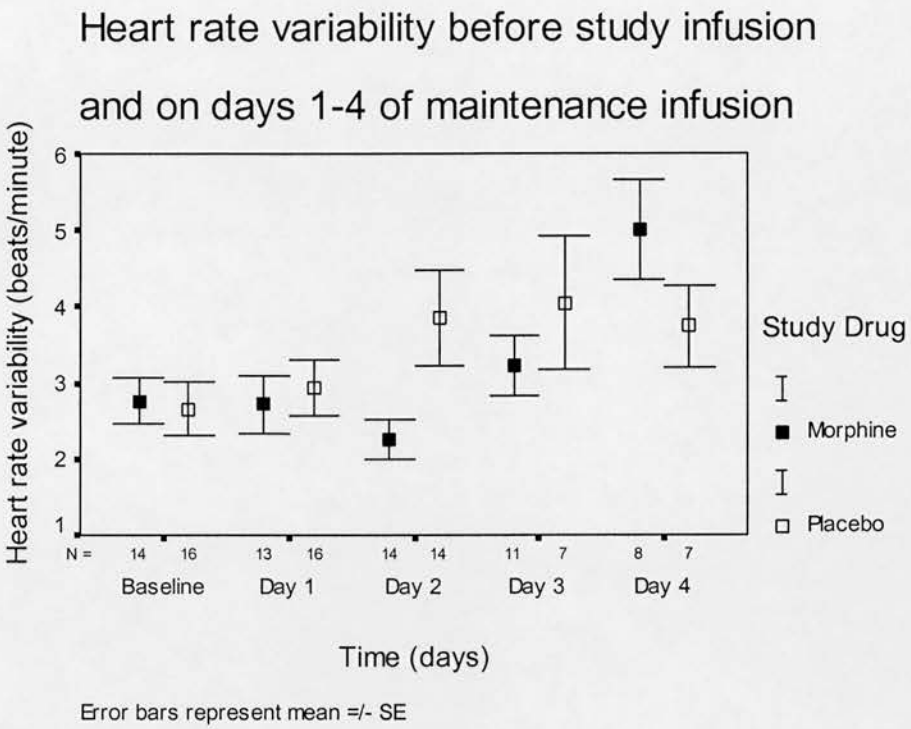


Figure 5.10 shows a plot of mean heart rate on days 1 and 2 of maintenance study infusion with baseline mean heart rate. Analysis of covariance indicates a statistically significant difference in the measurements between the morphine and placebo groups ($p = 0.024$) with the heart rate in babies treated with morphine falling with the onset of treatment more than in the placebo group. Figure 5.11 shows a similar plot for heart rate variability. Again, the level of variability during the first 48 hours of morphine therapy is reduced compared with that in the placebo group ($p = 0.065$). Although this result does not reach statistical significance, a trend is seen in this group of infants.

By days 3 and 4 of treatment with the maintenance infusion, this difference between the two study groups is no longer evident. Figures 5.12 and 5.13 show plots of day 3 and 4 results with baseline values of mean heart rate and heart rate variability respectively. Indeed, Figure 5.7 shows an increase in heart rate variability in the morphine group as compared to the placebo group after day 4. Using analysis of covariance to compare the two groups, there is no difference between baseline values and those obtained on days 3 and 4 for mean heart rate ($p = 0.521$) and heart rate variability ($p = 0.804$).

Figure 5.10

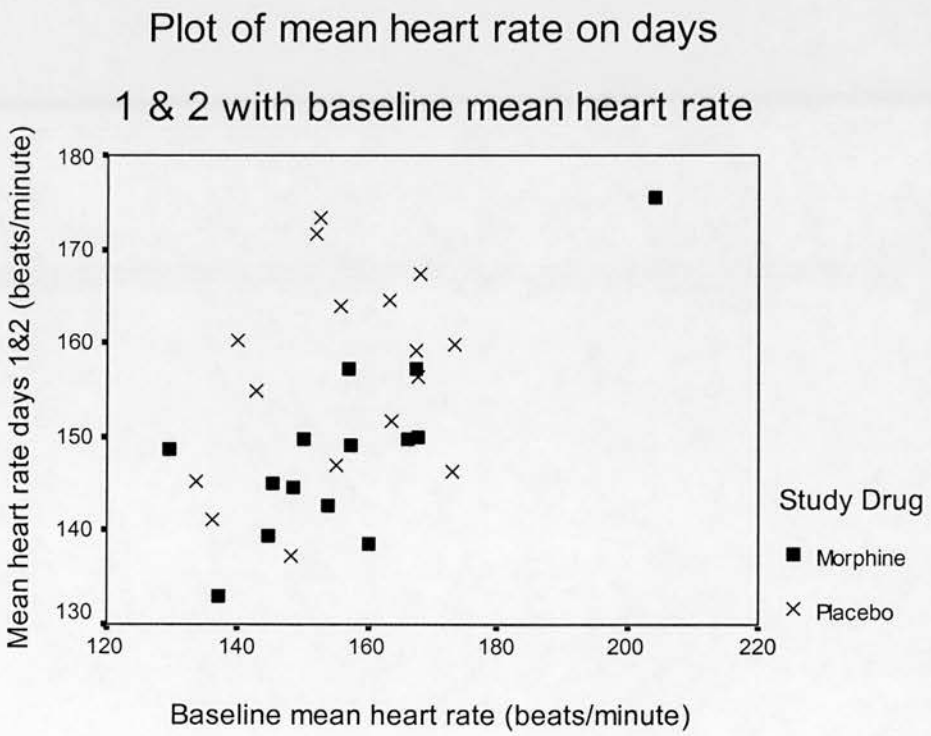


Figure 5.11

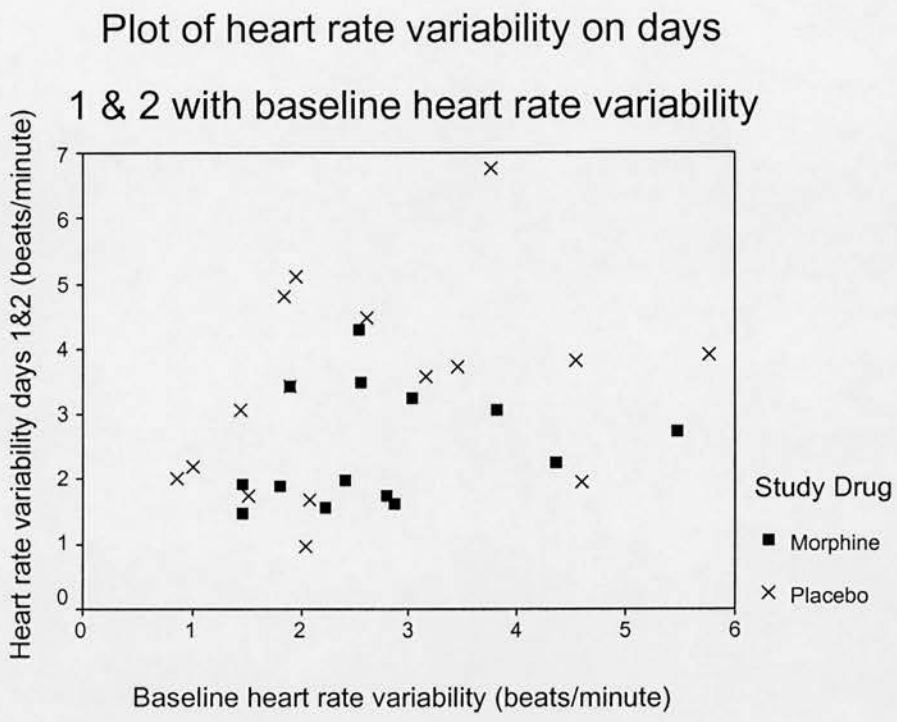


Figure 5.12

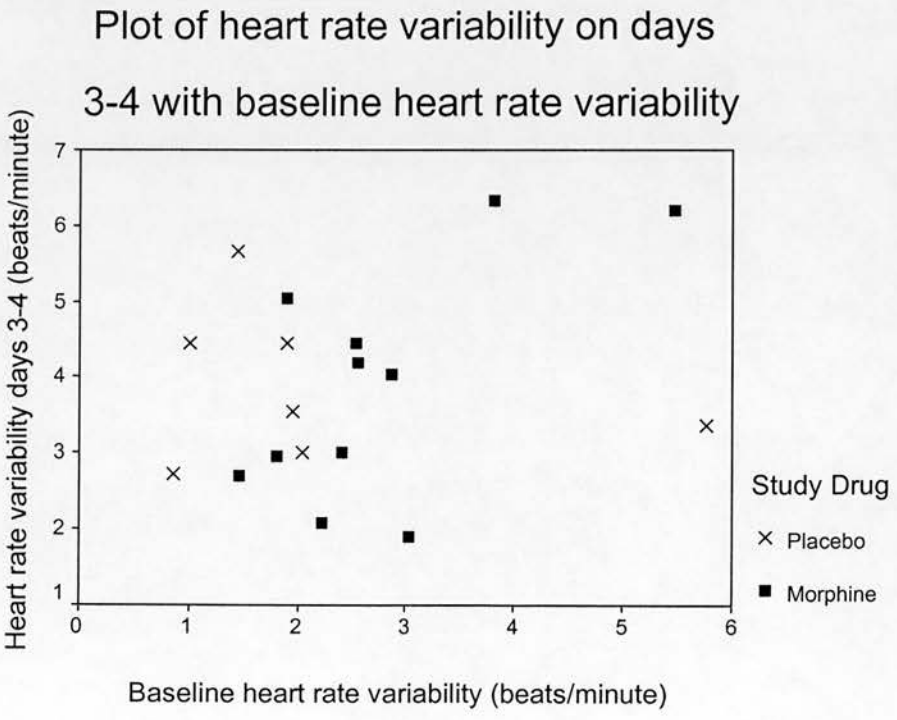
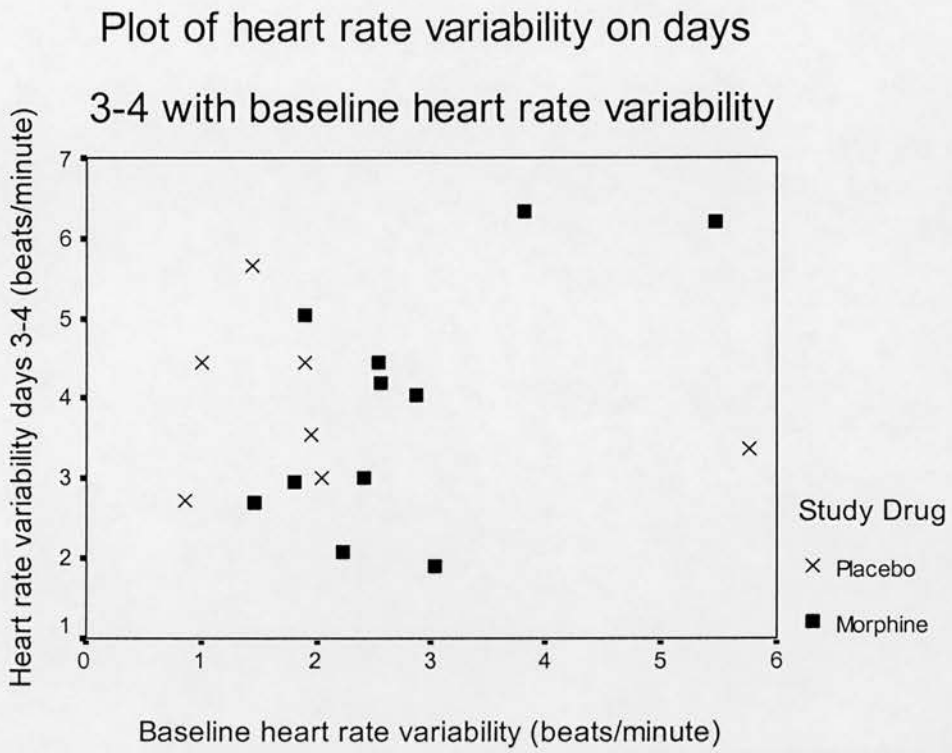


Figure 5.13



5.3.3 Comparison of mean heart rate and heart rate variability in babies after discontinuation of morphine or placebo

Figures 5.14 and 5.15 show available data for mean heart rate and heart rate variability in babies after discontinuation of morphine or placebo infusion. These results include values from the whole group of babies randomised into the study;

regardless of the length of time the infants received the study drug. The infusion will have been discontinued abruptly without a weaning period in those babies who received the infusion for 48 hours or less. In all babies who received the study drug for more than 48 hours, there will have been a weaning period of up to 48 hours.

Figure 5.14

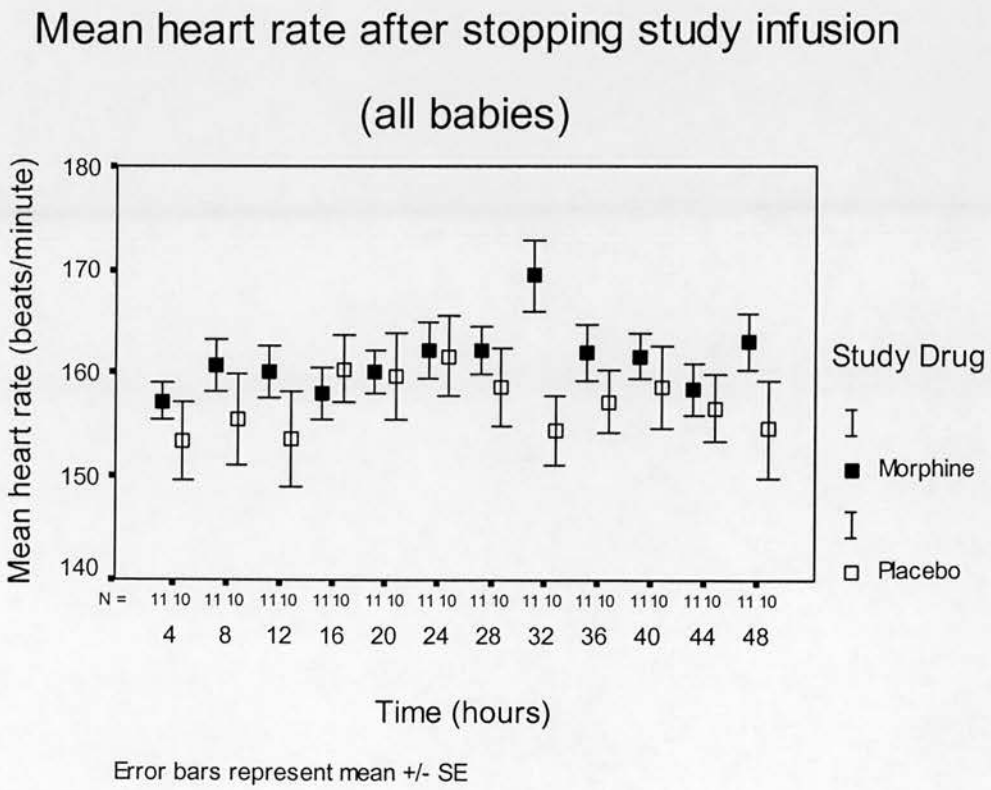
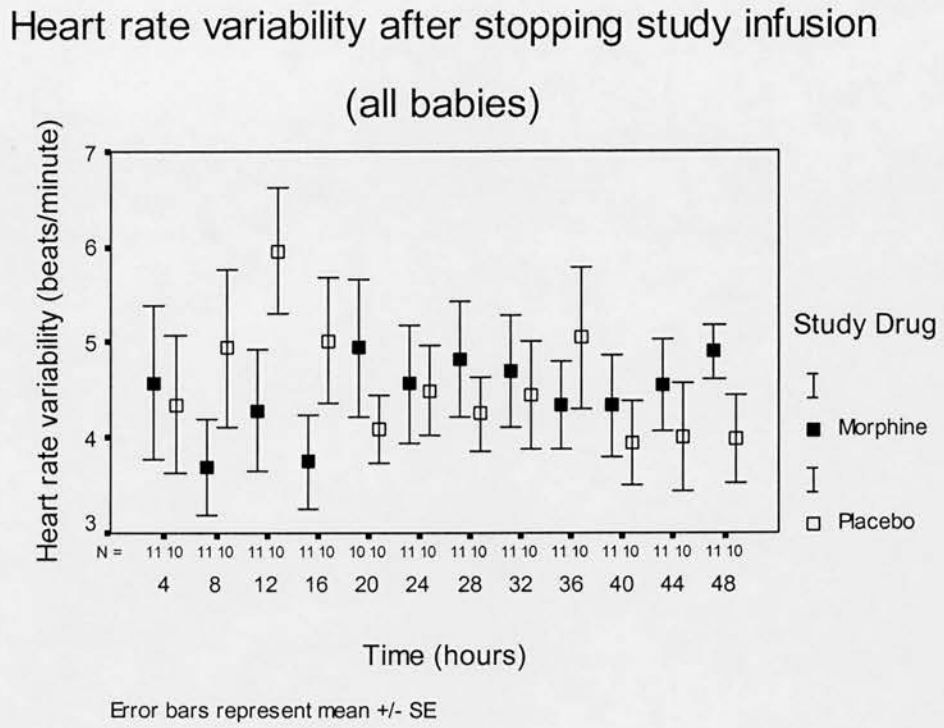


Figure 5.15



Analysis of variance would be the most appropriate method for analysis of the data showing the changes over time after stopping the study infusion. However, given the unequal numbers in the groups, the data are unsuitable. Likewise, multiple T testing is known to lead to erroneous results. The data were therefore analysed by calculating the slope of the line for each group and comparing these slopes using the independent samples T test[137].

There is no significant difference between the two groups of infants for either mean heart rate ($p = 0.616$) or heart rate variability ($p = 0.080$) during the first 48 hours

after stopping the study infusion. However it is interesting to note that the group of babies receiving morphine show relative tachycardia in comparison with the placebo group. This is a change from the time on maintenance infusion, where the mean heart rate in the morphine group was reduced compared with controls. Heart rate variability remains relatively reduced in the morphine group until around 20 hours of age. Thereafter, the values become similar again in the two groups toward the end of the 48-hour period.

5.3.4 The effect of weaning

Figures 5.16 and 5.17 show results after discontinuation in the infants in whom there was no weaning of the study infusion. There is a suggestion of higher mean heart rates within the morphine group in this plot continuing through the period after discontinuation of therapy. After discontinuation of the study drug, when the effect of weaning is included as an interaction in the analysis, there is no statistical difference between slopes for either the mean heart rates ($p = 0.544$) or the heart rate variability ($p = 0.615$) in the morphine and placebo groups. Weaning therefore does not appear to influence either variable.

Figures 5.18 and 5.19 show the mean heart rate and heart rate variability for the two groups during the period of weaning. The reduced mean heart rate seen in the

morphine group on maintenance infusion appears to persist into the weaning period although the difference in slopes does not reach statistical significance (p = 0.117). There is no significant difference in slopes for heart rate variability between the two groups (p = 0.123).

Figure 5.16

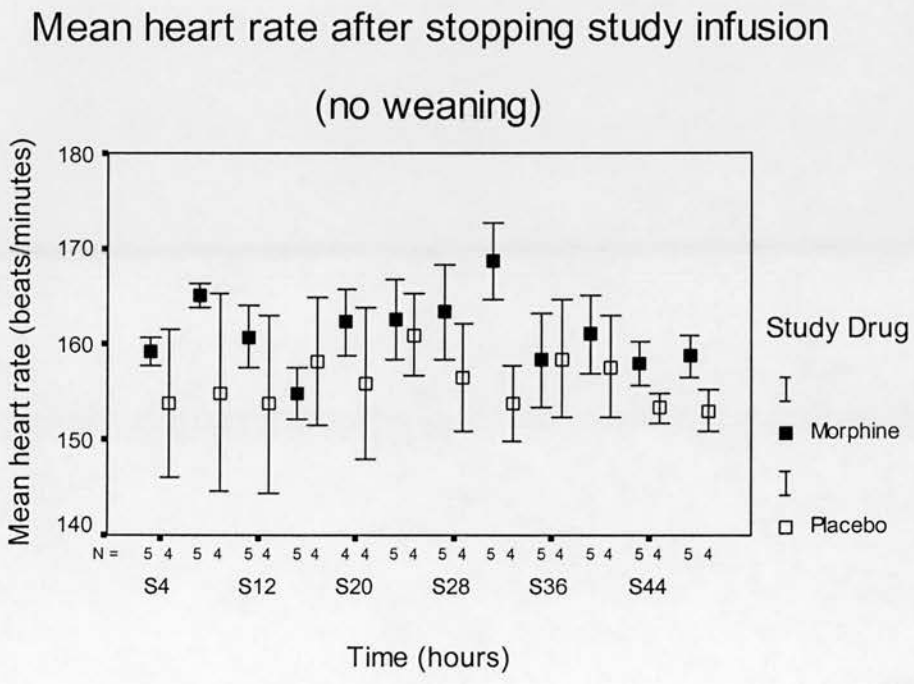


Figure 5.17

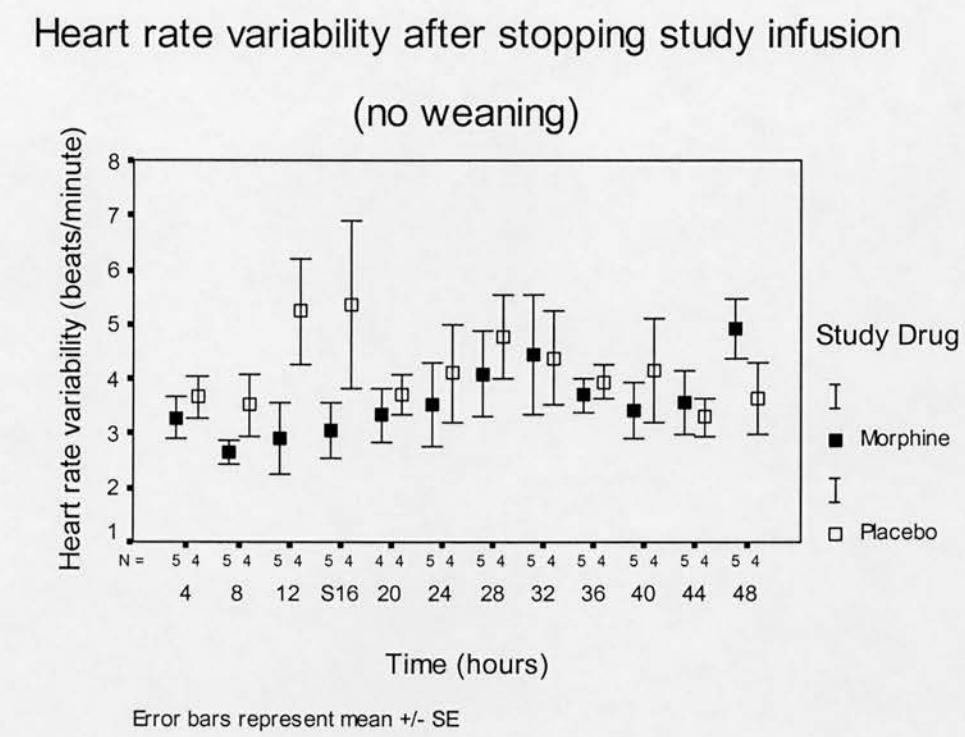


Figure 5.18

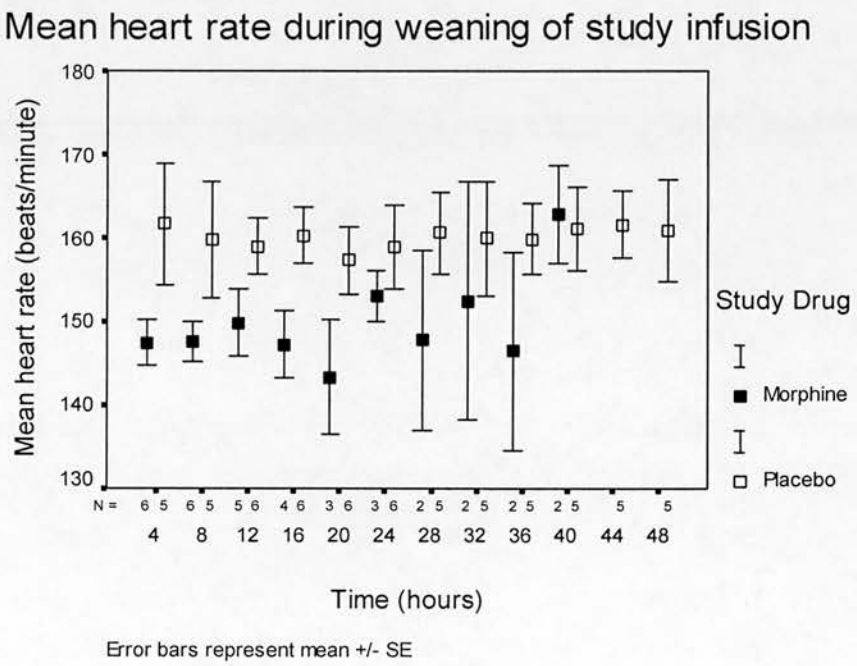
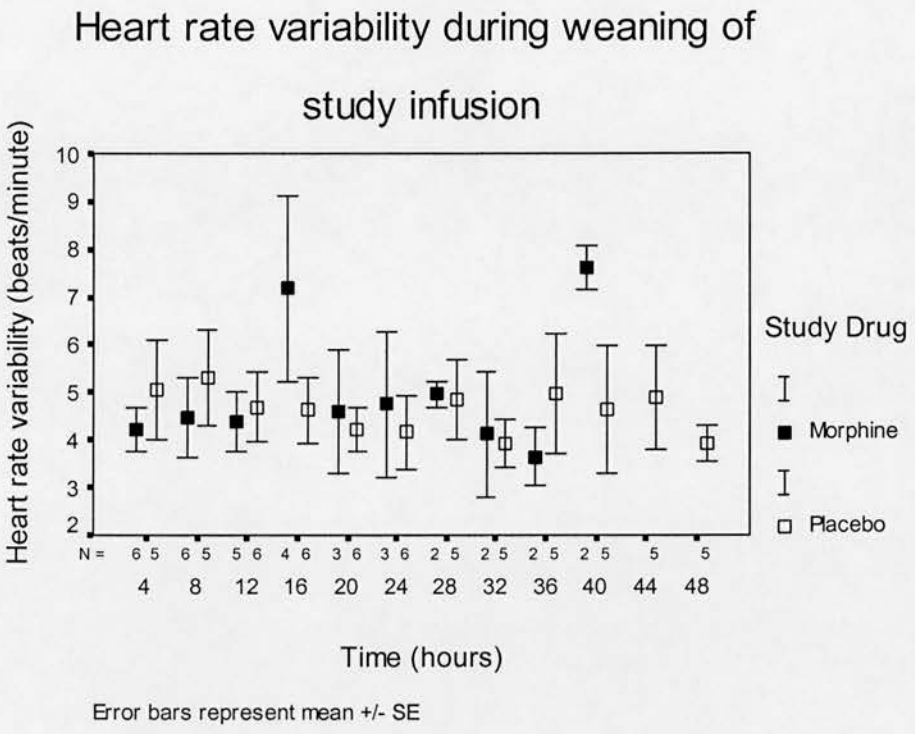


Figure 5.19.



5.3.5 The Effect of Gestational Age

For the purpose of data analysis, the infants are divided into the gestational age and morphine dosage groups that were chosen for stratification in the NEOPAIN Multicentre Trial. Therefore, in the following section, groups are analysed as follows:

1. Infants of 23-26 weeks' gestation who received 10mcg/kg/hour of morphine or equivalent volume of placebo (n = 16)

2. Infants of 27-29 weeks' gestation who received 20mcg/kg/hour of morphine or equivalent volume of placebo (n = 14)

3. Infants of 30-32 weeks' gestation who received 30 mcg/kg/hour of morphine or equivalent volume of placebo (n = 6)

5.3.5.1 Group 1

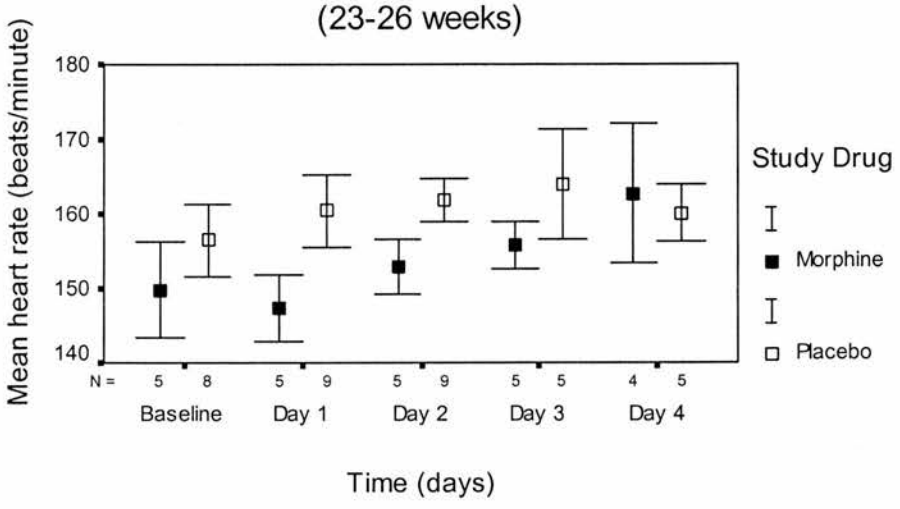
Figures 5.20 and 5.21 show the mean heart rate and heart rate variability in babies of 23-26 weeks' gestation during the first four days of the study. Figures 5.22 and 5.23 show plots of day 1 and 2 results with baseline results for both groups. Using analysis of covariance, the difference between the two groups on starting morphine trends towards statistical significance in this subgroup of infants ($p = 0.084$). There is no significant difference in heart rate variability with the onset of treatment ($p = 0.399$).

As with the whole study group any difference in mean heart rate from baseline disappears by day 3-4 (figures 5.24 and 5.25) of the study infusion in this subgroup ($p = 0.873$). No significant difference in heart rate variability is seen ($p = 0.814$).

Figure 5.20

Mean heart rate before study infusion and on days 1-4 of maintenance infusion

(23-26 weeks)

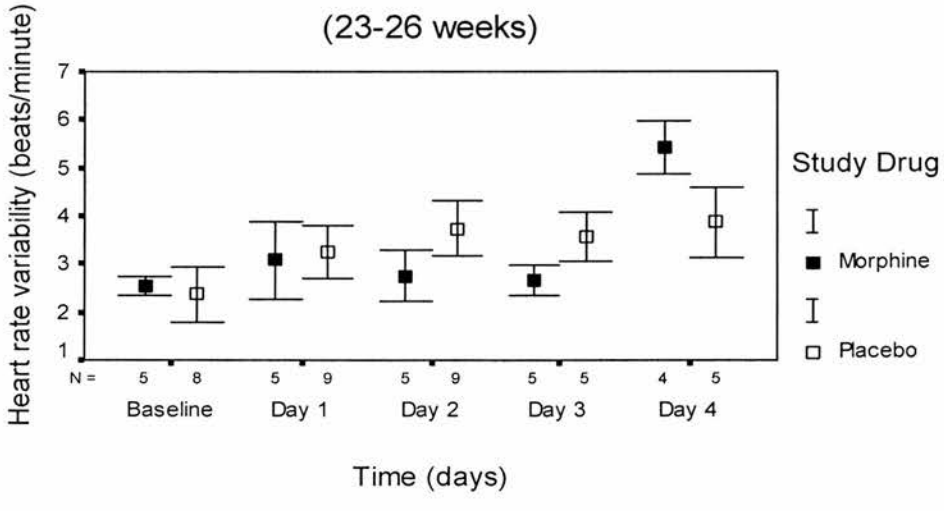


Error bars represent mean +/- SE

Figure 5.21

Heart rate variability before study infusion and on days 1-4 of maintenance infusion

(23-26 weeks)



Error bars represent mean +/- SE

Figure 5.22

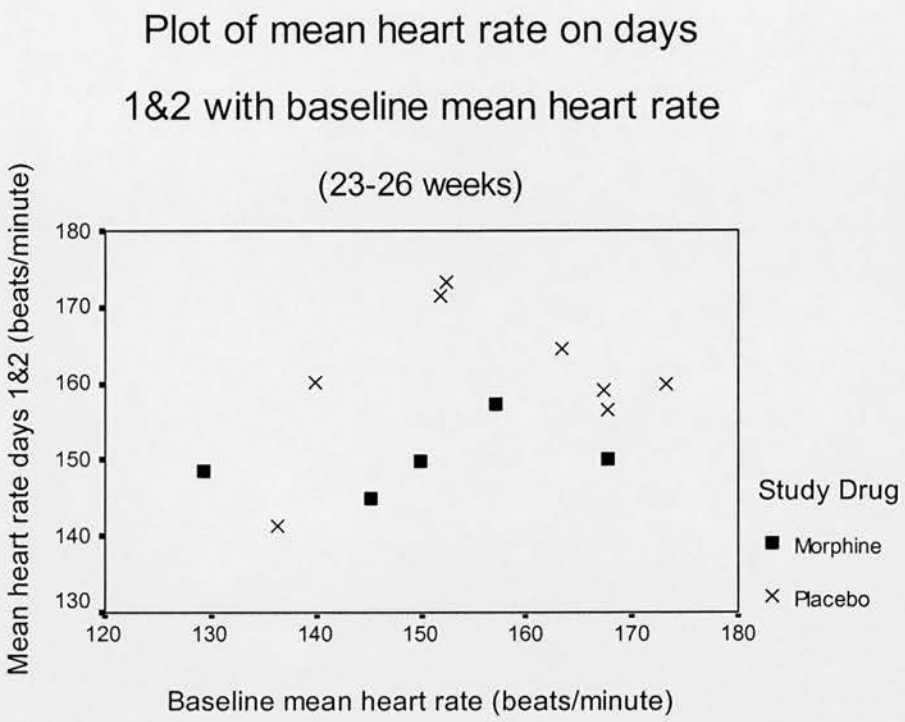


Figure 5.23

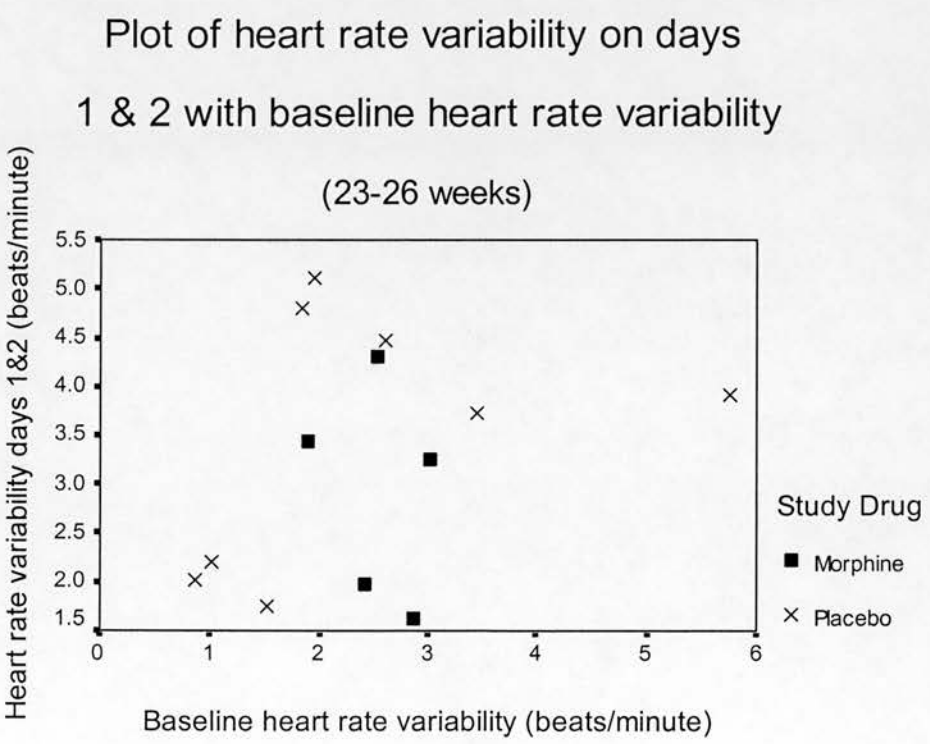


Figure 5.24

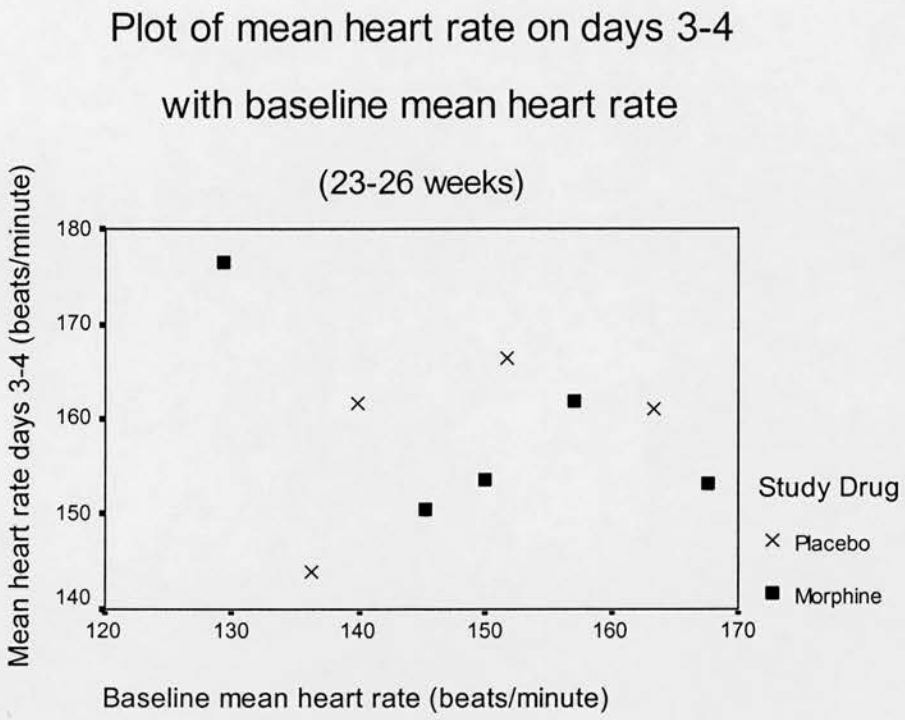
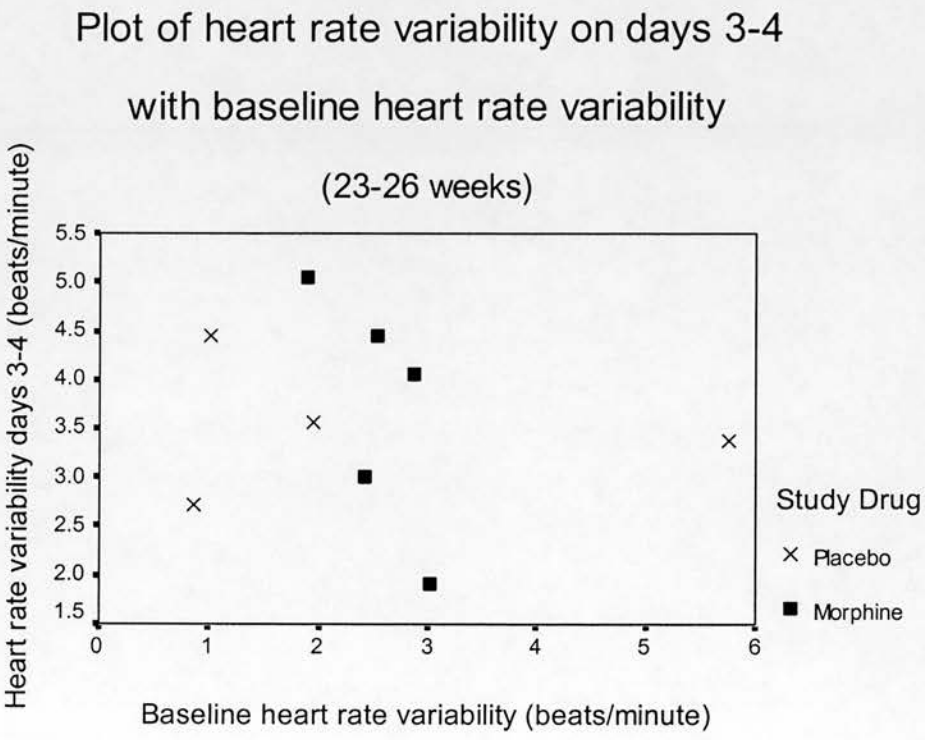


Figure 5.25



Mean heart rate and heart rate variability in all infants born at 23-26 weeks' gestation for the period after discontinuing the study drug are shown in Figures 5.26 and 5.27. Comparison of the slopes for both groups shows that there is no significant difference between them over the period of 48 hours after stopping the infusion for either mean heart rate ($p= 0.528$) or heart rate variability ($p = 0.828$). This analysis includes all babies whether or not the study drug was weaned before discontinuation. Weaning does not influence either mean heart rate ($p = 0.914$) or variability ($p = 0.835$)

Figures 5.28 and 5.29 show mean heart rate and heart rate variability data for those babies in the 23-26 week gestational group for whom the study infusion was weaned. There is no significant difference in slopes between the two groups for either mean heart rate ($p = 0.304$) or variability ($p = 0.508$)

Figure 5.26

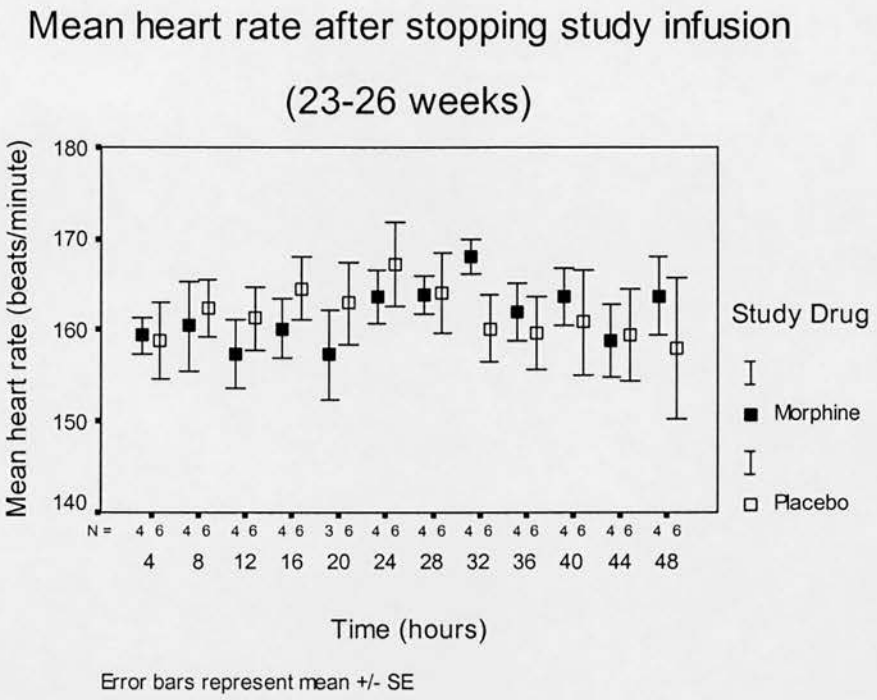


Figure 5.27

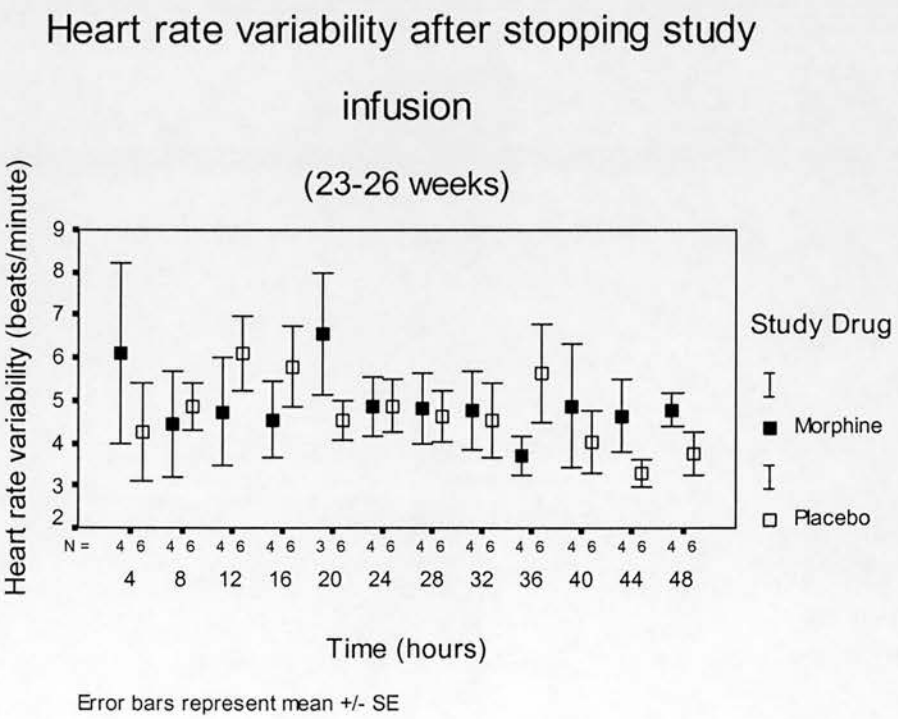


Figure 5.28

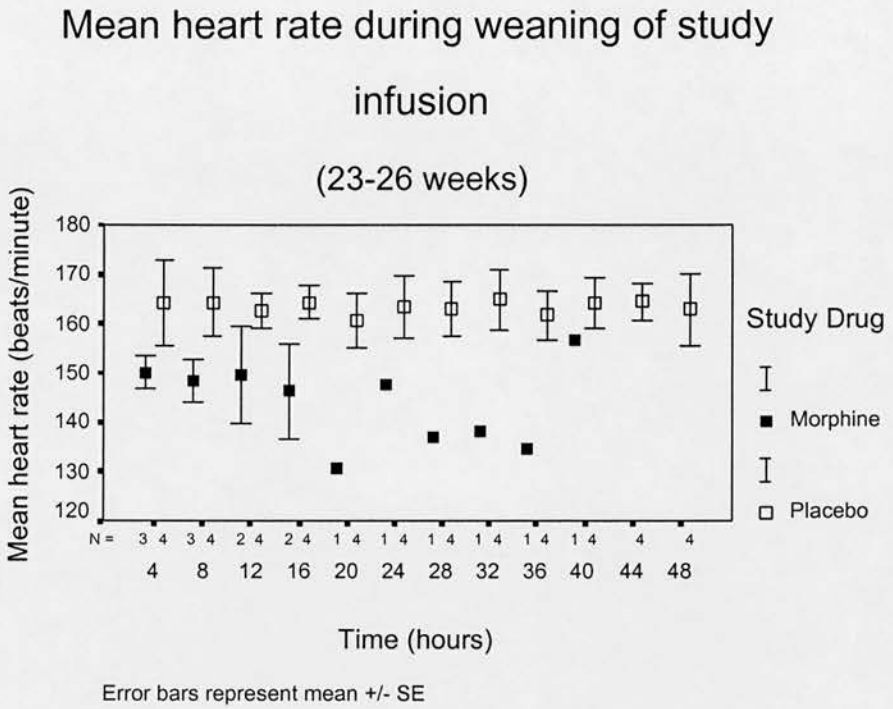
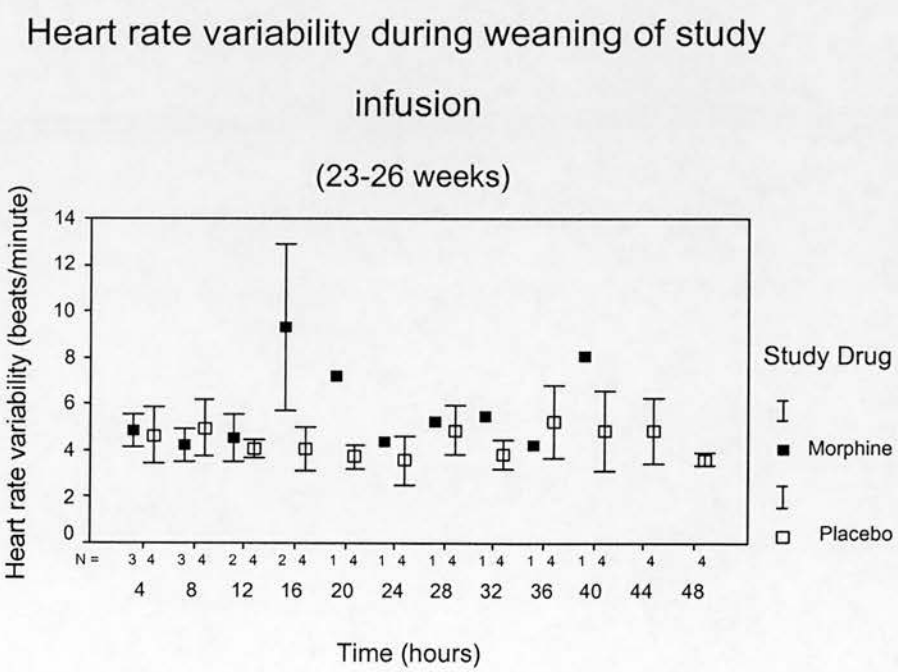


Figure 5.29



5.3.5.2 Group 2

Figures 5.30 and 5.31 show the mean heart rate and heart rate variability in babies of 27-29 weeks' gestation during the first four days that they received the study infusion. Figure 5.32 and 5.33 show plots of day 1 and 2 mean heart rate and heart rate variability with baseline values. Using analysis of covariance, the difference between the two groups on administration of the study infusion does not reach statistical significance for either mean heart rate ($p = 0.676$) or heart rate variability ($p = 0.357$)

Figure 5.30

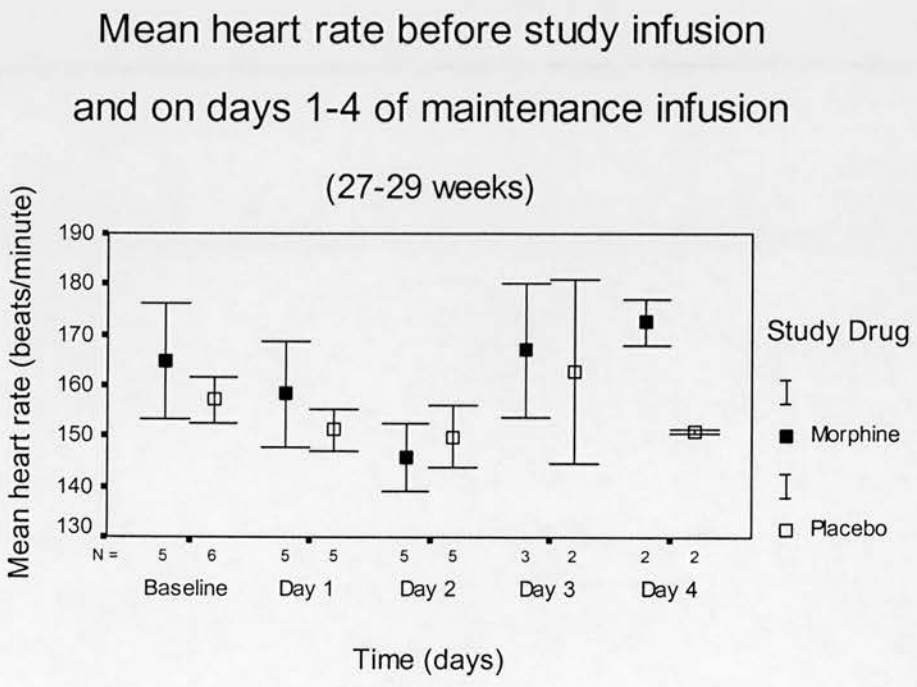
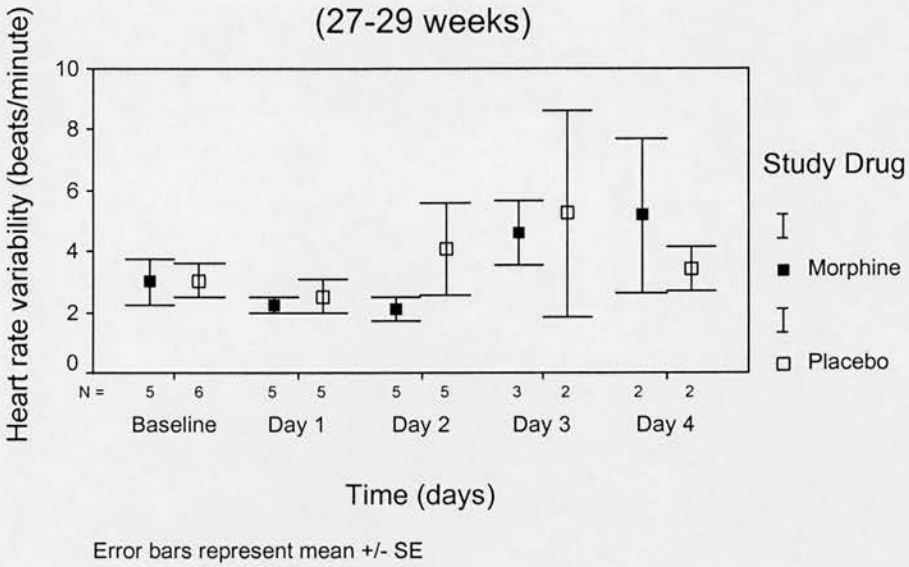


Figure 5.31

Heart rate variability before study infusion and on days 1-4 of maintenance infusion (27-29 weeks)



Error bars represent mean +/- SE

Figure 5.32

Plot of mean heart rate on days 1&2 with baseline mean heart rate (27-29 weeks)

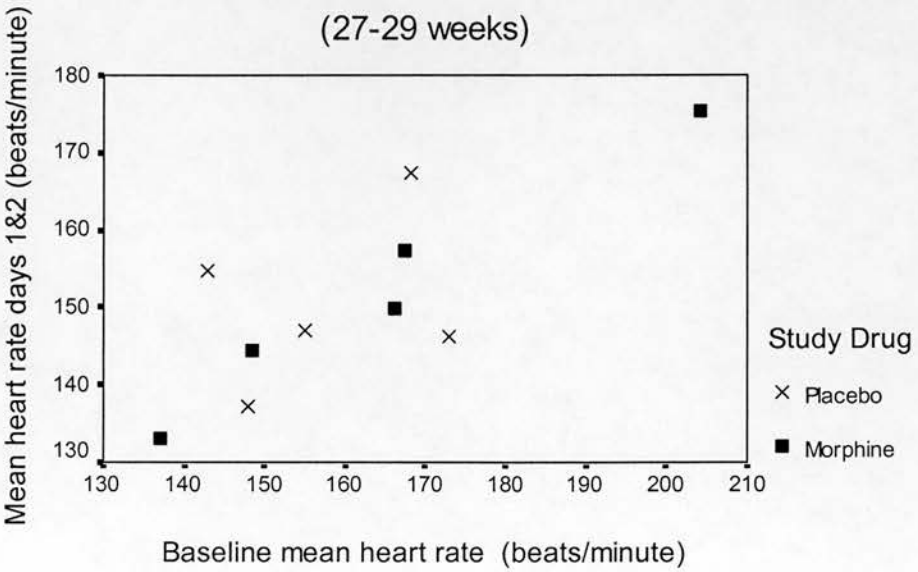


Figure 5.33

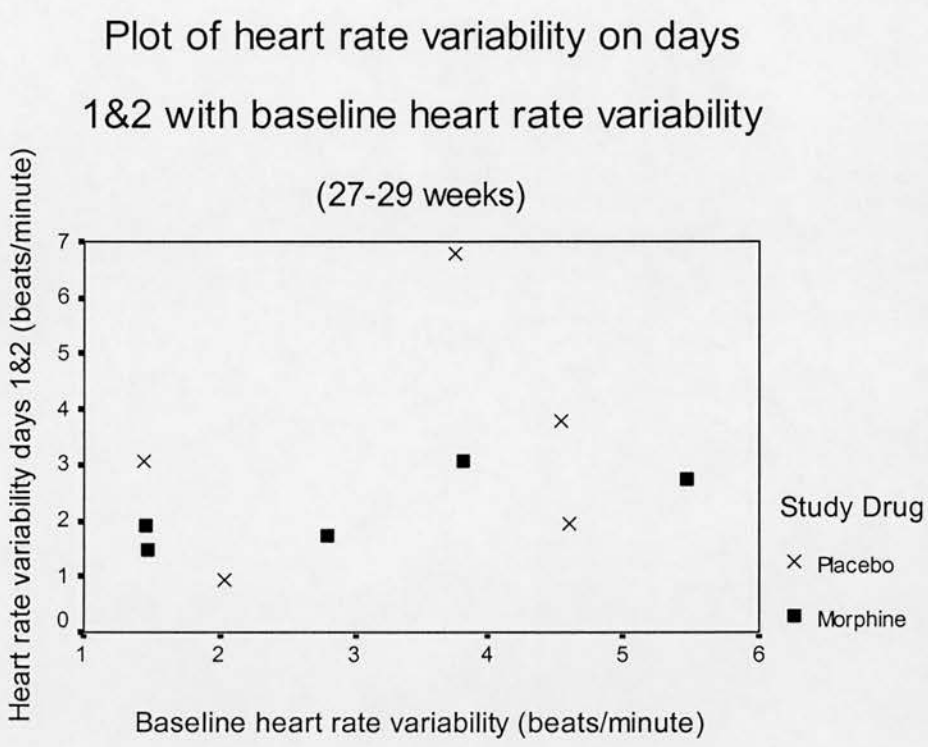


Figure 5.34

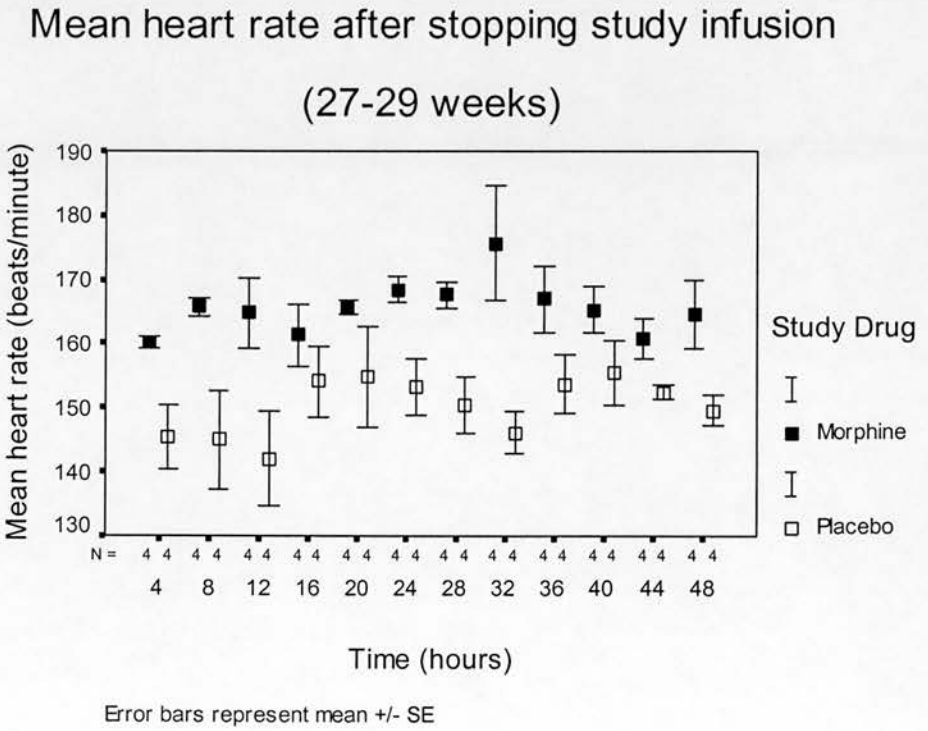


Figure 5.35

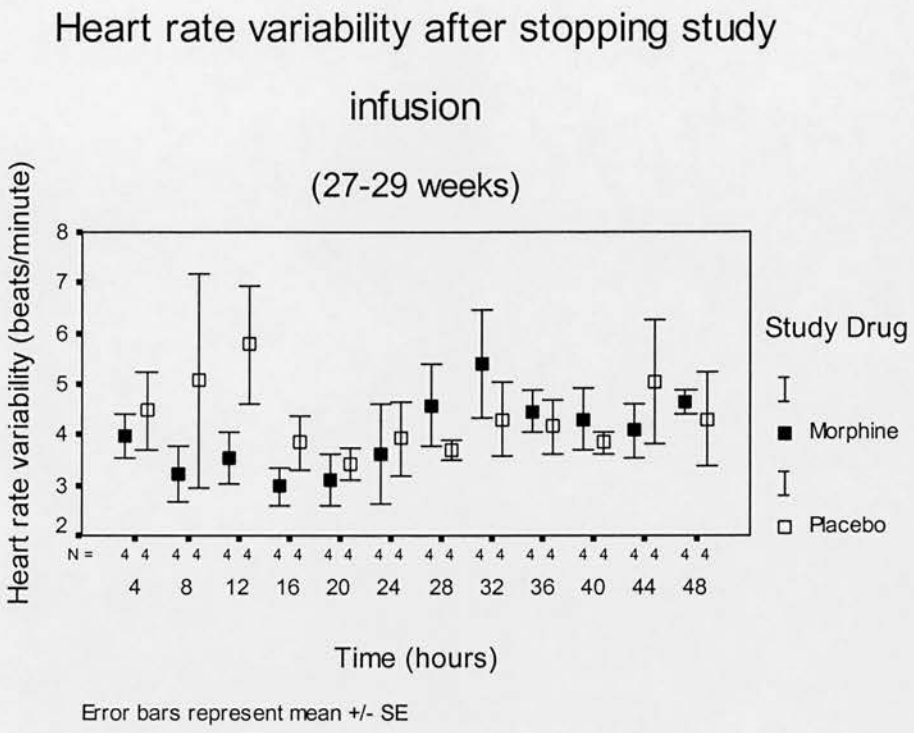


Figure 5.34 shows the mean heart rate during the 48-hour period immediately after stopping the study infusion. There is no significant difference between the morphine and placebo groups in terms of the change in heart rate over this time ($p = 0.918$). However there is clearly a relative tachycardia in the morphine group compared with the placebo group. There is no difference between the groups in heart rate variability (Figure 5.35) over this time ($p = 0.468$).

Figure 5.36

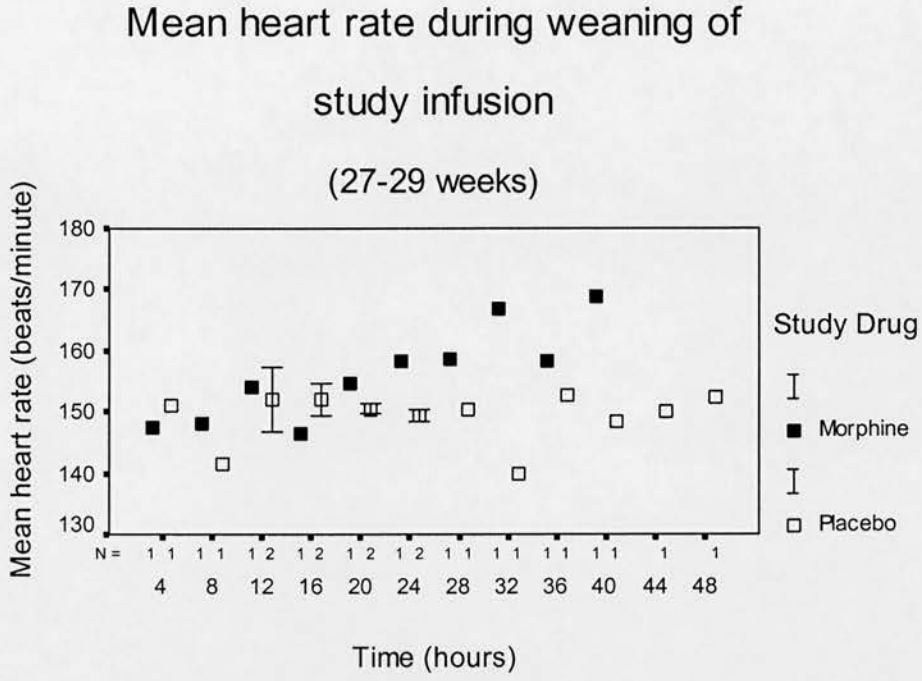
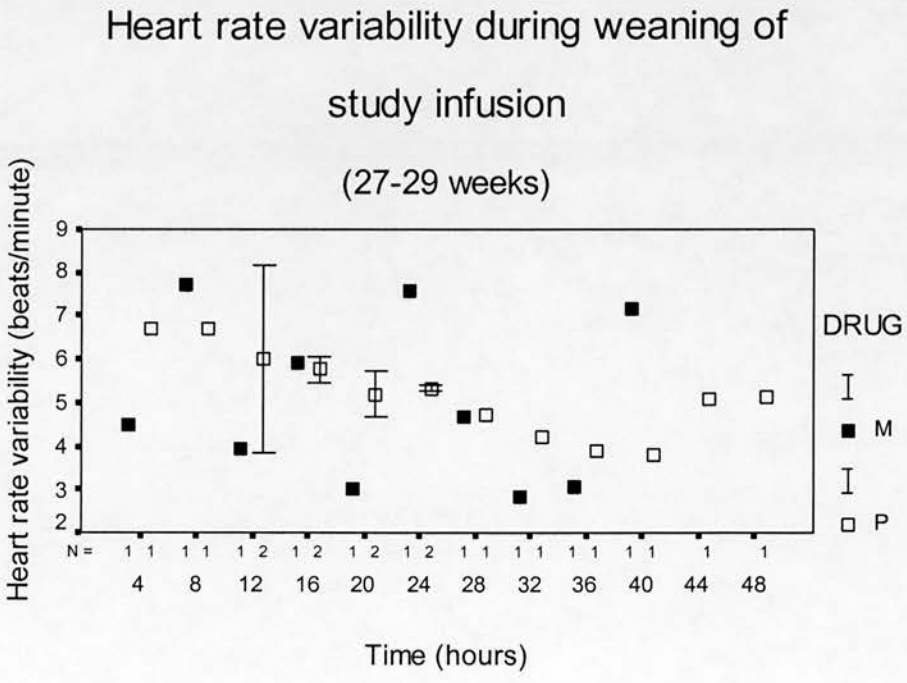


Figure 5.37



5.3.5.3 Group 3

The number of babies included in the study of 30 weeks gestation or more is extremely small. This means that no further analysis of these babies can be performed.

5.4 DISCUSSION

This study examined the differences in mean heart rate and heart rate variability between groups of preterm ventilated infants randomised to receive either morphine for analgesia, or placebo.

The analysis of the data is limited due to the repeated measures, small and unequal numbers within the groups and the amount of missing data. Analysis of covariance was used to examine the difference between baseline values and those obtained during the course of the infusion. Comparison of slopes was used to examine for any difference between the two groups over time for the period of weaning and the period of observation after stopping the study drug. This method of data analysis avoids the difficulties associated with multiple testing, and will show large and consistent differences between groups over time. However, it might fail to highlight

subtle trends in the data that might be present. Although numbers in this study are small, there are some significant results, identifiable trends and observations, which warrant further discussion.

If morphine were effective as an analgesic in this situation, a response would be expected soon after administration of the loading dose. A relative decrease in mean heart rate would be anticipated compared with infants receiving placebo. Heart rate variability is more complex and the expected changes are less easy to define. Although reduced variability has been observed in sick ventilated preterm newborns [13;14], increased variability has also been seen in response to handling and procedural pain and it has been considered as a measure of physiological instability. Previous work[138] has demonstrated a reduction in heart rate variability with morphine infusion. After discontinuation of the study drug, it would be expected that these parameters would become similar again within the two groups.

The heart rate is similar in the two groups before the study infusion is commenced. On initiation of treatment with morphine, there is an appreciable fall in mean heart rate compared with the placebo group that reaches statistical significance. This response may be an indication that morphine is effective in reducing pain and stress in this situation. Heart rate variability is similarly, though not significantly reduced in the morphine group, in keeping with previous work in this area[138]. This lends support to the hypothesis that increased heart rate variability indicates pain or stress that can be reduced by the use of analgesia. The change in mean heart rate from baseline does

not reach statistical significance on analysis of subgroups according to gestational age. However, results for the 23-26 week group show a trend towards significance. This might indicate that the more preterm infants, as well as being more sensitive to pain [18;139], have a greater response to analgesia than those of greater gestational age.

The disappearance of the difference between the groups on days 3 and 4 of the infusion raises the possibility of development of tolerance to the medication. Unfortunately the numbers of infants receiving the study drug for a prolonged time are too few to allow meaningful interpretation of later results.

The data were also analysed for indication of opiate withdrawal. This would be expected to be more likely to occur in infants who have received morphine for a prolonged period of time. However, the protocol for the NEOPAIN study required the study infusion to be weaned in these infants in order to avoid any potential adverse events on discontinuation. In this group, therefore, it would be expected that the weaning would attenuate any withdrawal effect seen. It is not thought that withdrawal phenomena are likely to occur following short-term opiate administration.

When the groups of babies are analysed as a whole, without taking into account any effect of weaning, there is no difference between the groups over time after stopping the study infusion. However, the infants whose mean heart rates were suppressed by morphine during the maintenance period are now relatively tachycardic when

compared with the infants receiving placebo. This possible rebound effect is even more marked in the 27-29 week infants (Figure 5.34), but is not seen in the 23-26 week group. This indicates a possible sign of withdrawal. The difference in response between the gestational age groups is interesting. In the older group, who received morphine 20mcg/kg/hour, the change in heart rate might indicate the increased effect of discontinuing a higher dose of the opiate in comparison with the 10mcg/kg/hour in the lower age group. Another possible explanation is that the infants of the lowest gestation group are not sufficiently mature to be able to alter heart rate variability in the same way as older infants. This group is also more likely to be more unwell. The lack of change in variability might therefore be a reflection of ongoing severity of illness exerting a greater effect than the withdrawal of medication. Although scores such as the CRIB score give an indication of severity of illness as measured within the first hours of life, no scoring system has been developed and validated for use during the subsequent course of neonatal intensive care.

Figure 5.15 shows persisting decreased variability in the morphine group for the first 16 hours of weaning, with the two groups becoming similar towards the end of the 48-hour period of weaning. This might also indicate an opiate withdrawal response. It is unfortunate that the low numbers of babies in the individual gestational age groups, and particularly in the 30-32 week group prevents analysis of this group.

Those infants who received the study infusion for more than 48 hours will fall into the weaning group. When the results from these infants are looked at separately, a

marked reduction in mean heart rate in the morphine group is clearly seen for the whole duration of the infusion. This holds true in the 23-26 week infants, but is not seen in the more mature group where numbers are too small to allow any inference to be made.

Infants who only received the study infusion for 48 hours or less did not have the drug weaned. They show a similar rebound increase in mean heart rate on stopping the infusion that persists until 32 hours after the drug has been discontinued. Thereafter, the two groups have similar mean heart rate. This raises the possibility of a withdrawal effect even after short periods of therapeutic opiate administration, though this has previously been thought unlikely. This change occurs at a time when, clinically, infants are sometimes reported by nursing staff as being more "active" or "agitated". The heart rate variability of these babies remains depressed for the first hours after discontinuation of the drug, but by 24 hours after, the variability of the two groups has become similar. This is in keeping with the pattern that would be expected, assuming that there is no withdrawal effect.

The numbers of babies studied are very small. The results must therefore be interpreted with great caution and no conclusions can be drawn from this work. There are, however, a number of factors as discussed above that bear consideration in future work on the subject of pain and analgesia in the newborn.

5.5 SUMMARY

Mean heart rate and heart rate variability were studied in 36 preterm ventilated newborn infants randomised to receive morphine or placebo infusion. There is evidence that morphine is effective in decreasing pain and /or stress associated with ventilation, shown by a decrease in mean heart rate and variability on starting the drug. The results also suggest possible development of tolerance after three or four days of treatment with a loss of these effects. Rebound tachycardia and increase in heart rate variability on weaning and discontinuation of the infusion support the hypothesis that there may be a recognisable phenomenon of opiate withdrawal associated with discontinuation of therapeutic doses of morphine. This is more obvious in infants who have received higher doses of morphine.

None of the results obtained in this small study reach statistical significance. Further investigation in a larger group of babies is necessary to confirm these findings.

CHAPTER 6

EFFECTS OF ANALGESIA ON THE CUTANEOUS FLEXOR WITHDRAWAL REFLEX IN PRETERM VENTILATED INFANTS

6.1 BACKGROUND

The cutaneous flexor withdrawal reflex or nociceptive flexion reflex is thought to be a polysynaptic reflex[140]. When a noxious stimulus is applied to the sole of the foot, pain receptors are stimulated and the resulting nerve impulse is conducted via unmyelinated C fibres and thinly myelinated A-delta fibres to the spinal cord dorsal horn[141]. This results in ipsilateral flexion at the ankle, knee and hip resulting in withdrawal from the stimulus. Studies in adults suggest that there is a linear correlation between the threshold for the cutaneous flexor withdrawal reflex and the subjective perception of pain[142]. The threshold for the maximal reflex response is similar to that for intolerable pain and the threshold is increased by the administration of analgesics[143]. The cutaneous flexor withdrawal reflex has been used in newborn infants to investigate and quantify response to pain[19]. Studies have

shown that flexion reflex thresholds in the newborn are much lower than those in adults and gradually increase with postnatal age[18]. Repeated stimulation of the skin in premature infants causes sensitisation and the flexor reflex can be induced by non-noxious as well as noxious stimuli.

In neonates, the flexor reflex threshold has been elicited using von Frey hairs. Although the cutaneous withdrawal reflex is associated with the anatomical structures related to perception of pain, stimulation using von Frey hairs to induce this reflex does not appear to be perceived as a painful stimulus and it can be induced by both noxious and tactile stimuli. In the adult, the threshold of the reflex has been shown to be useful as a measure of spinal cord excitability and is reduced with exposure to nociceptive stimuli. In the newborn infant it also appears to be useful as a measure of this excitability[19] Inhibition of the reflex has also been shown to occur with stimulation of the contralateral limb suggesting that even in preterm babies, nervous connections are functional. Although the withdrawal reflex cannot be regarded as a direct measure of pain, it can be a useful guide to the somatosensory state of infants undergoing intensive care.

Each von Frey hair is a single nylon filament, attached at right angles to a perspex handle. The set of von Frey hairs used in this work consists of 20 numbered hairs of different thickness, ranging from very fine (Number 1) to very thick (Number 20). The tip of the nylon filament is placed onto the skin of the subject and downward pressure is exerted in the direction of the hair until the hair bends. The weight

required to bend the hair is different for each one and it is quantifiable and reproducible. Hairs of increasing weight are applied to the foot in this way until the cutaneous flexor withdrawal reflex is seen. This point is the threshold of the reflex.

The reflex has been used extensively in pain research, but its potential for clinical application has not been assessed. This study investigates the change in the flexor withdrawal reflex threshold associated with administration of analgesia and withdrawal of therapeutic doses of analgesia in preterm newborn infants. The potential of the reflex as a method of assessing adequacy of analgesia and evaluating protocols for weaning of opiate analgesia is explored.

6.2 METHODS

6.2.1 Equipment

Two sets of von Frey hairs were used for the measurement of the cutaneous flexor withdrawal reflex. Each set consisted of 20 hairs as described above. In the group of neonates assessed, all thresholds were elicited with hair numbers 6 – 17. Hair numbers and the corresponding weights required to bend the hairs are shown in Table 6.1. The same set of hairs was used for all measurements on any individual

baby. The von Frey hairs were stored in the intensive care nursery of the Neonatal Unit for the duration of the study.

Table 6.1

Hair No.	Weight (g)
6	0.219
7	0.363
8	0.603
9	1.00
10	1.66
11	2.75

Hair No.	Weight (g)
12	4.57
13	7.58
14	12.6
15	20.9
16	34.7
17	57.5

Assessment of the threshold of the cutaneous withdrawal reflex was performed on 33 of the babies recruited to the NEOPAIN study. Babies were tested at the following times:

I. Prior to administration of the study drug
II. Daily for the duration of the maintenance dose of the study drug
III. Every 4 hours during the period of weaning of the study drug for up to 48 hours
IV. Every 4 hours after discontinuation of the study drug

On most occasions, testing followed the 15-minute period of ECG monitoring so that the baby had not been disturbed during that time. Where ECG monitoring was not carried out, testing followed a period of at least 15 minutes undisturbed rest. In the less stable babies, this was not always possible due to frequent nursing or medical procedures. Where essential nursing or medical procedures were needed at the time when testing should have taken place, it was delayed until the procedure was completed and there had been time for the baby to return to a state of rest.

The position of the baby was noted but was not altered for the purpose of the study. During the first days of life, when infants are unstable and require invasive blood pressure monitoring using umbilical catheters, babies are usually nursed in supine or lateral positions. Thereafter, position is altered at regular intervals and the babies spend some of their time prone. The behavioural state of the infant was also noted. At the time of testing, babies were quietly awake or asleep; babies who were crying, restless or in a very active state were not tested.

The foot was exposed and the infant was tested using von Frey hairs to assess the threshold for reflex withdrawal. Starting with von Frey hair number 6, the stimulus was applied to the lateral plantar surface of the foot. This was repeated using increasingly heavy graded hairs until the threshold was established. The threshold response was defined as clear withdrawal of the leg from the stimulus, involving flexion at the knee and hip, as well as movement at the ankle or foot. The number of the hair used to elicit this response was recorded.

6.2.2 Missing Data and Limitations of Data Collection Methods

6.2.2.1 Researcher availability

In order to minimise inaccuracies and interobserver error, most measurements were made by a single observer. However, it was impossible for any one person to be present at all times when testing was required. This was due mainly to the unpredictable nature of work involving newborn infants, unanticipated clinical decisions affecting study drug administration or discontinuation and the high intensity of work involved in obtaining 4 hourly data over a period of several days. This resulted in an unacceptably large amount of missing data for some babies. Therefore, two other researchers were instructed on the use of von Frey hairs. Since it is known that with repeated stimulation, a baby's response will change as hypersensitivity develops[18], it was not possible to test agreement between observers for any single measurement in a baby. The measurements of the additional researchers were observed to ensure that all observers agreed on the point at which the reflex was elicited.

6.2.2.2 Clinical reasons

In very sick babies, during the first few hours of life, after admission to the Neonatal Unit, many procedures are undertaken to achieve clinical stability and adequate monitoring. These include venous cannulation and insertion of umbilical arterial and venous catheters. Such procedures are carried out aseptically and require the baby to be covered with sterile drapes. In these circumstances, it was impossible to expose the foot to test the threshold. In some cases, the testing was delayed, but in others, data for that time period is unavailable. This also applies at times later in the course of intensive care when similar procedures are necessary.

Extremely premature babies have fragile blood vessels and the need for vascular access for administration of fluids and drugs and for invasive monitoring often presents difficulties. Resiting of venous and arterial cannulae is a frequent occurrence. The feet are common areas for these to be sited, meaning that sometimes neither foot is accessible due to the presence of cannulae, and securing tape or splints.

Data collection is discontinued early in babies transferred to other hospitals for their ongoing care. Similarly, in those babies for whom the prognosis is extremely poor, data is limited due to reorientation to compassionate care and the inappropriateness of continuing research in such babies.

6.2.2.3 *Technical Limitations*

The von Frey hairs used in this study were calibrated at the time of manufacture. Over time, it is possible that changes in the material may have occurred, which mean that the weight required to deform each hair may not be exactly the same as it was at the time of manufacture. Attempts were made to check this. Each hair was applied to a finely calibrated balance and the weight at which the hair bent was recorded. However, this was unsuccessful due to the inability to apply downward pressure in the direction of the hair without the hair tip moving across the smooth surface of the balance. More accurate and sophisticated equipment has recently been developed for applying differing pressures with a single electronic device, but such equipment was unavailable to us for this study.

6.3 RESULTS

Assessment of the cutaneous flexor withdrawal reflex was performed on 33 of the babies recruited to the NEOPAIN Study. Of these, 19 babies received morphine infusion and 14 were assigned to placebo. Table 6.2 shows that the study groups do not differ significantly at baseline in terms of birth weight, gestational age, number

of days on study infusion, severity of illness as defined by the CRIB score or threshold for the cutaneous flexor withdrawal reflex.

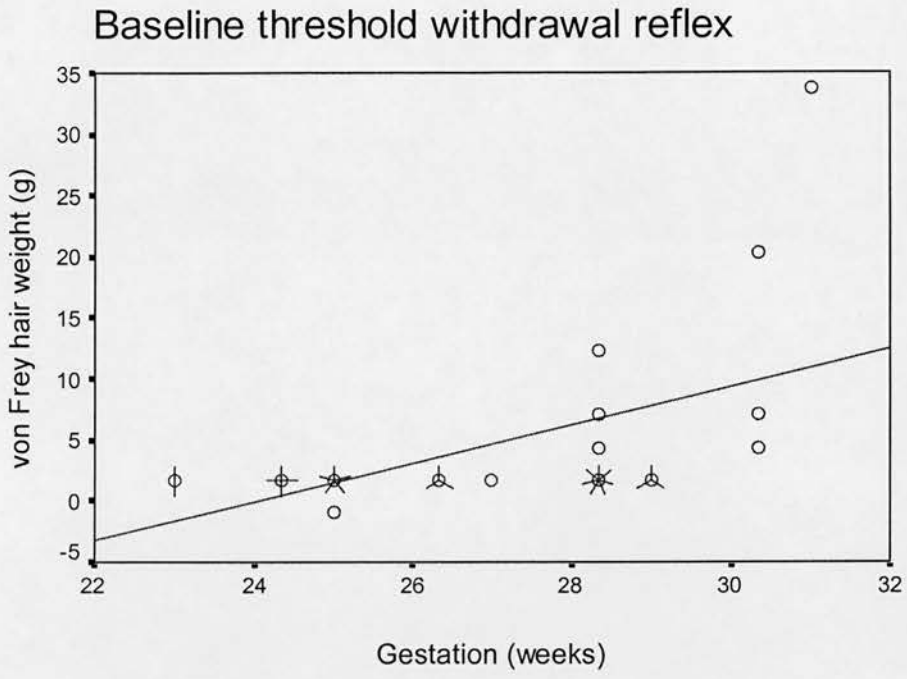
A scatter plot showing the baseline reflex withdrawal threshold for all babies confirms the relationship between the threshold and gestation (Figure 6.1). Babies were tested during the first few hours of life, so these values represent the threshold prior to commencement of the study infusion

Table 6.2: Characteristics of babies studied

	Morphine	Placebo	Significance*
No. of babies	19	14	
Male / female	11 / 8	7 / 7	
Birth weight (g)	1050 (590 – 1935)	857 (500 – 1500)	p = 0.423
Gestational age (weeks)	28 (24 – 31)	26.5 (23 – 30)	p = 0.300
No. days on study drug	4 (1 – 9)	3.5 (1 – 14)	p = 0.540
CRIB Score	4 (0 – 13)	4.5 (1 – 13)	p = 0.386
Baseline threshold (vFH no.)	10 (6 – 16)	11 (8 – 12)	p = 0.486
Baseline threshold (vFH weight(g))	1.66 (0.219 – 34.7)	2.75 (2.75 – 4.57)	p = 0.486

*Mann Whitney U; values are expressed as median (range)

Figure 6.1



6.3.1 Comparison of flexor reflex threshold sensitivity in babies receiving morphine and placebo

Table 6.3 parts A, B and C and Figure 6.2 show the babies for whom results are available. The cutaneous flexor withdrawal reflex thresholds for babies in each of the two study groups before starting the study infusion, during the maintenance infusion and weaning periods of the study, and for the first 48 hours after discontinuation of the study infusion are shown. Most infants discontinued or began weaning the study

infusion for clinical reasons after only four days. Others have very few results after this time for the reasons highlighted earlier. Meaningful interpretation of the small number of results obtained after day 4 of maintenance study infusion would be impossible; therefore only results for days 1 – 4 are considered in the analysis of babies during the time on maintenance infusion.

Figure 6.2

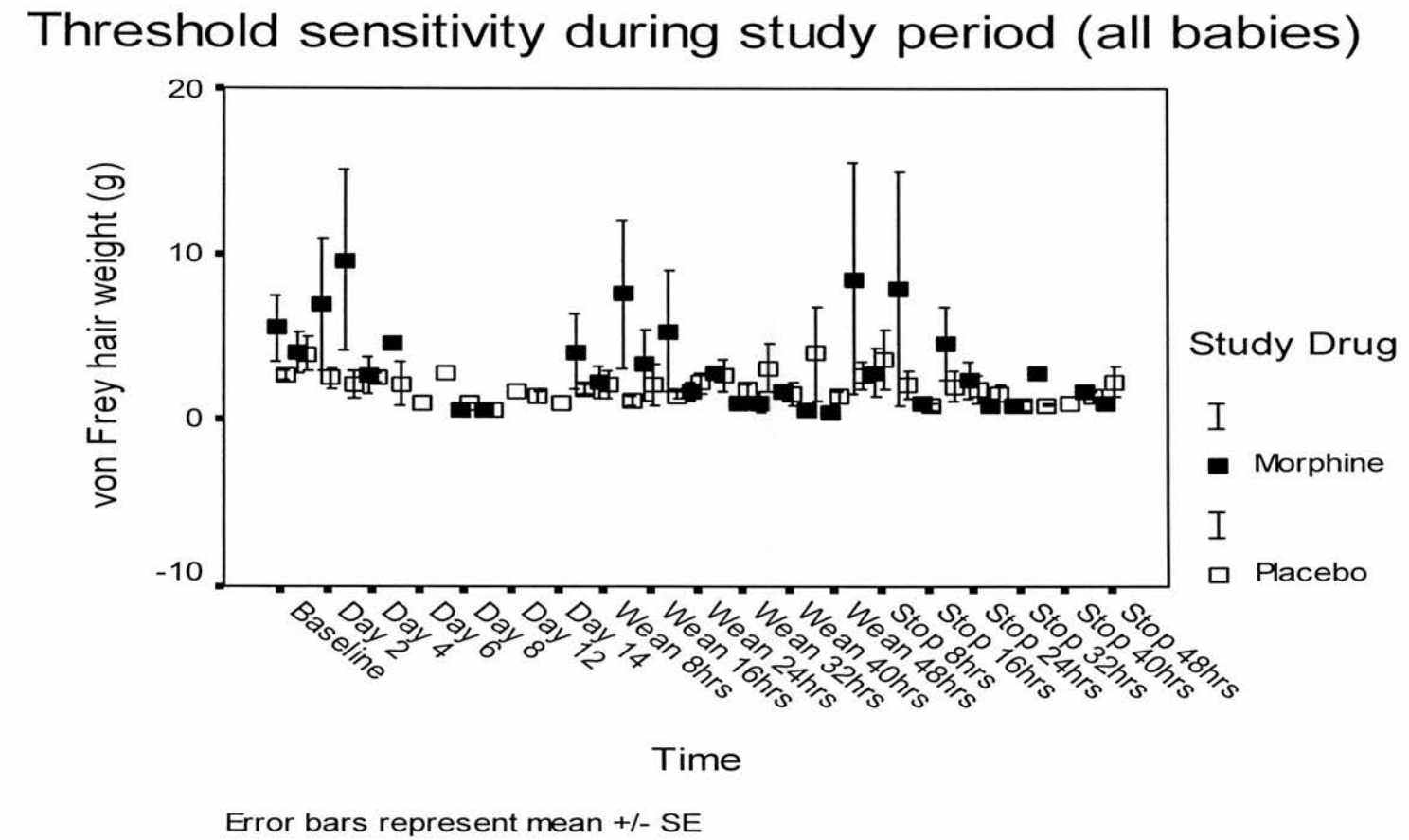

Threshold sensitivity during study period (all babies)
von Frey hair weight (g)
20
10
0
-10
Baseline
Day 2
Day 4
Day 6
Day 8
Day 12
Day 14
Wean 8hrs
Wean 16hrs
Wean 24hrs
Wean 32hrs
Wean 40hrs
Wean 48hrs
StoP 8hrs
StoP 16hrs
StoP 24hrs
StoP 32hrs
StoP 40hrs
StoP 48hrs
Time
Study Drug
Morphine
Placebo
Error bars represent mean +/- SE

Table 6.3: No. days treatment with study drug

A

Sample	Morphine (no. infants)	Placebo (no. infants)
Baseline	19	14
Day 1	12	10
Day 2	14	11
Day 3	10	4
Day 4	3	4
Day 5	1	3
Day 6	0	1
Day 7	0	1
Day 8	1	2
Day 9	1	1
Day 10	0	0
Day 11	0	0
Day 12	0	1
Day 13	0	2
Day 14	0	1

B

Wean 4 hours	5	6
Wean 8 hours	5	4
Wean 12 hours	4	3
Wean 16 hours	3	3
Wean 20 hours	3	3
Wean 24 hours	3	3
Wean 28 hours	1	3
Wean 32 hours	1	4
Wean 36 hours	2	2
Wean 40 hours	1	3
Wean 44 hours	1	4
Wean 48 hours	1	2

C

Stop 4 hours	8	8
Stop 8 hours	8	7
Stop 12 hours	8	8
Stop 16 hours	2	2
Stop 20 hours	5	4
Stop 24 hours	6	5
Stop 28 hours	3	5
Stop 32 hours	2	4
Stop 36 hours	2	4
Stop 40 hours	0	1
Stop 44 hours	1	2
Stop 48 hours	1	4

Figure 6.3

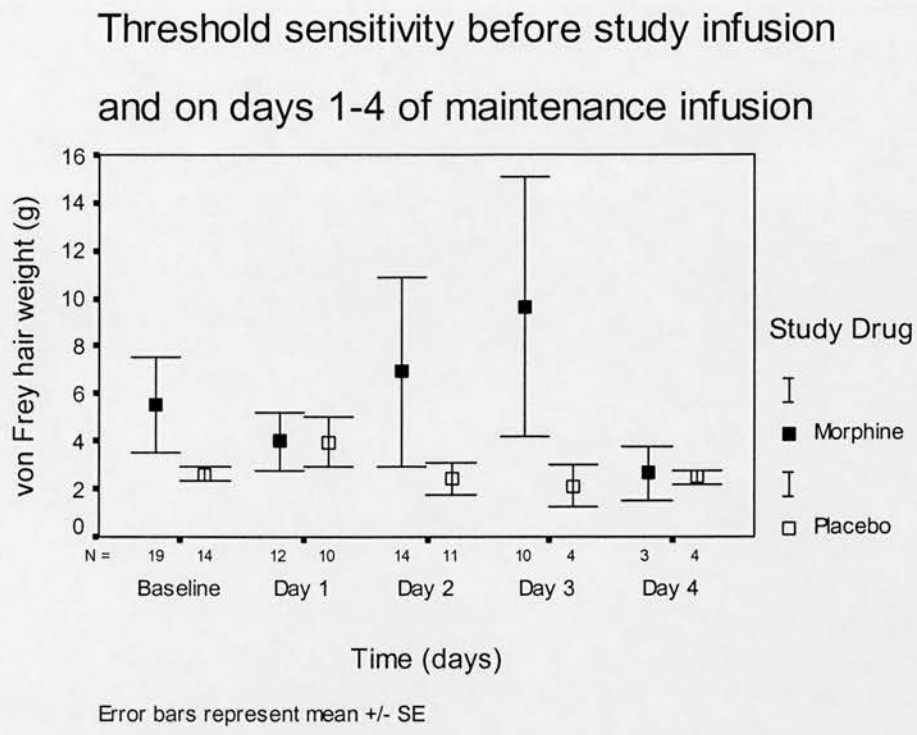


Figure 6.4

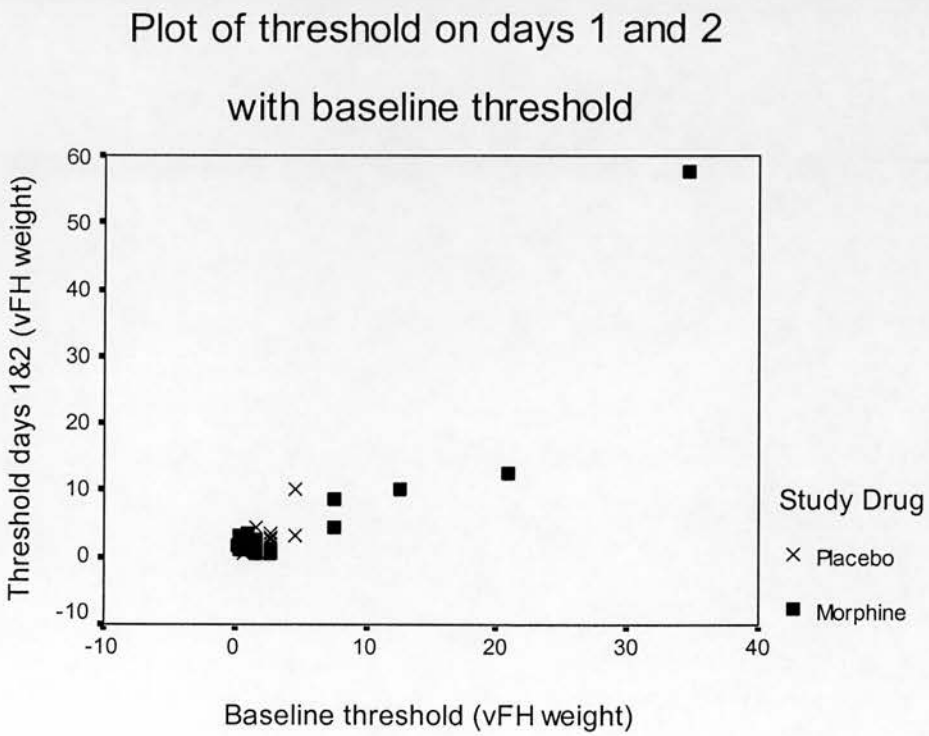
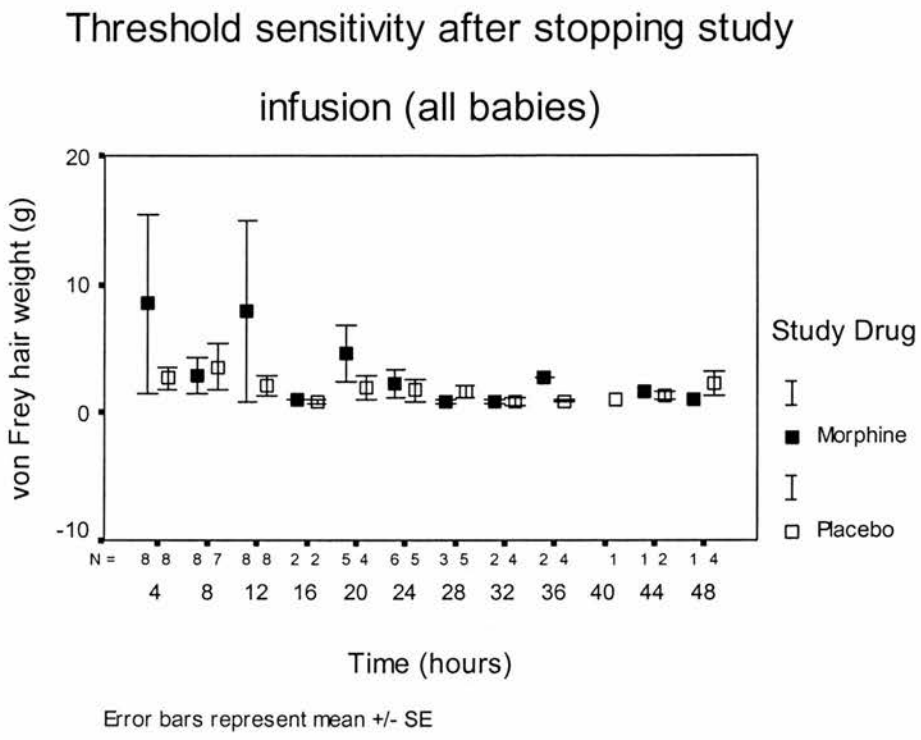


Figure 6.3 shows the threshold sensitivity for the cutaneous flexor withdrawal reflex before administration of the study infusion and during the first 4 days on maintenance infusion. To avoid problems associated with multiple T testing[137] and unequal numbers of subjects within different groups, comparisons of change in the cutaneous flexor withdrawal reflex threshold were made using analysis of covariance. Figure 6.4 shows a plot of mean threshold on days 1 and 2 of maintenance study infusion with baseline threshold. Comparing the morphine and placebo groups, there is no difference in the change from baseline values to those on days 1 and 2 ($p = 0.668$).

6.3.2 Comparison of flexor reflex threshold sensitivity in babies after discontinuation of morphine or placebo

Figure 6.5 shows the reflex threshold sensitivities after discontinuation of the study infusion. Results for all babies are included in this chart, whether or not there has been a period of weaning before stopping the infusion. These data were analysed using comparison of the slopes between the groups. There is no significant difference in the threshold between the morphine and placebo groups during the first 48 hours after discontinuing the study drug ($p = 0.847$)

Figure 6.5



6.3.3 The effect of weaning

Figure 6.6 shows the threshold for reflex withdrawal in the group of infants where there has been no weaning of the study drug. When the effect of weaning is considered in the analysis, there is still no significant difference between the two study groups after stopping the infusion ($p = 0.637$).

In the group of babies where weaning was necessary, there is a significant difference between the groups when comparison is made between the slopes of the groups ($p = 0.029$). Higher numbers of von Frey hair indicate decreased sensitivity to the stimulus. Figure 6.7 shows that from the time of initiation of weaning and for the next 20 hours, the threshold in the morphine group is generally higher than that in the babies receiving placebo. Thereafter, until discontinuation of the study drug, the infants receiving morphine therapy show increased sensitivity to the von Frey hair stimulus compared with the infants receiving placebo infusion.

Figure 6.6

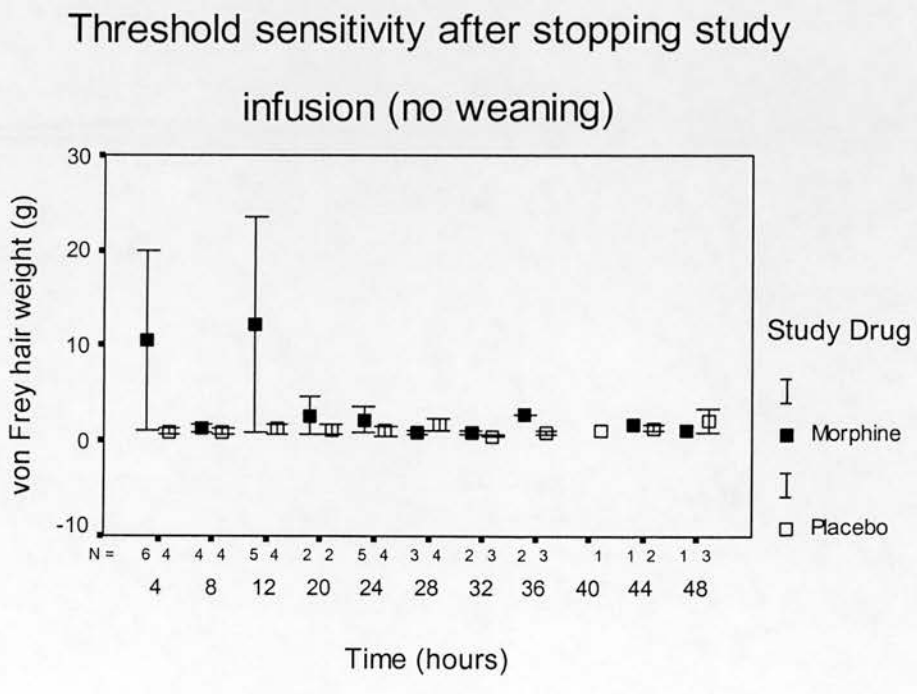
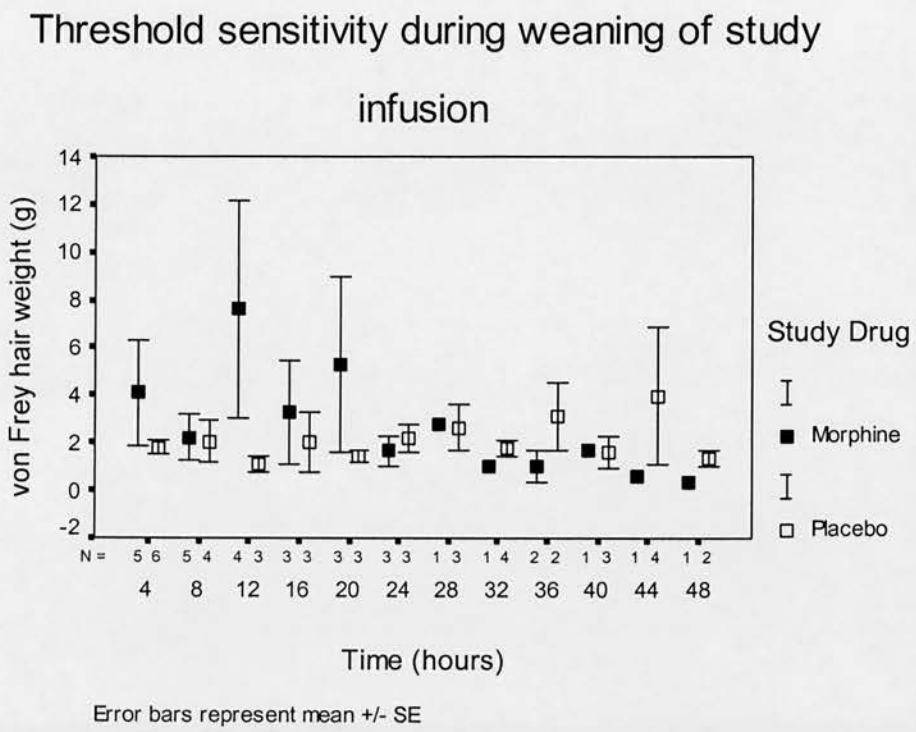


Figure 6.7



6.3.4 The Effect of Gestational Age

The small numbers of babies included in this study and the fact that no difference has been shown between the two treatment groups overall means that no meaningful subgroup analysis can be performed.

6.4 DISCUSSION

The threshold for the cutaneous flexor withdrawal reflex is thought to correlate well with pain threshold. In this study, it is used as a measure of pain threshold. The differences between the threshold in preterm infants randomised to receive morphine or placebo are explored.

The analysis of the data is limited due to the repeated measures, unequal numbers within the groups and the amount of missing data. Analysis of covariance was used to examine the difference between baseline values and those obtained during the course of the infusion. Comparison of slopes was used to determine any difference between the two groups over time for the period of weaning and observation after discontinuation of the study drug.

Initially, this study confirms the relationship described by Fitzgerald et al[18] between increasing gestational age and increasing reflex threshold. Since, in this study, almost all babies have discontinued the study infusion by the end of the first week of maintenance dose, the change with age is unlikely to affect the results.

If preterm infants experience pain in association with mechanical ventilation and morphine is effective as an analgesic, then it would be expected that the threshold for

the cutaneous flexor withdrawal reflex would be increased in ventilated infants receiving morphine compared with those receiving placebo infusion. On discontinuing the study infusion, this difference would be expected to disappear.

In this study, no significant difference compared with baseline values is seen in either mean heart rate or heart rate variability on administration of the study infusion. One possible explanation for this result is that the morphine dose was not adequate for pain relief. Although data on pharmacokinetics of morphine in the preterm neonate are few and suggest that there is great variability[144;145], previous studies have found morphine levels to be adequate with doses of 10 – 30mcg/kg/hour. Morphine has been shown to considerably reduce catecholamine levels in preterm ventilated infants[64;146]. Contributing factors to stress in sick newborn infants are many, and almost certainly include pain. It is possible, however, that the dose of morphine needed to produce a reduction in stress hormones may not be enough to alter the pain threshold in these infants. No indicator has been identified that is specific for pain. The most reliable indicators known are behavioural and these are less relevant in the situation of chronic pain than acute pain; high levels of stress hormones are not necessarily associated with behavioural indicators of pain[64].

No difference is shown between the morphine and placebo groups after discontinuation of the study infusion where the effect of weaning is not considered. In infants where weaning from the infusion was not required, the groups are similar throughout. The significant difference between groups over time during the weaning period might suggest the development of increased sensitivity after 20 hours of

weaning. The very small numbers, however, do not allow such conclusions to be drawn.

6.5 SUMMARY

The cutaneous flexor withdrawal reflex was explored in 33 preterm ventilated newborn infants randomised to receive morphine or placebo. There was no significant difference in the reflex threshold between the groups on starting treatment, during weaning of the infusion or after discontinuation of the drug. Small numbers, particularly later in the study mean that this work would be very unlikely to identify any differences present.

CHAPTER 7

EFFECTS OF ANALGESIA ON MEAN HEART RATE AND HEART RATE VARIABILITY IN INFANTS UNDERGOING OPHTHALMOLOGICAL EXAMINATION

7.1 BACKGROUND

7.1.1 Screening for Retinopathy of Prematurity

Retinopathy of prematurity is a condition exclusively affecting preterm babies. The incidence of disease has increased with the increasing survival of very low birth weight infants. It is a disorder in which there is interruption of the normal development of vasculature of the retina and it is characterised by formation of new and abnormal retinal blood vessels. The condition often resolves spontaneously over time, but can progress to severe disease with scarring, retinal detachment and visual impairment. It is a leading cause of blindness in infants born prematurely[147].

In infants whose disease shows progression to a level requiring treatment (threshold disease), laser therapy is effective in preventing further progression if initiated at this

stage[148]. Guidelines for screening have therefore been drawn up by a Joint Working Party of The Royal College of Ophthalmologists and the British Association of Perinatal Medicine[149]. In accordance with these recommendations, all infants at the Royal Infirmary of Edinburgh born at gestations below 32 weeks or with birth weight of less than 1500g receive ophthalmological examination on the Neonatal Unit. Screening begins at 6 weeks postnatal age, or 36 weeks corrected gestational age, whichever is earlier. Screening examinations are performed at regular intervals until the retina is fully vascularised. This aims to detect and monitor the disease in its early stages to allow for appropriate intervention.

7.1.2 Behavioural responses to eye examination

Examination of each eye for retinopathy of prematurity involves retraction of the eyelids using a lid speculum and manipulation of the eye using a muscle hook. Indentation of the eye often occurs during the examination. Such procedures have been reported to be painful by adults. When the eye examination begins and during the course of the procedure, infants display responses that are recognised indicators of acute pain in preverbal infants – brow bulge, nasolabial furrowing and crying[71]. These features disappear rapidly on completion of the examination. These pain behaviours are frequently observed in babies undergoing screening even with administration of local anaesthetic eye drops. No additional intervention,

pharmacological or non-pharmacological, is used routinely during eye examinations on the Neonatal Unit. This is a common practice throughout the United Kingdom.

7.1.3 Analgesic effects of Sucrose and non-nutritive sucking

A variety of interventions, both pharmacological and non-pharmacological are available for management of procedural pain in neonates. Sweet solutions have, for many years, been noted to produce a calming effect in crying babies and infants undergoing invasive procedures[150]. Sucking at the breast or on a pacifier has also been shown to reduce pain responses[151]. Administration of sucrose with or without non-nutritive sucking is the most widely studied non-pharmacological intervention for pain relief in infants. The analgesic effects of sucrose and non-nutritive sucking are thought to be mediated by both endogenous opioid and non-opioid receptors. A recent Cochrane Review concluded that oral sucrose reduces procedural pain in neonates[152]. It has been shown to decrease duration of crying and reduce behavioural and physiological pain responses in babies undergoing heelstick or venepuncture[153-155]. A wide range of doses has been investigated. Doses of less than 0.12g appear to be ineffective and little increase in effect has been shown with doses greater than 0.5g[156]. No adverse effects have been reported with administration of single doses of sucrose.

7.1.4 Pain Relief in screening for retinopathy of prematurity

Infants undergoing screening examination have usually progressed from the initial acute problems associated with prematurity and are often no longer receiving intensive care. Therefore, they rarely have a requirement for regular analgesic medication. Although local anaesthetic drops are administered prior to eye examination, the majority of infants score highly on validated pain scores such as the Premature Infant Pain Profile[12] during the examination. Since eye examination appears to be a painful procedure, there is a need to identify a safe and effective way of providing procedural pain relief and of assessing the adequacy of analgesia. In this group of more mature babies undergoing a brief, but acutely painful procedure, the use of sucrose with or without a pacifier might be expected to attenuate the pain response. This study was designed to study the effectiveness of sucrose as an analgesic for this procedure and to investigate the effects of analgesia for acute pain on mean heart rate and heart rate variability.

7.1.5 Study design and subjects

This was a randomised placebo-controlled study. 34 infants were included in the study. All met the criteria for screening for retinopathy of prematurity on the basis of

birth weight and/or gestational age. Babies receiving mechanical ventilation and those receiving nothing by mouth were excluded from the study. Any infant receiving concurrent alternative analgesic medication was also excluded. Babies receiving continuous positive airways pressure via nasal prongs (nCPAP) who were stable and comfortable on this form of airway support were not excluded.

Only the first eye examination for each baby was considered. This was to overcome potential confounding effects that might occur if a baby were to anticipate a procedure following previous exposure to the same painful stimulus. A single ophthalmologist carried out all examinations. The Lothian Research Ethics Committee granted ethical approval for the study and written consent was obtained from the parents of all infants.

7.2 METHODS

7.2.1 Allocation of treatment

Infants were randomised to receive one of the following four interventions two minutes prior to the start of one of the screening eye examinations. The start of the examination was defined as the time of administration of local anaesthetic eye drops:

1. 1ml sterile water given by mouth using a syringe.

2. 0.5ml Syrup BP (sucrose 66%) diluted with 0.5 ml sterile water (to give a dose of sucrose 0.33g) given by mouth using a syringe.

3. 1ml sterile water given by mouth using a syringe and pacifier put into the mouth.

4. 0.5ml Syrup BP (sucrose 66%) diluted with 0.5 ml sterile water (to give a dose of sucrose 0.33g) given by mouth using a syringe and pacifier put into the mouth.

Investigators, staff and parents were blind to the identity of the study medication, but could not be blind to the use of the pacifier.

7.2.2 ECG monitoring

ECG recording using the Braemar DL700 holter monitor (see Chapter 5, Section 5.2.1.2) connected to the baby's chest by five gel electrodes was started prior to

administration of local anaesthetic eye drops and continued for at least one minute after completion of the procedure. Monitoring electrodes were applied several minutes before monitoring was commenced, to allow the baby to return to a state of rest before study measurements were recorded. During the examination, the times of all events were noted: administration of study treatment, administration of local anaesthetic eye drops, completion of examination as defined by removal of the lid speculum following examination of the second eye.

7.2.3 Analysis of ECG data

Using the times recorded at the time of screening, periods before administration of the study solution and then during and after the eye examination were identified. These periods represent baseline, then deviation from the baseline during the painful procedure, and lastly, recovery after the procedure. Ideally, it would have been most appropriate, when examining the data, to identify the maximum deviation of heart rate from baseline during the procedure. However, since each examination lasted between two and nine minutes, the large amount of numerical data returned made this impracticable. Instead, a random number generator [www.random.org] was used to isolate a ten second block in the middle of the time spans before the study solution was given and the baby was at rest. A 20 beat period was chosen from this block. Similarly, a random block was chosen from the recovery period, between one and two minutes after completion of the examination. For the period representing the eye

examination itself 20 beat periods were analysed, at one-minute intervals. The maximum value obtained for mean heart rate was chosen to represent the peak deviation from baseline. The mean heart rate was calculated over the 20 beats and the heart rate variability was calculated using the standard deviation about the mean.

7.3 RESULTS

34 infants were recruited to the study. Table 7 shows the characteristics of the babies studied according to treatment allocation. Unfortunately, in spite of randomised allocation of treatment, the characteristics of the four groups were not the same at the time of entry to the study. There was a statistically significant difference between the groups with respect to gestation at birth ($p = 0.037$) with the babies who received placebo without pacifier, being of a lower gestational age than the other 3 groups. However, the groups were not significantly different in birth weight or in the corrected gestational age at the time of the eye examination. The lower gestational age group, not surprisingly, also had a higher percentage of babies who required intensive care and a higher percentage of them were given morphine during the first days of life. The difference in the number of babies previously receiving morphine reached statistical significance ($p = 0.022$). A number of the babies in each group were receiving caffeine as a respiratory stimulant at the time of eye examination.

Table 7.2 shows the behavioural states of the infants during the period immediately before the ophthalmological examination was performed. At that point, the heart rate leads had been positioned and time had been allowed for the babies to return to a resting state after this intervention. No study drug had been given at this stage and it therefore represents baseline measurements. Table 7.2 shows that some babies at this time were still in active states, although most were in quiet or active sleep states. Using Fisher's exact test, the difference in behavioural states between the groups is significant at the 1% level ($p = 0.004$). The group that later received both sucrose and pacifier included the greatest proportion of babies in awake states.

Babies who were receiving respiratory support via nCPAP were only included in this study if they were generally well otherwise and were considered to be comfortable and stable. Four of the 34 babies were receiving nCPAP. Three of these were randomised to placebo and one to sucrose. No infant on nCPAP was randomised to suck on a pacifier.

Table 7.1: Characteristics of babies studied

Study Drug	Placebo	Placebo + Pacifier	Sucrose	Sucrose + Pacifier	Significance*
n (%)	9 (26.5)	8 (23.5)	10 (29.4)	7 (20.6)	
Gestation at birth (weeks)	27 (24-30)	31 (25-36)	30 (25-34)	29 (25-31)	p = 0.037
Corrected age (weeks)	34 (30-36)	36 (31-39)	35.5 (30-40)	35 (30-36)	p = 0.175
Birth weight (g)	990 (640-1360)	1250 (750-1630)	1260 (850-1640)	1100 (640-1910)	p = 0.195

*One-way analysis of variance; Values are expressed as median (range)

Study Drug	Placebo	Placebo + Pacifier	Sucrose	Sucrose + Pacifier	Significance*
Previous ICU care n (%)	8 (88.9)	3 (37.5)	4 (40.0)	5 (71.4)	p = 0.085
Previous morphine therapy n (%)	4 (44.4)	3 (37.5)	0	0	p = 0.022
Caffeine therapy n (%)	5 (55.6)	3 (37.5)	2 (20.0)	3 (42.9)	p = 0.489
Nasal CPAP n (%)	3 (33.3)	0	1 (10.0)	0	p = 0.154

*Fisher's Exact Test

Table 7.2: Behavioural states of babies at baseline

Behavioural state	Placebo	Placebo + Pacifier	Sucrose	Sucrose + Pacifier
Quiet asleep n (%)	4 (44.4)	6 (75.0)	1 (10.0)	0
Active asleep n (%)	4 (44.4)	1 (12.5)	7 (70.0)	2 (28.6)
Quiet awake n (%)	0	1 (12.5)	0	3 (42.9)
Active awake n (%)	1 (11.1)	0	2 (20.0)	2 (28.6)

Table 7.3 shows the baseline mean heart rates and heart rate variability for each of the groups. There is a difference between both the mean heart rates and variability. Both groups who later receive a pacifier have lower resting baseline heart rates than the other groups. The group who receive placebo without pacifier have lower baseline heart rate variability than the other three groups. Neither difference reaches statistical significance.

Table 7.3: Baseline heart rate and heart rate variability

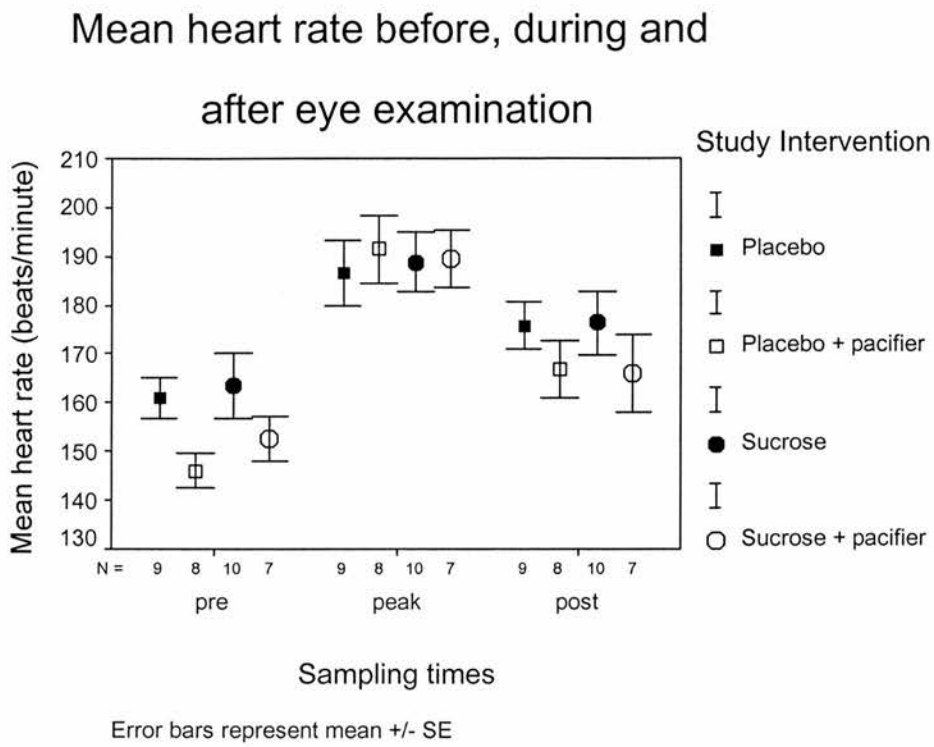
	Placebo	Placebo + Pacifier	Sucrose	Sucrose + Pacifier	Significance*
Mean heart rate (bpm)	160.97 (13.01)	146.05 (9.74)	163.46 (21.36)	152.76 (12.25)	p = 0.095
Heart rate variability (bpm)	2.38 (1.01)	3.76 (1.69)	3.21 (0.55)	3.57 (1.71)	p = 0.142

*One-way analysis of variance; Values are expressed as mean (SD)

7.3.1 Mean heart rate and heart rate variability during eye examination

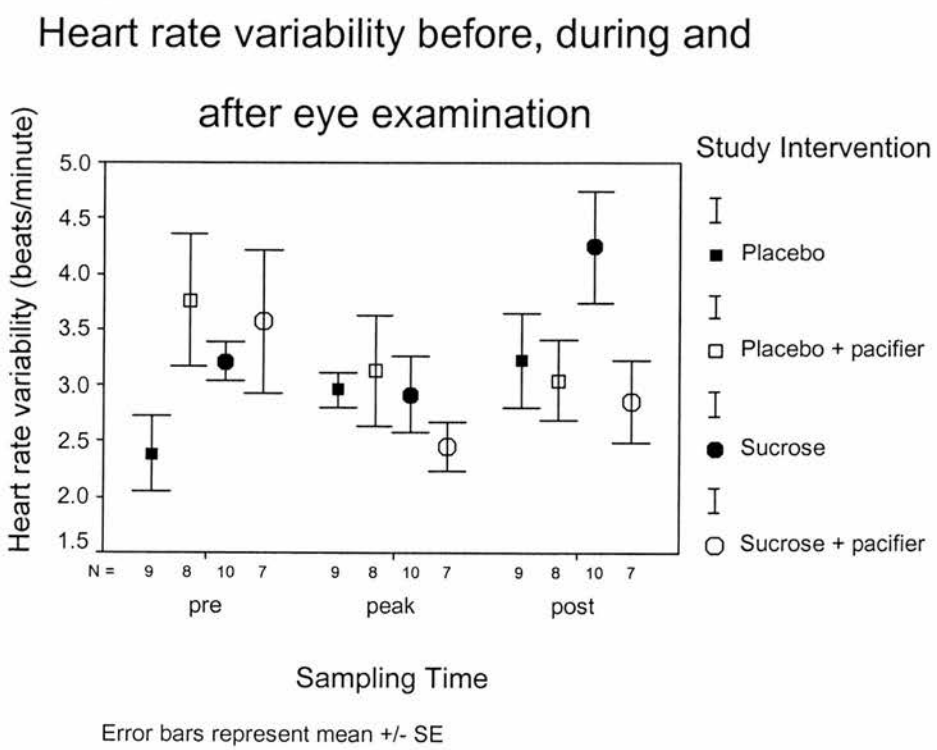
Figures 7.1 and 7.2 show the results for heart rate and variability before the examination (pre), during the examination (peak) and during recovery (post).

Figure 7.1



Error bars represent mean +/- SE

Figure 7.2



Error bars represent mean +/- SE

Table 7.4 shows the maximum deviation in mean heart rate from baseline during eye examination. In each group, there is a rise in mean heart rate that is highly statistically significant.

Table 7.4: Change in mean heart rate during eye examination

	Baseline mean heart rate (bpm)	Mean heart rate during examination (bpm)	Difference in means (95% CI)	Significance*
Placebo	160.97	186.51	25.54 (11.20-39.89)	$p = 0.003$
Placebo + Pacifier	146.05	191.40	45.35 (23.84-66.86)	$p = 0.002$
Sucrose	163.46	188.80	25.34 (19.28-31.40)	$p \leq 0.001$
Sucrose + Pacifier	152.76	189.57	36.81 (18.92-54.71)	$p = 0.002$

*Paired samples T- test

Table 7.5 shows the mean change in heart rate variability at the time of peak heart rate during eye examination. There is a fall in variability with the increase in heart rate in all intervention groups; on no occasion does this change reach statistical significance. The group who received placebo alone shows an increase in heart rate variability although this increase is not statistically significant.

Table 7.5: Change in heart rate variability during eye examination

	Baseline heart rate variability (bpm)	Heart rate variability during examination (bpm)	Difference in means (95% CI)	Significance*
Placebo	2.38	2.96	0.57 (0.22-1.37)	p = 0.135
Placebo + Pacifier	3.76	3.13	-0.63 (1.74-0.48)	p = 0.224
Sucrose	3.21	2.92	-0.29 (1.19-0.60)	p = 0.474
Sucrose + Pacifier	3.57	2.45	-1.12 (2.82-0.60)	p = 0.159

*Paired samples T- test

Since there are significant differences between the groups at baseline, it is more appropriate to analyse the groups according to change from baseline values for mean heart rate and heart rate variability, rather than by absolute values.

Table 7.6 shows the maximum change in mean heart rate during the examination and corresponding change in variability. The change is also shown as a percentage of the baseline value for each group. Using analysis of variance, there is no statistically significant difference between the four groups although there are trends in the percentage change for both mean heart rate and variability. Mean heart rate increases in all four groups during the examination. There is a decrease in variability in the three intervention groups, but the group receiving placebo alone show an increase.

Table 7.6: Change from baseline mean heart rate and heart rate variability during eye examination; comparison of study groups

	Placebo	Placebo + pacifier	Sucrose	Sucrose + pacifier	Significance*
Change in mean heart rate from baseline (bpm)	25.54 (11.20-39.89)	45.35 (23.84-66.86)	25.34 (19.28-31.40)	36.81 (18.92-54.71)	p = 0.095
% Change in mean heart rate from baseline	16.21 (6.72-25.71)	31.88 (15.97-47.78)	16.06 (11.34-20.80)	24.79 (11.74-37.83)	p = 0.058
Change in heart rate variability from baseline (bpm)	0.57 (-0.22 -1.37)	-0.63 (-1.74 -0.48)	-0.29 (-1.19 -0.60)	-1.12 (-2.82 -0.58)	p = 0.104
% Change heart rate variability from baseline	41.18 (-0.57 -82.93)	-10.32 (-40.60 -19.96)	-6.73 (-32.87 -19.41)	-12.23 (-57.63 -33.16)	p = 0.052

*One-way analysis of variance; Values are expressed as mean (95% CI)

7.3.2 Mean Heart Rate and Heart Rate Variability during Recovery from Eye Examination

Tables 7.7 and 7.8 show the difference between the baseline heart rate and that during the recovery period, between one and two minutes following eye examination.

The mean heart rate does not return to the pre-examination level in any of the study groups. In all four groups, the mean heart rate remains significantly elevated compared to baseline, but has decreased with respect to the maximum rate during examination.

Table 7.7: Mean heart rate during recovery from eye examination

	Baseline mean heart rate (bpm)	Mean heart rate during recovery (bpm)	Difference in means (95% CI)	Significance*
Placebo	160.97	175.70	14.73 (3.37-26.09)	p = 0.017
Placebo + Pacifier	146.05	166.90	20.85 (10.00-31.70)	p = 0.003
Sucrose	163.46	176.35	12.89 (-1.68 -24.10)	p = 0.029
Sucrose + Pacifier	152.76	165.94	13.18 (-0.36-26.01)	p = 0.046

*Paired samples T- test

Table 7.8: Heart rate variability during recovery from eye examination

	Baseline heart rate variability (bpm)	Heart rate variability during recovery (bpm)	Difference in means (95% CI)	Significance*
Placebo	2.38	3.23	0.84 (0.13-1.82)	p = 0.081
Placebo + Pacifier	3.76	3.04	-0.71 (-2.50-1.07)	p = 0.374
Sucrose	3.21	4.24	1.03 (0.14-2.21)	p = 0.078
Sucrose + Pacifier	3.57	2.85	-0.72 (-2.50-1.06)	p = 0.361

*Paired samples T- test

Table 7.9 and 7.10 show the mean differences in heart rate and heart rate variability for the groups from the height of the examination to the recovery phase. In the infants receiving sucrose without pacifier, the fall in heart rate from peak level to

recovery is statistically significant. This is not the case in the other three groups. Heart rate variability in three of the groups increases, though this is only statistically significant in the infants receiving sucrose without pacifier. The change in variability is not significant with any other intervention.

Table 7.9: Change in mean heart rate from examination to recovery

	Peak heart during examination rate (bpm)	Mean heart rate during recovery (bpm)	Difference in means (95% CI)	Significance*
Placebo	186.51	175.70	-10.81 (-22.63-1.01)	p = 0.068
Placebo + Pacifier	191.40	166.90	-24.5 (-50.70-1.70)	p = 0.063
Sucrose	188.8	176.35	-12.45 (-23.37--1.53)	p = 0.030
Sucrose + Pacifier	189.57	165.94	-23.63 (-49.27-2.01)	p = 0.065

*Paired samples T- test

Table 7.10: Change in heart rate variability from examination to recovery

	Heart rate variability during examination (bpm)	Heart rate variability during recovery (bpm)	Difference in means (95% CI)	Significance*
Placebo	2.96	3.23	0.27 (-0.74-1.27)	p = 0.614
Placebo + Pacifier	3.13	3.04	-0.09 (-1.49-1.32)	p = 0.886
Sucrose	2.92	4.24	1.33 (0.40-2.25)	p = 0.010
Sucrose + Pacifier	2.45	2.85	0.40 (-0.46-1.26)	p = 0.302

*Paired samples T- test

7.4 DISCUSSION

Randomisation in this study was carried out using a sealed envelope method. However, differences in the baseline characteristics between the four groups are clearly apparent. This makes it difficult to determine whether any differences in response are due to the effect of the study treatment or to inherent differences in the subjects studied. It would only be possible to eliminate such differences by recruiting larger numbers of babies into the study. The study was designed primarily to explore the efficacy of sucrose as an analgesic in eye examination. Power calculations were performed to detect a difference in the behavioural response to examination, since this is the most sensitive known indicator of acute pain. In view of the baseline differences that have occurred by chance, this part of the study will be continued to recruit additional babies. Although research has shown a change in heart rate variability with acute pain[16;134], there are only few data exploring the effect of analgesia on heart rate and heart rate variability[17]. It would, therefore be impossible to determine the number of babies required to show a difference between the groups. This work was carried out as an opportunistic study and must therefore be regarded only as an exploration of the subject.

It is possible that some of the pre-study differences may have affected the results in a number of ways. Altered behavioural and physiological responses to pain have been demonstrated in infants who have undergone intensive care. Johnston and Stevens[3] studied the effect of postnatal age on pain responses and showed that babies exposed to intensive care had decreased behavioural responses to pain and higher heart rates

when compared with newly born infants of similar gestation. This was also noted by Grunau et al[157] and may explain the higher baseline heart rates in the two groups with lower gestational age at birth.

Little work has been done to investigate the effects of previous opiate analgesia on physiological parameters later in life. Grunau et al demonstrated that infants treated with morphine in the first weeks of life might have increased cardiac autonomic responses to acute pain. [157] They suggest that these infants, as a result of previous stress, may be in a state of ongoing stress. This concept is in keeping with the hypothesis that a state of heightened arousal exists in these babies as a result of the wind up phenomenon[43].

In this study, there is a trend in the placebo only group towards a different reaction to eye examination. In contrast to the other groups, heart rate variability, in this group of infants, increases rather than decreases with the painful stimulus, though in none of the groups does the change reach statistical significance. Characteristics of this group that may play a part in this include the lower gestational age at birth, previous morphine therapy and the lack of any active treatment intervention. Unfortunately, it is not possible to clarify the relative contributions of each. The increase in variability would not have been expected from previous work that has shown decreased heart rate variability in infants with painful interventions such as heel stick[16] and venepuncture[17].

Behavioural state is known to influence both heart rate variability and pain responses. In sleeping babies, variability is higher in active sleep states than quiet sleep and is higher again in infants when awake. The period of rest after positioning of the ECG leads was intended to allow the babies to settle into a state of quiet sleep. However, the majority of babies were in active states before the intervention suggesting that a longer interval would have been beneficial for standardisation of methods. As well as having an effect on baseline values, the state differences might also affect the change with the painful stimulus. Grunau and Craig demonstrated that facial activity in response to pain differs with the behavioural state prior to the noxious stimulus[73]. Awake infants show greater response than sleeping infants and those in quiet sleep had smaller facial responses than those in active sleep. It is not known whether there is a similar differential response in heart rate or heart rate variability. Given the small numbers in this study, it would be unlikely to detect such an effect.

With the onset of the eye examination, the pattern in mean heart rate is that which would be expected. All groups show a significant tachycardia in response to pain and there is no difference in the extent of this between the treatment groups. If the analgesic intervention were effective, attenuation in the pain response might be anticipated with the effective therapy. The fact that this is not evident implies that no analgesic effect is seen. This may be because sucrose and/or pacifier are not useful as analgesics, though research into pain responses on heelstick and venepuncture indicate otherwise. An alternative explanation is that the pain caused by this procedure is so severe that sucrose is not a potent enough analgesic to be appropriate

in this situation. Increase in heart rate is seen with many different kinds of stress. It is likely, given the highly significant change in the heart rate of the infants in all groups, that mean heart rate is not a sensitive enough indicator of pain to be useful in detecting a difference. The response in heart rate variability is interesting in that it alters in the placebo group in the opposite direction from all groups of infants receiving active intervention to alleviate pain suggesting a possible treatment effect in the other groups. However, since the change is contrary to what would be anticipated and the difference is not statistically significant, it is difficult to interpret this information and it must be acknowledged that the difference in baseline characteristics of this group may have influenced this response. The small numbers in the groups also make spurious results possible. Additional information on the behavioural responses of these infants may shed further light. Since behaviour is still acknowledged as the most sensitive indicator of pain, differences not identified by the study of heart rate and heart rate variability may become apparent.

Following completion of the examination, heart rate would be expected to fall as the infant returns to a resting state. It is interesting to note that clinically, most infants fell into a deep state of sleep immediately the examination was over and the eye speculum was removed. However, this study shows that even one or two minutes later, the heart rate is still significantly higher than baseline levels in all groups, indicating that the infants are still in a state of stress. However, in the infants receiving sucrose without pacifier, the fall in heart rate from peak level to recovery is statistically significant. This is not the case in the other three groups. Heart rate variability increases as stress diminishes. In the group given sucrose, the rise in

variability from peak levels reaches statistical significance, while in the other three groups there is no significant change following the procedure. The possibility exists, therefore, that sucrose is effective and that this is seen as a more rapid and smooth return to baseline levels once the painful experience has finished.

7.5 SUMMARY

Mean heart rate and heart rate variability were studied in 34 infants undergoing screening examination for retinopathy of prematurity. The infants were randomly allocated to four groups receiving sucrose or placebo with or without a pacifier. There were significant differences between the different intervention groups at baseline. There was no significant difference between the groups for heart rate response during examination. Heart rate variability fell in three treatment groups, but increased in the group who received placebo alone. This finding is in contrast to previous work and is difficult to explain. Mean heart rate and heart rate variability in infants receiving sucrose fell towards pre-examination levels more rapidly than in the other groups. Investigation of behavioural responses may be more helpful in clarifying the role of sucrose as an analgesic for this procedure.

CHAPTER 8

DISCUSSION

This work set out to explore the relationships between acute/procedural and subacute/chronic pain, heart rate variability and analgesia in premature infants in the intensive care situation. The larger part of the work consisted of opportunistic studies of infants in Edinburgh involved in a large multicentre study. The other part included heart rate and heart rate variability monitoring as part of a small study to determine the efficacy of sucrose as an analgesic. The small numbers of infants recruited to the studies has limited the extent to which it has been possible to realise these aims. The shortfall in actual numbers, compared with anticipated recruitment predictions is not, however, due only to poor levels of recruitment of infants. Rather, there are a number of contributing factors, most of which relate to change and advancement of clinical practice.

The last 20 years has seen vast changes in the field of neonatology. Infants of lower and lower gestational ages, which would previously not have been considered viable, are now surviving[158;159]. Advances in intensive care methods and in particular ventilation strategies have undoubtedly played a great part in this change. With greater numbers of immature babies spending time receiving ventilatory support, and successfully being discharged home following intensive care, research extended from

exploration of measures to improve survival, to include investigation of the effects of interventions that have now become commonplace. The fact that infants on ventilators almost certainly experience discomfort, pain or distress has been a source of anxiety and a spur for researchers. Anand, a number of years ago, conceived the idea for the N.E.O.P.A.I.N. Multicentre Trial. It was designed to address this concern by investigating the use of morphine in preterm ventilated newborn infants and its effect on neurological outcome. Feasibility for the definitive study was established with the NOPAIN Trial, a pilot study, and the results of this were published in 1999[160]. During the time at which the pilot study was in progress, infants of less than 29 weeks gestation in Edinburgh were routinely intubated at delivery and frequently remained ventilated for a number of days or weeks. Recruitment of such infants in the first hours of life was relatively unproblematic and Edinburgh's contribution to the study, in terms of numbers, was substantial.

During the time since the pilot study and the definitive study, some aspects of both obstetric and neonatal care have changed almost beyond recognition. These changes have had significant implications not only for practice, but also for research in extremely premature infants. Two such advances have been the more frequent use of antenatal steroids in anticipated preterm delivery[161] and the use of early surfactant therapy[162] which, in Edinburgh is now often administered in the delivery room and if possible, before the first mechanical breath. Both of these have contributed to a decline in the severity of lung disease in these high-risk neonates. Increasing confidence in the use of nasal continuous positive airways pressure as alternative respiratory support has meant that infants remain on ventilators for shorter periods of

time and frequently for less than 24 hours[163;164]. In some cases, babies who may have been ventilated for a significant length of time in the relatively recent past, now escape ventilation completely[165;166].

Whilst this sea change is remarkable and can only be applauded as a success story for perinatal medicine, it has significant implications for the study of conditions and treatments in the smaller group of infants now requiring full intensive care. The number of infants available for recruitment to major studies such as the N.E.O.P.A.I.N. Trial has been considerably reduced. The implications for smaller, single centre research initiatives are even greater. This study has been adversely affected by this phenomenon, with initial recruitment of babies being limited and early withdrawal of infants from the study being common due to rapid improvement that might not previously have been anticipated.

Another possible contributing factor to the low numbers of infants recruited for both parts of this work is the number of parents declining participation on behalf of their infants. In the United Kingdom, media coverage of recent high profile "scandals" has done much to weaken confidence in the medical profession and may have led to reduced willingness to enter into research trials[167;168]. However, this probably did not make a significant difference in this case. The percentage of parents consenting to involvement was high overall with recruitment of more than 70% for the Edinburgh component of the N.E.O.P.A.I.N. study and around 90% for the study of sucrose analgesia.

The results and conclusions of the study must, therefore be interpreted with caution. Studies including small numbers and incomplete data are well recognised both to present difficulties in choice of statistical methods of analysis and to increase greatly the chance of error when testing the significance of findings. Type I errors occur when too low a level of significance is accepted to make an assertion that a difference between populations exists. Throughout this work and according to convention a p-value of 5% is accepted as statistically significant. Type II errors occur when the sample size is too small to detect a difference that may in fact be present and it is likely that this may have exerted an effect on the results. Power calculations were not performed for any of the outcomes discussed in these studies. Indeed, this would have been impossible given the nature of the work. This unfortunately means that no conclusions can be drawn from any of the study results. There are, however, some findings that reach or show a trend towards statistical significance and these may warrant further investigation.

Decrease in heart rate and heart rate variability with morphine therapy indicates that both may be useful measures of relief of pain or stress. Little is known about the development of tolerance in the preterm neonate, but the disappearance of effects seen with morphine suggests that this may be a factor. The study sought to identify possible indicators of opiate withdrawal. The rebound tachycardia and increase in heart rate variability seen in the morphine treated babies is worthy of further research. It is possible that behavioural factors may provide additional evidence if they are also considered in any further studies. There are interesting differences in the responses of the infants according to gestational age. Numbers in the study

prevent clarification of this, but one can speculate that this may reflect a dose effect, since the doses in the groups were different depending on gestational age. This possibility could be ruled out if morphine levels for all infants were available. Further exploration of the effects of morphine should include this, or standardise the dosing regimen across the gestational ages to avoid confounding.

The study of the cutaneous flexor withdrawal reflex, disappointingly, failed to identify any significant differences in threshold between the groups other than during the period of weaning. In further investigation of the phenomenon of opiate withdrawal, this may prove a useful tool. However, modern electronic devices would be more accurate than the nylon hairs used in this research.

The final study, looking at the effects of sucrose analgesia suffered from the difficulties of small numbers and consequent on this, significant differences between the groups at entry to the study. No significant effects were found on the basis of this work, and in spite of the recognised difficulties in this particular study, it is likely that heart rate and heart rate variability are not the most effective way of assessing the response to an intense stimulus such as eye examination. It is likely that the effects of eye examination on cardiovascular parameters are significantly influenced by factors other than pain. For, example, it is known that indentation of the eye, which may, but does not necessarily occur during such an examination can have a profound vagal effect and induce bradycardia. It is impossible to know how much this may have affected the results of this investigation. Further investigation of the

effects of pain relief in eye examination should probably be directed towards behavioural responses.

Although there are significant limitations associated with this work, it is the only work to date specifically investigating the effects of analgesia on heart rate and heart rate variability in preterm neonates. As such, it contributes to the body of knowledge in the study of pain in the newborn. A number of hypotheses have been generated which suggest that further research is warranted, particularly on the subjects of opiate withdrawal and appropriate morphine dosing according to gestational age. The extent to which future research concerning morphine therapy may be carried out will depend to a certain extent on the results of the N.E.O.P.A.I.N. trial yet to be published. If morphine therapy is shown either to afford unquestionable benefit or harm in this population, then it is unlikely that such research will continue to be regarded as ethical. The importance of taking any opportunities as they rise to conduct even small studies to learn about conditions and treatment is evident, whilst recognising the obvious limitations of this approach. However, any future research in this field should, if possible, be in the form of large randomised controlled trials to avoid the problems associated with small study sample sizes.

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