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DEPARTMENT OF WOMAN AND CHILD HEALTH
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FACTORS MODULATING NEONATAL PAIN RESPONSIVENESS

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Cover of René Descartes, L'homme de Rene Descartes Paris. Acute physiological pain from burning injury, by Charles Angot (1664).
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To my family Göran and Ida

Lyssna, alla barn har ett budskap som vi kan lära av

Every child has a message we can learn from

By my supervisors

ABSTRACT

To relieve pain in newborn infants particularly preterm infants is essential in modern neonatology. However, there are a number of innate difficulties related to pain assessment and pharmacological treatment, which includes in this thesis work. It emanated from our participation in an international team that designed and conducted the NEOPAIN multicenter trials, testing the effects of morphine analgesia in 898 preterm mechanically ventilated neonates. Primary neurological outcomes were defined as death, severe IVH and PVL that were not affected, respectively. However, intermittent doses with morphine increased the incidence of composite outcome 24% vs. 15 %. The pre-emptive morphine infusion did not improve short-term pulmonary outcomes; it extended the mechanical ventilation time with one day. Additional morphine doses aggravated the respiratory outcomes in preterm neonates with RDS and severity of illness. Because these neonates were "sicker" and had greater physiological instability, they required more frequent procedures and pain assessments by caregivers, consequently an increased use of additional doses of morphine analgesia. However, the pre-emptive morphine infusions reduced pain responsiveness, measured by changes in heart rate (HR) $p<0.005$, blood pressure $p<0.001$, and decreased PIPP scores ($p<0.02$), as compared with placebo.

The ability of professional NICU staffs to correctly estimate analgesia treatment is limited. Neither education (63% to 54%, $p=0.60$) nor experiences (65% to 55%, $p=0.28$) in the NICU affected their ability to assess whether the neonates had received analgesia or not, highlighting the difficulties in assessment of prolonged neonatal pain.

Term newborn infants delivered vaginally (VD) reacted with less responsiveness (facial expression 36.6%, cry 21.6%, and HR 4.2 %, $p=0.04$-0.001) after injection of vitamin K as compared to the infants delivered by caesarean section (CS). VD infants showed increased physiological reactivity to graded painful stimuli in the first hours following birth ($p<0.001$ and $p=0.04$, for high- and low-intensity stimuli, respectively) but no such increase occurred in the CS newborn infants ($p=0.96$ and $p=0.52$). These findings suggest that vaginal delivery leads to a foetal inhibition of pain and thermal sensory processing soon after birth.

Both painful and tactile stimuli resulted in hemodynamic responses in the parietal (somatosensory) cortex, monitored by NIRS in preterm neonates, but did not occur in the occipital (visual) cortex. This sensory perception was accentuated responses in preterm neonates with male sex ($p<0.05$), lower gestational ages ($p<0.05$), or greater postnatal ages ($p<0.001$). These nuanced responses of preterm infants at the cortical level indicate conscious perception of pain.

LIST OF PUBLICATIONS

This thesis is based on the following five papers. The papers will in the text be referred to by their Roman numerals (I-V).

I. *Anand KJS, Hall RW, Desai N, Shephard B, Bergqvist LL, Young TE, Boyle EM, Carbajal R, Bhutani VK, More MB, Kronsberg SS, Barton BA, for the NEOPAIN Trial Investigators Group.*
Effects of morphine analgesia in ventilated preterm neonates: primary outcomes from the NEOPAIN randomised trial.
Lancet, 2004, 363: 1673-1682.

II. *Bhandari V, Bergqvist LL, Kronsberg SS, Barton BA, Anand KJ.*
Morphine administration and short-term pulmonary outcomes among ventilated preterm infants.
Pediatrics 2005, 116: 352-359.

III. *Bartocci M, Bergqvist LL, Lagercrantz H, Anand KJ.*
Pain activates cortical areas in the preterm newborn brain.
Pain 2006, 122: 109-117.

IV. *Bergqvist L, Eriksson M, Kronsberg S, Barton B and Anand K.*
Seeing through the blind! Ability of hospital staff to differentiate morphine from placebo, in neonates at a placebo controlled trial.
Acta Pædiatrica 2007, 96: 1004-1007.

V. *Bergqvist LL, Anand KJS, Katz-Salamon M, Hertegård S and Lagercrantz H.* Mode of delivery modulates physiological and behavioural responses to neonatal pain.
Journal of Perinatology, 2008, Sep 4, Epub.

CONTENTS

1 LIST OF ABBREVIATIONS

$paCO_2$	Partial pressure of arterial carbon dioxide (kPa)
ACC	Anterior Cingulate Cortex
AGA	Appropriate weight for Gestation Age
Apgar score	After Virgina Apgar also explained by **A**ppearance (skin colour), **P**ulse (heart rate), **G**rimace (reflex, irritability), **A**ctivity (muscle tone), and **R**espiration
APIB	Assessment of Preterm Infant´s Behaviour
BIIP	Behavioral Indicators of Infant Pain
bpm	Beats per minute (heart)
BW	Birth weight
CBF	Cerebral blood flow
CHEOPS	Children's Hospital of Eastern Ontario Pain Scale
CNS	Central Nervous System
CRIB	Clinical risk index for babies
CRIES	Crying, Requires Oxygen, Increased VS, Expression, Sleepless (pain scale)
CS	Caesarean section
DPF	Differential path-length factor
DRG	Dorsal root ganglion
DSVNI	Distress Scale for Ventilated Newborn Infants
ECG	Electro cardiogram
EMEA	European Medicines Agency
GA	Gestational age, weeks
HbH	Deoxy-haemoglobin
HbO_2	Oxy-haemoglobin
Hbtot	Total haemoglobin
HR	Heart rate
HRV	Heart Rate Variability
M3G	Morphine 3-Glucuronide
M6G	Morphine 6-Glucuronide
N_2O	Nitrous oxide
NEC	Necrotizing entercolitis
NEOPAIN	Neurologic Outcomes and Pre-emptive Analgesia in Neonates
NFCS	Neonatal Facial Coding System (pain scale)
NGF	Nerve growth factor
NICU	Neonatal Intensive Care Unit
NIPS	Neonatal Infant Pain Scale
NIRS	Near infrared spectroscopy
NMI	Neonatal medical index
OSBD	Observational Scale for Behavioural Distress
PAT	Pain Assessment Tool
PCA	Postconceptional age, weeks

PD	Pharmacodynamic
PNA	Post Natal Age, weeks
PICU	Paediatric Intensive Care Unit
PIPP	Preterm Infant Pain Profile
PK	Pharmacokinetic
PRBS	Procedural Behavioural Rating Scale
PUMA	Paediatric Use marketing Authorisations
ROP	Retinopathy of Prematurity
SaO_2	Arterial oxygen saturation
SUN	Scale for Use in Newborns (pain scale)
UGT-2B7	Uridine 5'-diphosphate Glucuronosyl Transferase-2B7
VAS	Visual analogue scale
VD	Vaginal delivery
VLBW	Very Low Birth Weight
VS	Vital Signs, including heart rate, blood pressure

2 INTRODUCTION

The capability of the newborn infant to feel and remember pain has been underestimated for a very long time, leading to unnecessary pain experiences in this population. Infants and neonates are even more sensitive to pain due to immaturity of endogenous modulation. Furthermore the newborn infants' limited ability to communicate their pain; they should have the same human right to be alleviated from pain.

The infants brain particularly the preterm brain undergoes an enormous development with regard to the branching of neurons, synpatogenesis, wiring and myelination, see Levene et al. 2001, Lagercrantz et al. 2002. This development is not only genetically programmed but also influenced by input from the sensory organs and spontaneous activity (Fransson et al. 2007). Pain and particularly chronic pain may mainly affect the making of the brain and cause permanent injuries (Anand et al. 1999b; Anand 2000a; Fitzgerald 2005; Anand et al. 2007).

The importance of understanding the development of pain mechanisms is further corroborated by the increased survival of extremely preterm infants, who may sustain multiple painful and distressing procedures during early life. In Sweden more than 50 % of infants born during the 23rd- 24th gestational week survive nowadays (Finnström 1998; Serenius 2004).

The overall objectives of this thesis were to estimate pain by objective methods in preterm and newborn infants. More specifically:

- To assess the short-term neurological and pulmonary effects of pre-emptive analgesia with morphine to preterm infants.
- To record cortical activity in infants sustaining painful procedures.
- To assess the sensitivity to pain stimuli (vitamin K injection) in infants delivered vaginally versus those born after caesarean section.
- To examine the ability of the neonatal staff to estimate the effect of analgesia treatment.

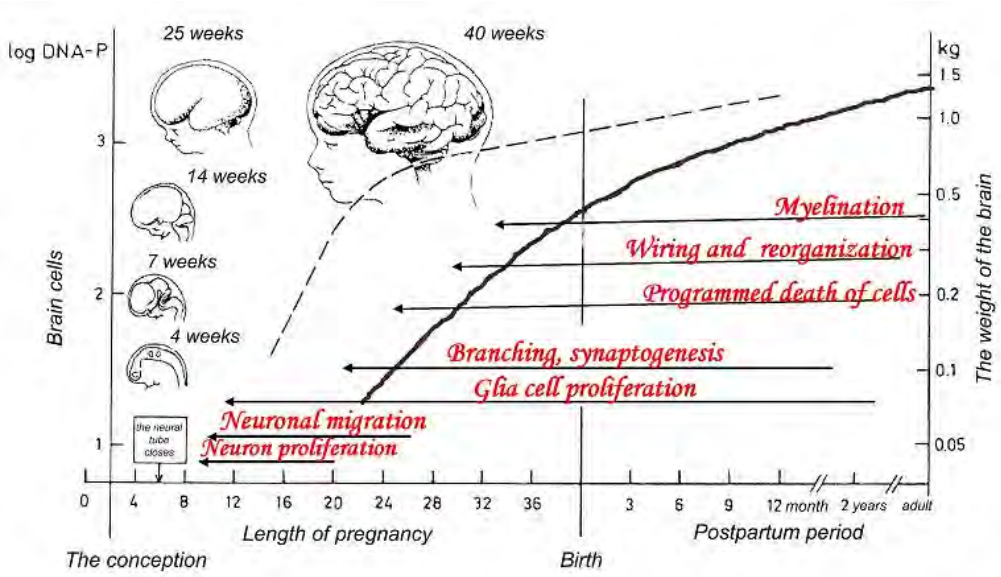


Figure 1. Brain development, *by courtesy of Hugo Lagercrantz.*

3 BACKGROUND

3.1 DEFINITION OF PAIN

The International Association of the Study of Pain (IASP) defines that pain is "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of damage". According to the IASP "pain is always subjective. Each individual learns the application of the word through experiences related to injury in early life" (International Association for the Study of Pain 1979; Merskey 1991).

However, this definition of pain by the IASP does not apply humans incapable of self-reporting newborn and older infants. Consequently, the "relationships between *feeling pain* and *reporting pain* are highly context-dependent. They reflect who is eliciting the self-report, the methods used to assess pain" (Anand and Craig 1996). The new perspective of the pain definition, as suggested by Anand and Craig, has been of immense importance for the development of structured pain assessment tools to evaluate individual pain in infants with reduced capabilities to communicate their experience. Furthermore, the IASP statement that pain is learned through experiences in early life requires additional definitions within the newborn population. Anand and Craig suggest that the ability to experience pain is innate and appears early in ontogeny with individual development to serve as a signalling system in case of tissue damage. Their theory is based on empirical evidence that purports that both behavioural and physiological responses are valid pain indicators. Consequently, Anand and Craig propose "behavioural alterations caused by pain are the infantile forms of self report and are not, surrogate measures, of pain". They also stated that "infants, children and the cognitively impaired do not have to know, or be able to express the meaning of an experience to have the experience of pain" (Anand and Craig 1996). The newborn infant and even foetuses respond with programmed withdrawal reflex, grimaces and crying as will be discussed in this thesis.

In the early 2000s, AD Craig described pain as a homeostatic emotion, like itch, visceral distension, muscle ache, hunger, thirst, "air hunger" and sensual touch. According to AD Craig, pain can either be unpleasant (commonly), or pleasant (as when it relieves an intense itch) or a mixture of such sensations. The human feeling of pain is both a distinct sensation and a motivation, a specific emotion that reflects the behavioural drive located in different areas of the anterior insular cortex and anterior cingulate cortex (ACC) (Craig 2003). Emotional responses to pain may be quantitatively or qualitatively different in young children as compared to older subjects.

The United Nations Convention on the Rights of the Child recognizes that children belong to a vulnerable population and that they are entitled to special consideration in all respects, including healthcare. Despite this recognition, inadequate prevention and relief of children's pain is still widespread. This failure reflects shortcomings in recognizing children's ability to perceive, respond to, and be harmed by pain; exaggerated fear of the side effects of analgesics and anaesthetics in children; and lack of resources to provide training for clinicians and treatments for children (Levene and Quinn 1992; Harrop 2007).

The ability of the newborn infant to feel and remember pain has been underestimated and under treated for a very long time, leading to unnecessary pain and discomfort experiences in thousands of neonates. Due to the newborn infants limited ability to communicate pain, several myths and misconceptions regarding neonatal pain sensitivity have been prevalent (Finnström and Schollin

1998). Consequently, it has been generally accepted that infants tolerate pain and discomfort better than adults, and that infants do not experience pain as adults since their nervous system is immature. The overall awareness that newborns do feel pain and that pain can elicit long-term adverse effects is now increasing. Still, many newborn infants undergo repeated painful procedures without adequate analgesia (Carbajal et al. 2008; Finnström et al. 1998). There is also a myth that newborns do not have a memory of pain, which has contributed to undertreatment pain and negative long lasting effects from early painful stimuli. The clinical and experimental pain research over the last 20 years has discredited these myths and incorrect opinions, but they still do exist (Levene 2005). Repetitive mechanical stimulation shows in animal studies with a risk to develop hypersensitivity (Eriksson et al. 2005).

There are strong indications that chronic pain may affect the wiring of the neuronal networks possibly leading to attention deficit disorders. Whit diffusion tensor imaging showed that white matter microstructure of ex-preterm infants lesions in the brain persist at long term follow up at 11 year. The preterm infant had a lower fractional anisotropy values in the posterior corpus callosum and bilaterally in the internal capsules (Nagy et al. 2003). In the newborn infants, functional magnetic imaging (fMRI) was used to study the resting-state networks driven by spontaneous signal fluctuations (Fransson et al. 2007). How does early pain affect these spontaneous activities with cerebral pain processing following NICU care?

Thus it is obvious as stated by IASP, 2007 "The inability to communicate verbally does not negate the possibility that an individual is experiencing pain and is in need of appropriate pain-relieving treatment." – Certainly a welcome change for clinicians and researchers dealing with infant pain! (IASP ; Johnston et al. 1996) 2007.

The neonatal pain problem is significant, not least in the preterm population. In Sweden the annual birth rates is around 100,000 infants per year, see table 1 for definitions of pregnancy duration. Almost 1000 infants (1%) are born very preterm in Sweden (Lindam 2008). These infants usually need hospital care for 2-4 months, and are at high risk for being exposed to repeated painful procedures as part of the medical care. Particularly infants with birth weight below 1000 grams survive more often now (Finnström et al. 1998; Serenius et al. 2004; Högberg et al. 2006).

Table 1. Data from the Swedish Medical Birth Register 2006.

DEFINITION	**GESTATIONAL AGE (WEEKS)**	**NUMBER OF BIRTHS (N)**	**ALL BIRTH (%)**
Full term	≥ 37+0	97 965	93.9%
Preterm	32+0 – 36+6	5 433	5.2 %
Very preterm	28+0 – 31+6	662	0.6 %
Extremely preterm	< 28 (live born)	308	0.3 %

3.2 HISTORICAL PERSPECTIVE ON NEONATAL PAIN

Opium, produced from the immature seed pods of opium poppies (*Papaver somniferum*) has been used for pain relief for thousands of years. The Egyptian Ebers Papyrus, ca 1500 BC., describes a way to "prevent the excessive crying of children" using grains of the poppy-plant strained to a pulp. In these early times, humans believed pain was related to evil and magic, hence the responsibility of providing relief was left to sorcerers and priests. Also the Greece Hippocrates, (460 BC-370 BC), noted that the gingiva surfaces in teething infants was highly sensitive to pain and concluded at this time, that pain was tolerated less in infants than adults (Chadwick 1951). Cornelius Celsus (1st century A.D.), described the use of opium and alcohol as a common anaesthetics drugs during surgical operations and pain relief in young children, at that time, mainly to avoid stomach problems (Spencer 1994). The Greek physician Galen (130-200 A.D.), was experienced in trauma surgery and advised the use of alcohol to relieve pain. He described the crying in the newborn infant as associated with pain and discomfort (Still 1931). Nils Rosén von Rosenstein (1706-1773) is considered to be the father of modern paediatrics. In his book "The diseases of children, and their remedies" (1764, translated to English in 1776) he described various drugs to alleviate pain in children.

René Descartes, the French mathematician, scientist and philosopher who died in Stockholm 1650, presented some of the earliest theories of pain perception. He described acute physiological pain, such as burning injury, as a bell ringing in the brain, a view that has been completely discarded by modern science (Harrop 2007), see the cover to this thesis.

Morphine was first synthesized from opium in 1806 by Sertürner (Brownstein 1993), a German pharmacist, who named the drug *morphium* after Morpheus, the Greek god of dreams. Morphine came into more general use half a century later (Brownstein 1993). In 1848, Henry Bigelow at the American Medical Association stated that pain treatment was completely unnecessary in newborn infants, since infants did not remember their suffering. Pain was thus associated with memory, intelligence and rationality, following the discovery of anaesthesia. At this time the status of newborn infants was placed at the level of primates (Pernick 1985).

In 1872, Darwin described in his book "The Expression of the Emotions in Man and Animals", that the emotions necessary for survival, they appear early, and pain is the first part of the sensory system that is affected by evolution. For example, the signalling of hunger and tissue damage is obviously the most necessary emotions for survival of the newly born, and therefore will appear earlier than any other adapted behaviours during development (Darwin 1872).

Until recently, it was questioned whether newborns can perceive pain and whether specific pain receptors and pathways were sufficiently developed at birth to result in a response to pain. The "Liverpool technique" was introduced in the end of the1950s, for newborn infants, which used high doses of muscular relaxants with low doses of nitrous oxide (N2O), i.e. none or very low-dose of analgesia was given to the surgical paediatric patients. In Smith's "Textbook of Anaesthesia" from 1963 the cerebral cortex of infants was regarded as underdeveloped. Cortical activity was equivalent to a deeply anesthetized patient lacking brain activities, and consequently the newborns were incapable of feeling pain.

The medical literature before the 1970s is essentially lacking any formal reviews or research regarding the management of pain in children. A report from 1968 describes that only two out of 60 postoperative patients received any analgesia (Swafford 1968).

During the late 1980´s, a series of studies demonstrated that preterm infants undergoing surgery with minimal anaesthesia, which was at that time a standard practice, exhibited significant negative responses to surgery which affected both morbidity and mortality (Anand and Hickey 1987; Anand et al. 1987; Anand and Aynsley-Green 1988; Anand et al. 1990). These studies were "eye openers", leading to a public debate initiated by parents', which culminated in a series of editorials in medical journals during the late 1980s. The impact of this research was almost immediately evident and a significant progress in postoperative pain treatment from 1974 to 1991, was demonstrated, with an increase from 24 to 968 administered doses of analgesia in one institution (Asprey 1994).

At the same time Fitzgerald et al. described pain transmission in the neonate and also identified serious negative consequences from undertreatment of neonatal pain. These studies had a dramatic impact on the neonatal intensive care units (NICU), with changed practices towards increasing pain treatment (Fitzgerald et al. 1989; Reynolds and Fitzgerald 1995).

3.3 PHYSIOLOGY OF PAIN

The nature and purpose of pain is to act as a protective mechanism for the body. The pain system consists of receptors that transmit pain sensation to peripheral nerves which end in the central nervous system. The developing nervous system in the newborn infants is characterized by functional and structural plasticity, which is especially sensitive to the centre of sensory and pain processing (Narsinghani and Anand 2000; Evans et al. 2001; Levene 2001; Lagercrantz 2002).

Pain itself stimulates a catabolic state that will increase tissue injury, delay and prevent healing, and may also lead to increased morbidity and mortality in critically ill newborns.

Infants have lower pain thresholds, their responses are less synchronized, and they have longer lasting spinal reflex muscle contractions as response to mechanical skin stimulation than adults (Fitzgerald et al. 1988; Andrews and Fitzgerald 1994). The cutaneous reflex responses may result in pain responses involving movements from the whole body (e.g. wriggling, rolling), and simultaneous responses from other extremities, although as the infants mature the responses become more restricted to defined parts of the body.

The ill or very preterm newborn may become exposed a large numbers of painful and distressing procedures as part of medical diagnostic and therapeutic interventions. Typical interventions are venipuncture, heel lancing, lumbar puncture and vaccination. Such skin breaking procedures trigger immediate pain responses, as a strong warning of bodily injury, which may also be observed by caregivers. It is important to remember that the pain response always occurs but that it may not at all times be recognized by the observer (Barker and Rutter 1995a; Carbajal et al. 2008).

Experimental data suggest a window of increased sensitivity for permanent changes in the immature nervous system as response to nociceptive stimuli in the newborn period (Anand et al. 1999c).

Improved knowledge on development of neurobiological pathways and mechanisms associated with pain will increase our possibilities to understand and develop methods for assessing and measuring pain in the newborn, as well as extend our methods for adequate pain control in this population.

Pain is a multi-layered phenomenon, across all age groups and all animal species that have been studied so far. There are different types of pain:

- Physiological pain resulting from tissue injury,
- Inflammatory pain caused by inflammation, cytokine release, or changes in tissue pH,
- Neuropathic pain secondary to nerve damage or neuroinflammation, or
- Visceral pain originating from hollow and solid viscera.

Each of these types of pain appears to have unique receptors and mechanisms, although as knock-out mice experiments show, there is considerable overlap and redundancy within the pain processing system. Nevertheless, some types of pain have specialized nociceptors, nerve fibres, neuronal and glial processing in spinal and supraspinal areas. Some to these mechanisms are responsible for phenomena like primary and secondary hyperalgesia, allodynia, temporal or spatial summation, sympathetically maintained pain, referred pain, or pain of central origin (Levene 2002).

Peripheral Pain Mechanisms

Peripheral sensory input to the nervous system is provided by receptors that react to exogenous sensory stimuli such as touch, pain, cold, heat, light, sound, smell and olfaction. These receptors are highly sensitive to one type of stimulus.

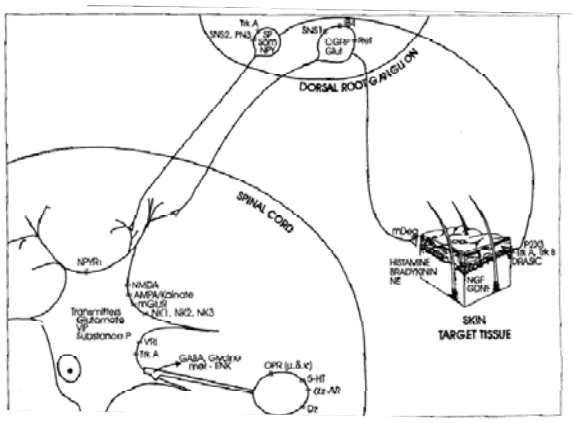


Figure 2. Connections between the dorsal horn of the spinal cord, dorsal root ganglia (DRG) and target tissue, showing expression of neurotransmitters and receptors, *from Narsinghani, Anand, et al. Lab Animal 2000*.

Nociceptors

The nociceptors adapt poorly to painful stimuli, instead the fibres become progressively thicker and more sensitive as the stimuli continues. This increased pain sensitivity resulting from activation of nociceptors is called, hyperalgesia.

Very little is known about the developmental regulation of nociceptors. Sensory nerve fibres in nociceptors and low-threshold mechanoreceptor endings grow out from the dorsal root ganglion prenatally and innervate the skin, by birth in the rat and during the 2nd trimester in the human they have reached the distal skin of the foot (Payne et al. 1991; Reynolds et al. 1991). The larger A-fibres

develops first, the nerve fibres reach the skin and form the initial cutaneous nerve plexus before the C-fibres (Jackman and Fitzgerald 2000).

Nerve growth factor (NGF) is produced in the skin and plays an important role in the normal receptor development. NGF also plays a major role later in life, since peripheral release of NGF following injury lead to enhanced pain sensation, but also to changes in receptor functions and hyperinnervation resulting in pain or hypersensitivity at the site of injury long time after the original wound has healed (Arthur 1991).

Inflammation

An inflammatory response following tissue damage, may persist for hours and days, is associated with increased pain sensitivity (hyperalgesia) around the injured area, see figure 3. Ruda et al have developed an animal model based on persistent hind paw peripheral inflammation (lasting 5-7 days with symptom). The study included behavioural and physiological assessment in addition to evaluation of structural changes in the spinal cord. The study demonstrated that inflammatory pain during the neonatal period permanently alters pain processing in the spinal cord (Ruda et al. 2000). Inflammation increase the sensation in the skin around the injury, and touch will then cause pain in this area, expressed as Allodynia.

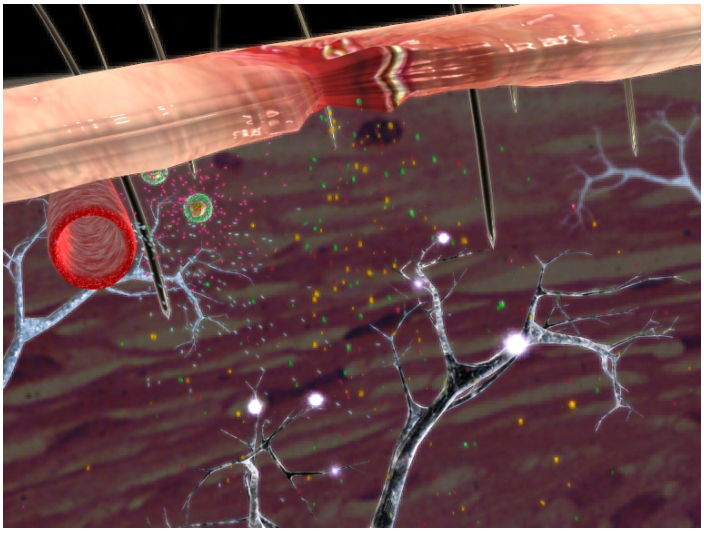
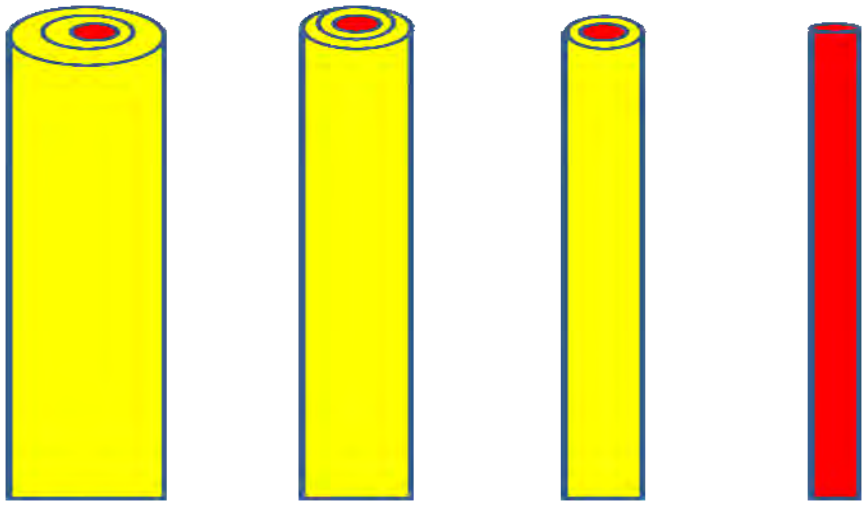


Figure 3. Inflammation in the skin activates nociceptors, *from the website* *http://www.georgiapainphysicians.com/downloads/m2_slides/2.Pain%20inflammatory%20mediators.jpg*

Afferent nerve fibres

Painful stimuli are transmitted to the central nervous system through two types of fibres. Myelinated Aδ-fibres are fast (velocity about 5-25 m/sec), with a diameter 1-3 μm and, while the unmyelinated C-fibres are slow (0.1-2 m/sec), very thin with a diameter 0.1-2 μm (Arthur 1991), see figure 4.



AXON TYPE	Aα	AB	Aδ	C
Diameter (µm)	13-20	6-12	1-3	0.1-2
Speed (m/s)	80-120	35-75	5-25	0.1-2.0
Function	Muscle (spindle and golgi tendon)	Muscle (spindle, hair, vibration, touch)	Deep pressure, **pain**, **cold**	Touch, pressure, tickle, aching **pain**, **cold**, warm

Figure 4. Primary afferent axons, *modified from http://faculty.washington.edu/chudler/axon.gif*

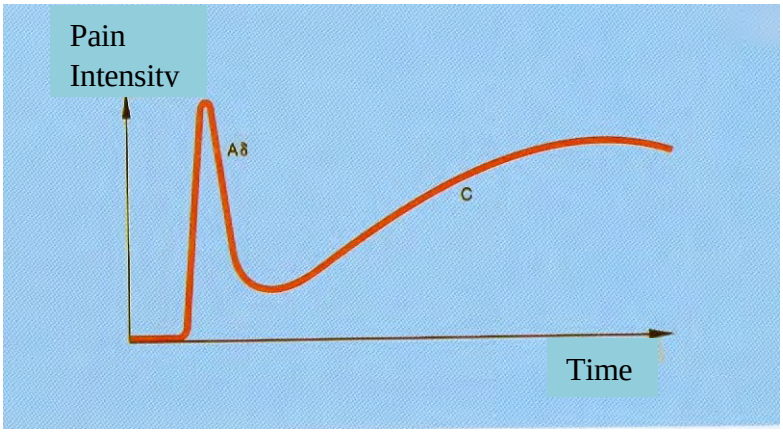


Figure 5. The different conduction velocities of pain fibres result in a "double" pain response. First the fast, sharp and focused pain transmitted through the Aδ-fibres, than the diffuse burning pain resulting from activation of the slower C-fibres (acute and chronic pain). Modified *from Per Hansson et al, Kabi Pharma now Pfizer in the book "Smärta fysiologiska aspekter"*

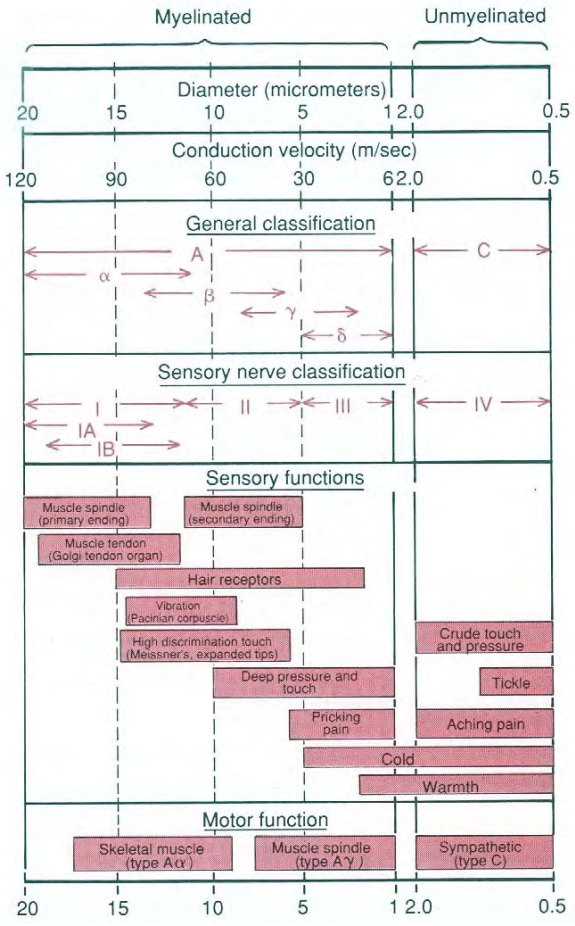


Figure 6. Physiological classifications and functions of nerve fibres, *from Guyton figure 46-6 Textbook of Medical Physiology 1991.*

The high-threshold Aδ-fibres are mechanoreceptors responding to pain, while the low-threshold Aβ mechanoreceptors respond to touch. Both pathways play important roles to initiate an immediate reaction to move away from a noxious stimulus. The low-threshold Aβ mechano-receptors are most immature at birth. The number of sensory afferents responding to a needle prick, with pattern and amplitude of a response, depends on level of neurotrophic factors in the skin (Snider 1994).

Tissue damage in early postnatal period causes a profound and lasting sprouting response at local A- and C-fibres sensory nerve terminals. This results in temporary hyperinnervation that recovers after a few weeks in neonates (Reynolds and Fitzgerald 1995). Description of peripheral mechanisms related to the development of chronic injury-induced pain (Fried et al. 2001).

Neurotransmitters

Inflammation produces substances that directly activate or sensitize nociceptors. This activation results in decreased pain thresholds, which in turn activates nociceptors and increases their impulse activity. Neurotransmitters and signalling molecules are involved in pain pathways that are expressed early in the developing nervous system, during sensitive periods corresponding to the time frames when the preterm neonates experience NICU treatment. That gives the possibility for the newborn infants to respond to pain, with a lack of inhibition and expression result in increased sensitivity with risks for injuries (Lagercrantz 2002).

Central Pain Mechanisms

Spinal cord level

The afferent pain signals to the brain utilise two pathways in the lateral spinothalamic tract a) the neospinothalamic tract and b) the paleospinothalamic tract.

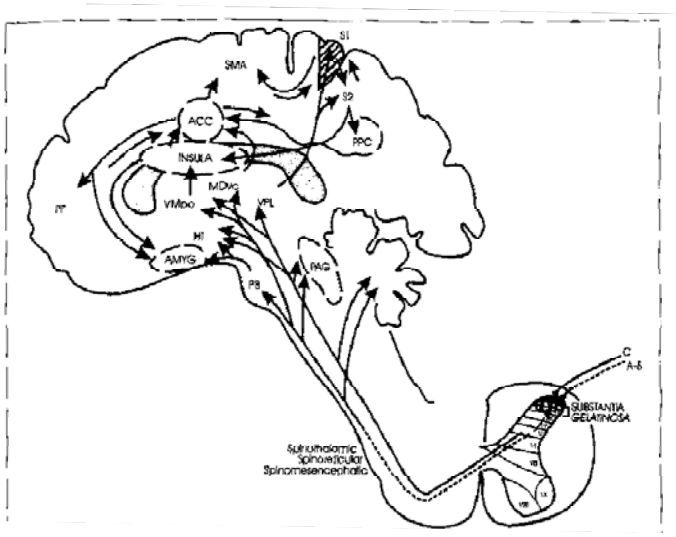


Figure 7. Supraspinal pain pathway and structures involved in pain processing and their spinal connection *from Narsinghani, Anand, et al., Lab Animal 2000.*

The "fast" type Aδ-fibres transmits mechanical and thermal pain from the periphery to lamina I (lamina marginalis) of the dorsal horn. Here the Aδ-fibres synapse with the dendrites of the neurons in the neospinothalamic tract. At the same level, the axon of these neurons cross over to the contralateral side of the cord through the anterior commissure, and then passes up toward the brain in the anterolateral columns (Levene 2002).

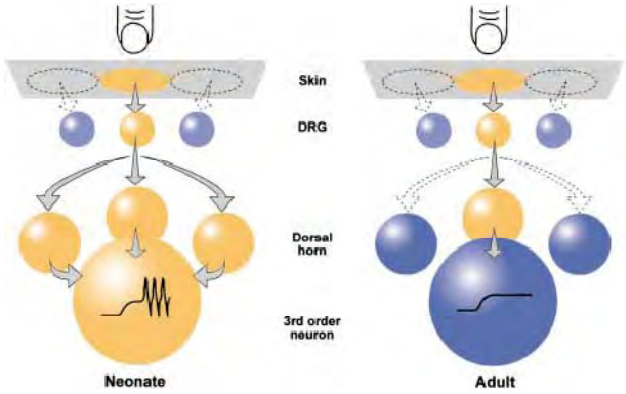


Figure 8. DRG in neonate and adult, *from Maria Fitzgeral, The Neuroscientist 2001.*

The change in total cerebral white-matter proportion was significantly greater than the change in total cerebral gray-matter proportion maturation between childhood and adolescence, suggesting that the relative gray-matter reduction is probably due to significant increases in white matter (Sowell et al. 2002).

The balance of excitation and inhibition differs in the neonatal dorsal horn compared with that in the adult. Although inhibitory transmission is present, it may be less targeted in the neonate than in the adult. This is depicted as a possible absence of specific afferent and descending control of inhibitory interneurons. In the neonate, C-fibre synaptic transmission is weak and the frequency of miniature excitatory postsynaptic currents (mEPSCs) is low (Levene 2002).

The paleospinothalamic tract transmits pain sensation from the peripheral type C fibres and some type Aδ-fibres. The peripheral fibres terminate mainly in laminas II and III of the dorsal horn, in an area called substantia gelatinosa which contains neurons with little myelin. The signal is transmitted to lamina V after interconnection through short interneurons within the dorsal horn. Here, the interneurons synapse with neurons in the paleospinothalamic tract. The axons pass through the anterior commissure to the opposite side of the spinal cord, and then run upwards towards the brain in anterolateral pathways. A few of these fibres do not cross over to the contralateral side and instead passes ipsilaterally to the brain. In the human, the nociceptive nerve tracts are myelinated up to the thalamic level at about 30 weeks (Fitzgerald 2005).

Substance P

A neuropeptide at the synapse in the dorsal horn released and metabolized slowly. Consequently, substance P persists after the painful stimuli, the concentration increasing, which might explain the progressive increase in intensity of slow-chronic pain with time (Lagercrantz 2002; Levene 2002).

The flexor reflex

At 26 gestational weeks, the human foetus/preterm infant has developed a measurable flexion withdrawal reflex to noxious stimuli. The threshold for these reflexes is lower, such that activation does not always require a noxious stimulation as in adults. The reflex muscle contractions are also more synchronized with a longer duration than in more mature subjects. The flexor reflex threshold increases with postconceptional age (PCA). Repeated skin stimulation results in considerable hyperexcitability or central sensitization with generalized whole body movements of all limbs. This phenomenon diminishes after 29-35 weeks PCA Sensitization is demonstrated by the cutaneous flexor reflex threshold, that is significantly reduced followed repeated pain stimuli (Andrews and Fitzgerald 1994; Fitzgerald 1988).

Lower brain level

The medulla, pons, mesencephalon, hypothalamus, thalamus, cerebellum, and basal ganglia are activated by pain at a subconscious level; these regions also control some pain reflexes without engaging the cerebral cortex (Arthur 1991).

A few fibres of the neospinothalamic tract terminate in the reticular areas of the brain stem, but most fibres pass all the way to the thalamus, terminating in the ventrobasal complex with the dorsal column, medial lemniscal tract. A few fibres terminate in the posterior nuclear group of thalamus transmitted to other basal areas and the somatic sensory cortex.

Only 10-25% of the paleospinothalamic tract fibres pass all the way to the thalamus. Instead these fibres terminate in other areas: a) the reticular nuclei of the medulla, pons and mesencephalon; b) the tectal area of the mesencephalon deep to the superior and inferior colliculi; and c) the periaqueductal gray region. These regions are all very important for the dynamic regulation of pain

thresholds in real-time (via spinal-brainstem-spinal loops) as well as the modulation of pain. Activation of the slow, chronic pain fibres in the reticular areas of the brain stem and the intralaminar nuclei of the thalamus is associated with strong arousal effect. This explains why an individual with severe pain has great difficulties to sleep (Levene 2002).

Cortical level

The cerebral cortex plays an important role for interpreting the quality of pain, whereas pain perception is a function of lower brain levels. Consequently, removal of the somatic sensory areas of the cerebral cortex does not destroy the ability to perceive pain. Recent studies suggest that early pain memories are not accessible to conscious recall, but may be associated with abnormal behavioural patterns or altered sensory processing in later life (Porter et al. 1999; Anand 2000b; Ruda et al. 2000).

Thermal Sensation

At least three types of sensory receptors in the skin can discriminate thermal gradation: a) the pain receptors, (nociceptors) are only stimulated at extreme degree of cold and heat, "freezing cold" and "burning hot" sensations; b) cold receptors and, c) warm receptors. There are three to ten times as many cold receptors as warm receptors. The receptors are stimulated by changes metabolic rates which are modified by the temperature described in figure 9. The thermal receptors adapt to a stimulus, i.e. when skin temperature decreases, the sensation is that the individual feels much colder than when skin temperature remains unchanged. The opposite sensation is also present, if the temperature is increasing a person feels much warmer than at constant temperatures (Arthur 1991).

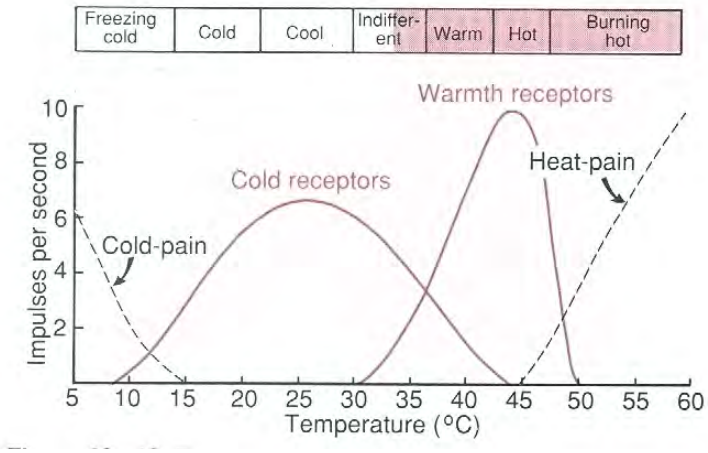


Figure 9. Temperature receptor response, *from Guyton figure 48-10, Textbook of Medical Physiology 1991.*

There are four different nerve fibres that discriminate temperatures at different levels: a) pain fibres stimulated by cold: b) cold fibres c) warm fibres and, d) pain fibres stimulated by heat. The afferent thermal nerve fibres are located in deep layers of the dorsal horn in the spinal cord, parallel to the spinothalamic pathways that transmit pain.

3.4 INHIBITION OF PAIN

The potential of the pain control system varies individually as a result of difference in the brain´s capacity to control the degree of afferent pain signals. The three major components included in pain inhibition are: a) the periaqueductal gray area of the mesencephalon and upper pons surrounding the aqueduct of Sylvius; b) the raphe magnus nucleus, lower pons and upper medulla. From these areas the signals are transmitted down in the dorsolateral columns in the spinal cord to; c) a pain inhibitory complex located in the superficial dorsal horn of the spinal cord. At this last point the analgesia signals can block the pain before it is relayed on to the brain.

Several transmitter substances are involved in the endogenous analgesia system, especially enkephalin and serotonin. The brain opioid system operates through endorphins, dynorphins and enkephalins that act at multiple areas in the brain.

Simons et al discuss the descending inhibitory controls are immature at birth (Simons and Tibboel 2006). During foetal life these pathways develop in an efferent direction from the brainstem, via the dorsalolateral funiculus of the spinal cord, to the dorsal horn in the spinal cord. The collateral branches become functionally effective first at postnatal day 10 in rats (Fitzgerald and Koltzenburg 1986). The lack of central inhibition in the immature subject leads to exaggerated and generalized responses to all sensory inputs, affecting low as well as high threshold stimuli. Specific pain responses may require convergent afferent inputs building up over time to become clinically apparent.

Opioids

There are three subclasses of opioid receptors (μ-, δ- and κ-receptors) within the central nervous system. The endogenous opioid peptides enkephalins, dynorphins and endorphins bind to these receptors. There is sufficient evidence that the endogenous opioid system contribute to functioning analgesic mechanisms in the early postnatal period (Marsh et al. 1997). The distribution of μ-opioid receptors has a high density the first two postnatal weeks in rat the spinal cord (Kar and Quirion 1995; Rahman et al. 1998). The μ-opioid receptors mediate stimulation of neurotensin release in the PAG (Stiller et al. 1997).

3.5 PAIN DEVELOPMENT IN HUMANS

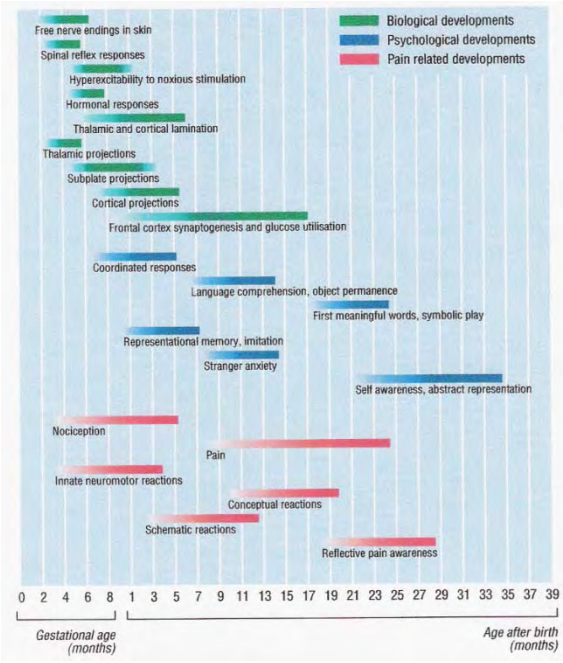


Figure 10. Developmental stage before and after birth, *from Derbyshire, et al. BMJ 2006*.

3.6 PAIN IN FETUS

In uterus, the human foetus responds to pain by pronounced hormonal (Giannakoulopoulos et al. 1994), and hemodynamic (Teixeira et al. 1999; Fisk et al. 2001) changes. When fentanyl analgesia was administered to the foetus during invasive procedures at 20 to 35 gestational weeks, the attenuated stress response to painful stimuli were seen, indicating that these responses were indeed induced by pain and stress (Anand and Maze 2001; Fisk et al. 2001).

3.7 EFFECTS OF PAIN IN NEONATES

Repeated skin stimulation results in hyperexcitability or central sensitization with generalized movements of all limbs. This phenomenon decreases after 29-35 PCA (Andrews and Fitzgerald 1994). A neonatal animal model of persistent peripheral inflammation, exhibited spinal neuronal circuits with increased input and segmental changes in nociceptive primary afferent axons and altered responses to sensory stimulation (Anand 2000b; Ruda et al. 2000).

The negative long term effects of human neonatal pain experience are described in some studies. Altered damped behavioural and autonomic pain scores following blood collection at 32 weeks postconceptional age were associated with greater numbers of experienced invasive procedures. Previous exposure to morphine was associated with "normalized" (i.e. increased) rather than diminished responses (Grunau et al. 2001).

Male neonatal circumcision is associated with a greater pain response with observer VAS and behavioural score to routine vaccination at 4 or 6 months (Taddio et al. 1995).Topical anaesthesia, using EMLA during neonatal circumcision attenuated the accentuated responses to the vaccination occurring 4-6 months later (Taddio et al. 1997).

Preterm infants following routine NICU medical care (i.e., endotracheal suctioning, chest physical therapy, diaper change, or nasogastric feed) were observed using the NIDCAP system. These procedures proceeded changes in heart rate, sleep/waking state, followed with extensor movements as finger splay and leg extension. Facial brow showed a function of the number of invasive procedures in the past 24 hours, might reflect sensitization (Grunau et al. 2000).

Anand et al describe the exposure to repetitive neonatal pain may cause permanent or long-term changes because of the developmental plasticity of the immature brain. That human preterm neonate is subjected to repetitive pain during neonatal intensive care. Neonatal rat pups were stimulated the NICU care with needle prick and tactile stimulation, respectively. Pain thresholds tested with hot-plate test, withdrawal reflex, alcohol preference, air-puff startle, and social discrimination followed by Fos expression in the somatosensory cortex and weight gain. Tactile group had better weight gain. The noxious group had decreased pain latencies indicating pain sensitivity, increased preference for alcohol, increased defensive withdrawal behaviour, prolonged chemosensory memory and decreased Fos expression. These findings suggest that repetitive pain in neonatal rat pups may lead to an altered development of the pain system associated with decreased pain thresholds during development. Increased plasticity of the neonatal brain may allow these and other changes in brain development to increase their vulnerability to stress disorders and anxiety-mediated adult behaviour (Anand et al. 1999c).

Research groups studying neuronal apoptosis in rodent models concluded that aesthetic agents are harmful for the neonatal rat brain although the current consensus is that these data do not apply to the developing human brain and should not alter the clinical use of Ketamine or other anaesthetic agents (Simons and Anand 2006; Anand 2007; Bhutta et al. 2007).

3.8 PHYSIOLOGICAL ASPECTS OF NEONATAL PAIN

Physiological indicators of pain in infants include; a) increased heart rate, respiratory rate and blood pressure, respectively, as well as decreased heart rate and intracranial pressure variability, respectively: b) decreased oxygenation and peripheral blood flow, and increased palmar sweating: c) autonomic changes affecting skin colour, and causing nausea, vomiting, gagging, hiccoughing, perspiration and dilated pupils (Stevens et al. 2000). These signs of pain have been studied in a large numbers of clinical settings (Quinn et al. 1992). However, isolated changes in these indicators can be difficult to interpret as they are also influenced by non-noxious stimuli, disease states, or therapeutic interventions particularly in the ill and preterm infants. Pain responses may also be variable in the immediate post stimulus period as a result of inconsistent and unsustainable activation of the immature sympathetic nervous system.

Physiological indicators alone may not determine the presence or absence of pain or the efficacy of an intervention. In combination with other behavioural indicators of pain it will add important information regarding the infant's response to acute pain.

Heart rate responses
A change in heart rate (HR) is the most frequently reported physiologic indicator of pain. Significant increases in HR were reported as response to tissue damage at circumcision, heel lancing and immunization in both term and preterm infants. Significant changes in HR, mainly decreases, were also reported with use of analgesia and/or comfort measures during painful procedures (Grunau and Craig 1987; Porter et al. 1988; Grunau et al. 1990; Benini et al. 1993; Craig et al. 1993; McIntosh et al. 1993; 1994; Stevens et al. 1994; Taddio et al. 1995; Johnston and Stevens 1996; Taddio et al. 1997). Endotracheal suctioning is one of the most common invasive procedures in ventilated preterm neonates, which may result in transiently increased or decreased HR (Simons et al. 2003). Heart rate variability (HRV) reduction in total HRV and power in the low frequency band in preterm infants occur heel lancing (Lindh et al. 1997).

Oxygen saturation
Painful procedures are associated with transient significant decreases in oxygen saturation (SaO_2), as shown in several studies on newborn infants at different gestational age (Maxwell et al. 1987; Masciello 1990; Benini et al. 1993).

Respiration
A change in respiratory rate is registered in several studies, but the direction of change has not been consistent, in most cases an increase occurs but also a decrease and even apnoea can occur (Craig et al. 1993; McIntosh et al. 1993).

Pain significantly affects respiration and may result in tachypnoea, apnoea and irregular breathing. The direction of the changes in respiratory pattern is not consistent or predictable (Craig et al. 1993; McIntosh et al. 1993).

Cerebral blood flow
There are few studies investigating possible changes in cerebral blood flow (CBF) as response to pain in neonates. However, endotracheal suctioning (a procedure that has been used in several studies of newborns to estimate pain responses) is associated with large fluctuations in cerebral blood flow and oxygenation, as demonstrated by near infrared spectroscopy in preterm infants although the nature of these changes were related to changes in carbon dioxide levels and oxygenation (Shah et al. 1992; Skov et al. 1992).

3.9 BEHAVIOURAL ASPECTS OF NEONATAL PAIN

Charles Darwin described facial expressions and crying responses following pain, and took this as important evidence for of emotional expression in the newborn infant (Darwin 1872). Since then, his observations have been confirmed by a considerable amount of research.

Behavioural indicators of pain include changes in facial expression, crying, body movements, and changes in behavioural state and functions (e.g. sleep and eating pattern). The challenge when evaluating newborn infants is to determine if the behaviour is specific for pain or an expression of discomfort, fear, fatigue, illness, or hunger (Craig et al. 1993).

Facial expression

Facial activity in response to pain is less variable and more consistent in infants than crying or body movements (Johnston and Strada 1986; Rushforth and Levene 1994). The most frequently described responses to painful procedures includes specific facial actions, such as squeezing of the eyes, bulging of eye brows, open lips, and retraction of the upper lip causing a nasolabial furrow. Such facial expressions have been demonstrated in newborn infants at all gestational ages (Craig et al. 1993; Stevens et al. 1994; Taddio et al. 1997) as well as in older infants (Johnston et al. 1993). Facial activity is the most reliable and consistent indicator of pain, related to the unidimensional approaches across situations and populations (Grunau and Craig 1987; Grunau et al. 1990; Stevens and Johnston 1994) and should be considered the golden standard of behavioural responses for pain in infants (Craig 1998).

Vocalization response

Morphology and size of the vocal system differ between the newborn and others ages (Campos Banales et al. 1995). Crying is a unidimensional indicator that signals the infant's distress to care providers. The quality of cry can give information about the infant's state and biological integrity (Lester 1984). Crying includes several domains, e.g. fundamental frequency (pitch), cry duration, latency to cry, and cry intensity. Crying as a response to pain is more commonly present in term infants as compared to preterm infants. In very low birth weight (VLBW) infants, crying occur only about 50% of the time following pain (Michelsson et al. 1983; Porter et al. 1986; Barr 1993).

Acoustic analysis of the infant's vocalization response is also used frequently to assess pain and discomfort, mainly in research projects. Acoustic parameters that have been found to be relevant for estimation of pain are: the latency, the duration and the fundamental frequency (F_0) of the first cry. Other parameters which may indicate a pain response are the chaotic changes in spectrograms from sound recordings (Grunau and Craig 1987; Grunau et al. 1990; Stevens et al. 1994; Michelsson and Michelsson 1999; Runefors et al. 2000; Runefors and Arnbjonsson 2005).

Perceptual evaluation

Clinical routinely used parameters were suggested as the F_0, the pitch that correlates with F_0, sound level (dB), aphonic/intermittent aphonics, instability, and press (Hammarberg and Gauffin 1995; Hammarberg 2000). However, the clinical use of cry analysis is cumbersome, likely to be of limited benefit among preterm neonates, all mechanically ventilated neonates, or neonates with limited energy reserves because of critical illness. Pain cries can be identified by adult listeners as the most urgent and intense; cries from similarly painful procedures are judged to have the same degree of urgency and intensity along 3 dimensions described by the harmonic, temporal, and pitches characteristics of the infant's cry (Porter et al. 1986).

Body movements

General body movements have been found to vary in response to pain, being diminished in preterm neonates in general, relative to full-term newborns (Craig et al. 1993). Some neonates respond without movements. Typical of pain responses are the finger split, leg extension in preterm infants (Grunau et al. 2000).

3.10 PAIN ASSESSMENT IN THE NEWBORN

Pain assessment provides a clinical estimation of pain severity. The terms "pain assessment" and "pain measurement" are frequently used interchangeably. Although, pain assessment is a more comprehensive term than pain measurement, it includes both quality and quantity of pain estimates which can form a basis for clinical decision making. Pain measurement includes quantification and answers the question "how much pain?" The quality of pain, its frequency, duration, periodicity, and other contextual factors are included in pain assessment.

Several factors contribute to difficulties in adequate pain assessment of the newborn infant. These include lack of basic information on the developmental neurobiology of nociception and pharmacokinetics of analgesics in the newborn period, combined with their inability to communicate verbally. That resulted in newborns often being undertreated, as they did not appear to experience pain.

Pain assessment scales

Pain assessment can be performed both with unidimensional or multidimensional scales. Measures with several composite parameters have been developed during a numbers of years for assessing pain in infants. Pain scales often include both physiological responses using monitor recordings or readings, and behavioural responses that are video-recorded or visually observed such as changes in facial expression, body movements and skin colour. Some of the pain assessment scales are developed to estimate procedural, acute pain, while some are profiled at evaluating persistent pain and distress. Today there are numerous mostly inadequately validated pain scales in the literature. The Children's Hospital of Eastern Ontario Pain Scale (CHEOPS) (Beyer et al. 1990) was one of the first developed for assessing postoperative pain in children aged 1-7 years of age (Gaston-Johansson and Asklund-Gustafsson 1985).

An overview of the most commonly used pain assessment scales is given below, and also includes the pain scales (NFCS and PIPP) that were used in the present thesis. Scales evaluating broader concepts of behavioural distress in children such as anxiety, fear and/or depression are excluded since these scales cannot be used for assessing newborn babies. The COMFORT score (Ambuel et al. 1992) was organically developed to measure psychological distress in patients in paediatric intensive care units (PICU) patients, including newborn infants up to 24 months of age, but has been modified to also study pain in neonates. This scale is regarded as too complicated to use because of its multiple scale gradations and non-symmetric design. It includes five levels of responses for eight parameters, such as alertness, calm/agitation, type of respiratory response, movement, muscle tone, facial tension, BP and HR (Blauer and Gerstmann 1998).

The Neonatal Infant Pain Scale (NIPS) (Lawrence et al. 1993) was adapted for preterm and term infants and includes six pain indicators, five with behavioural (facial expression, cry, arms and legs relaxed or tense, and arousal state) and one physiological breathing patterns. This scale is primary used for research purposes, although some hospitals also used it for clinical assessment of postoperative neonatal pain (Taylor et al. 2006).

The Neonatal Facial Coding System (NFCS) is a fine grained, anatomically based measure of the behavioural pain response, which has consistently show high reliability, construct validity in term and preterm infants (Grunau and Craig 1987). The origin NFCS scale was used to describe the responses to acute pain occurring across different sleep/wake states, see figure 11, and included nine facial expressions. Later versions also differentiate acute pain from non-painful events, tissue

damage (painful) from non-tissue damage (stressful) phases of a procedure, therapeutic efficacy of pharmacologic analgesia or sucrose during invasive procedures, and non-pharmacologic interventions to manage pain (Grunau et al. 1990). The NFSC score decreased more following painful venipuncture than heel lancing (Larsson et al. 1998).

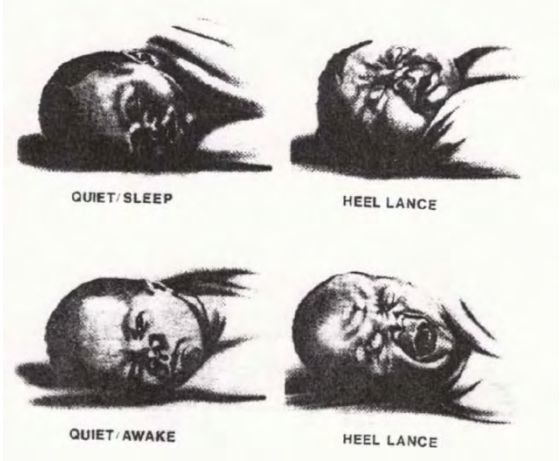


Figure 11. The facial expression in two newborn infants during a heel stick, upper picture shows a sleeping infant with less expression and the lower pictures, an awake infant, (Grunau and Craig 1987) *from Grunau, Craig. Pain 1987*.

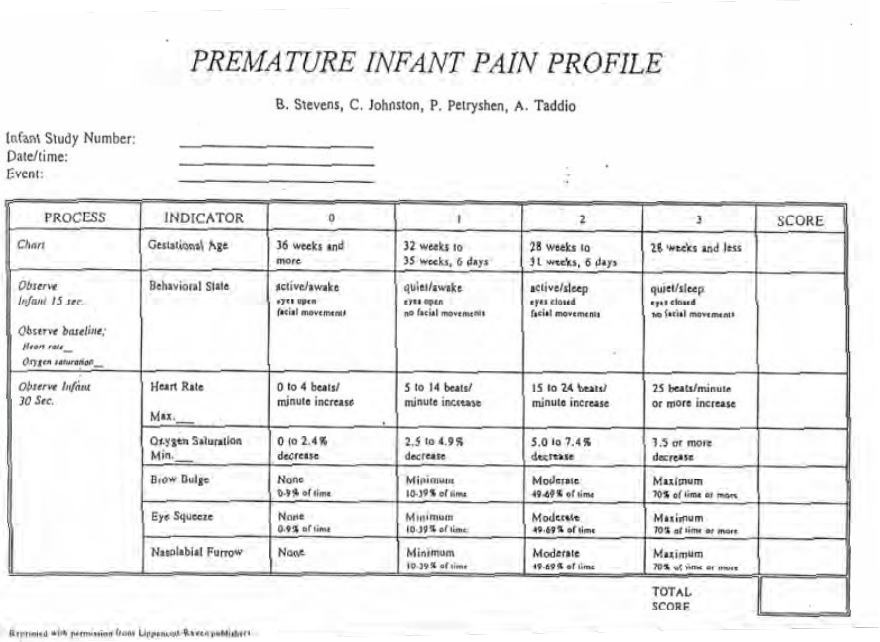
The CRIES pain scale (Krechel and Bildner 1995) is a 10-point scale similar to the APGAR score (Apgar 1953; Apgar and Kreiselmen 1953). This scale was developed for preterm and term neonates, with physiological (requires O_2, HR and BP) and behavioural variables (crying, facial expression and sleepless) previously shown to be associated with neonatal pain following surgery. The CRIES scale refers to Crying, Requires oxygen for saturation above 95, Increased vital signs, Expression, and Sleepless.

The Scale for Use in Newborns (SUN) (Blauer and Gerstmann 1998) was developed via comparison with NIPS and the COMFORT scales for pain assessment during four routine procedures (intubation, intravenous catheter insertion, endotracheal tube suctioning, and diaper changes). The SUN scale was a preferred tool, because it was easy to use, with scale symmetry, and scoring consistency. It includes seven parameters, four physiological (level of consciousness, breathing, heart rate, mean blood pressure) and three behavioural (movement, tone and facial expression).

The Distress Scale for Ventilated Newborn Infants (DSVNI) (Sparshott 1995) was developed to assess behavioural (facial expression, body movement and colour) and physiological responses to painful procedures in the ventilated newborn infant. The scale is based on the Neonatal Behavioral Assessment Scale (NBAS) (Brazelton 1978), the Assessment of Preterm Infant´s behavior (APIB) (Als and Brazelton 1981), the NFCS (Grunau and Craig 1987; Grunau et al. 1990) described above, the IBCS (Craig et al. 1984) and the Gustave-Roussy Child Pain Scale developed by Gauvain-Piquard in 1987. It lacks adequate psychometric validation and has limited clinical utility, because neonatal distress may result from pain or various other causes.

The Neonatal Pain Assessment Tool (Friedrichs et al. 1995), includes both behavioural and physiological parameters with seven items (state, cry, activity, heart rate, blood pressure, respiratory rate and oxygen saturation). The specific characteristic of this scale is the inclusion of descriptive information such as hours since the last pain medication was administered, the type and dose of pharmacological interventions, and the type and frequency of non-pharmacologic interventions.

The Preterm Infant Pain Profile (PIPP) (Stevens et al. 1996) was developed to assess acute procedural pain in preterm and term neonates in both research and clinical practice. The PIPP is a measure that includes both physiological and behavioural indicators, and contextual factors (figure 12). Each indicator is evaluated on a four point scale (0, 1, 2, and 3). Gestational age and behavioural state of the infant are taken into consideration in the scouring such that the total scores range from a total of 21 for infants of lower gestational age (GA) than week 28 and a total score of 18 for infants of higher GA week 36. The PIPP scale is validated for pain assessments of neonates at all gestational ages and is probably the most common clinically used pain scale (Stevens et al. 1999).



PREMATURE INFANT PAIN PROFILE

B. Stevens, C. Johnston, P. Petryshen, A. Taddio

Infant Study Number: _____
Date/time: _____
Event: _____

PROCESS	INDICATOR	0	1	2	3	SCORE
Chart	Gestational Age	36 weeks and more	32 weeks to 35 weeks, 6 days	28 weeks to 31 weeks, 6 days	28 weeks and less	
Observe Infant 15 sec. *Observe baseline:* *Heart rate__* *Oxygen saturation__*	Behavioral State	active/awake eyes open facial movement	quiet/awake eyes open no facial movements	active/sleep eyes closed facial movements	quiet/sleep eyes closed no facial movements	
Observe Infant 30 Sec.	Heart Rate Max.__	0 to 4 beats/ minute increase	5 to 14 beats/ minute increase	15 to 24 beats/ minute increase	25 beats/minute or more increase	
	Oxygen Saturation Min.__	0 to 2.4% decrease	2.5 to 4.9% decrease	5.0 to 7.4% decrease	7.5 or more decrease	
	Brow Bulge	None 0-9% of time	Minimum 10-39% of time	Moderate 40-69% of time	Maximum 70% of time or more	
	Eye Squeeze	None 0-9% of time	Minimum 10-39% of time	Moderate 40-69% of time	Maximum 70% of time or more	
	Nasolabial Furrow	None	Minimum 10-39% of time	Moderate 40-69% of time	Maximum 70% of time or more	
					TOTAL SCORE	

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Figure 12. The PIPP scale, *from Stevens, Johnston, et al. Clin J Pain 1996.*

Slater et al. recently evaluated the PIPP scale using regional changes in cerebral hemodynamics, as measured by Near infra-red spectroscopy (NIRS). They found good correlation between PIPP scores and the level of cortical activity, but facial expression correlated best with cortical activity (correlation coefficient = 0.74; $p < 0.0001$). Cortical pain responses still occurred in some babies with no changes in facial expression and low pain scores (Slater et al. 2008), thus suggesting the limitation of clinical pain assessments

There are few scales for evaluating chronic pain and distress in newborns of different gestational ages. The EDIN scale (Echelle Douleur Inconfort Nouveau-Né, neonatal pain and discomfort scale) is a validated French scale for assessing prolonged pain in premature infants. It includes five behavioural indicators of prolonged pain: facial activity, body movements, quality of sleep, quality of contact with nurses, and consolability (Debillon et al. 2001). Although, acute pain can be measured reliably using a variety of methods, but measurement of chronic pain in newborns is difficult and is currently the focus of much research activity. For example, Boyle et al. found that 4 factors most frequently identified babies receiving placebo analgesia: facial expressions of pain, high activity levels, poor response to handling and poor ventilator synchrony (Boyle et al. 2006b). Karolinska Hospital and Lund University Hospital in Sweden are currently collaborating in a project that is developing, validating and comparing two pain scales for chronic pain, with the newly constructed Astrid Lindgren Children's Hospital Pain Scale (ALPS 1 and 2 for full-term infants and children). Another new pain scale to use in the NICU is the Behavioural Indicators of Infant Pain (BIIP) that combines sleep/wake states, 5 facial actions and two hand actions. In the initial study BIIP shows to be a reliable, valid scale for assessing acute pain in preterm infant. (Holsti et al. 2008)

3.11 TREATMENT OF NEONATAL PAIN

Treatment of pain is a basic human right that exists regardless of age. This was stated by the United Nations Child Declaration of the Rights of the Child (proclaimed by General Assembly resolution 1386(XIV) on 20th November, 1959). This is also supported by the Nordic standards for children and young people in hospitals and the European Association for Children in Hospital, EACH is the umbrella organisation for member associations involved in the welfare of all children before, during or after a hospital stay. Presently, 18 associations from sixteen European countries and from Japan are members. The Nordic EACH organisation is NOBAB, Nordiska föreningen för syke barns behov, an umbrella organisation with local and national representatives has the welfare of the child as the highest priority in Nordic paediatric hospitals and "Barnens bästa i främsta rummet" (SOU 1997:116).

Sweet solutions

The specific mechanisms by which sweet solutions reduce pain perception in neonates is not clear; however the most common explanation is that the sweet solution activates endogenous endorphin pathways (Blass et al. 1987). Elliot Blass and colleagues are the pioneers within this research area.

Sucrose

Sucrose is a disaccharide (fructose and saccharose). The analgesic effects have been extensively investigated, will only be mentioned here in brief . A Cochrane review concluded that sucrose was safe and effective for reducing procedural pain for single painful events in neonates. The review comments that use of repeated doses would need further investigation, advocates combination of sucrose with behavioural and conventional pharmacological measures. Sucrose treatment is relatively contraindicated in unstable preterm infants, or those who have conditions such as necrotizing enter-colitis (NEC). A commercially available 24% sucrose solution (Sweet-Ease®) can be given as drops, or on a pacifier. Sucrose is commonly given during or after a painful procedure to infants, but is not suitable as regular analgesia for ongoing pain (Stevens et al. 2004). There is an ongoing discussion that the fructose might act as hypersensitive allergic activator.

Glucose

In Sweden glucose is more commonly used, often as a 30% solution for oral administration. This solution is available in the pharmacies throughout Sweden. Glucose administrated before a painful stimulus, reduces pain responses, i.e. decreased crying, lower HR increase and lower PIPP after venipuncture in newborn infants (Eriksson et al. 1999a; b). However, when parents were asked to grade their infants pain by VAS assessment after glucose administration, there was low agreement with PIPP score and cry duration (Gradin et al. 2004). No tolerance was observed after repeated doses with glucose 30% administration. Clinically important is that repeated doses of glucose do not decrease the pain-relieving effect (Eriksson and Finnström 2004). When compared to the local analgesia cream EMLA, 30% glucose solution was found more effective in reducing pain responses, as assessed by lower PIPP scores and shorter crying time, but HR responses were similar in both groups (Gradin et al. 2002).

Non-nutritive sucking

Non-nutritive sucking of a pacifier has been described as a method to reduce distress during routine neonatal eye examination for retinopathy of prematurity (ROP). It decreases distress as assessed by an age appropriate, validated pain scale (PIPP), with no additional benefit from dipping the pacifier in sucrose (Boyle et al. 2006a).

Breast milk/breastfeeding

A Cochrane review has examined the contribution made by breastfeeding, or by administering oral breast milk to neonatal procedural analgesia. Neonates in the breastfeeding group had reduced heart rate response and reduced durations of crying, compared to the swaddled or pacifier group. Administration of sucrose had similar effectiveness as breastfeeding for reducing acute pain in neonates (Shah et al. 2006).

NIDCAP and environmental

The Newborn Individualized Developmental Care and Assessment Program (NIDCAP) is based on a detailed behavioural observation of the infant, which is the starting point for individualized care-giving based on the infant's current functioning and developmental goals, which changes neonatal care from a protocol-based to a relationship-based care (Als and Gilkerson 1997). The NIDCAP also includes family support to promote the infants' medical, developmental, and emotional well-being aspects. It has been shown that NIDCAP treatment is associated with better brain organization, as measured by diffusion tensor imaging MRI, and EEG (Als et al, Pediatrics 2004). In ex-preterm infants NIDCAP treatment was associated with a positive behavioural trend at preschool age (Westrup et al. 2004). Preterm infants randomized to a NIDCAP intervention during eye screening examinations for ROP showed similar PIPP scores as infants receiving standard care. However, salivary cortisol returned faster to baseline in the infants subjected to NIDCAP (Kleberg et al, Pediatrics 2008). Venipuncture gives less response to pain then capillary blood sampling, EMLA® did reduce response (Larsson 1998)

Opioid Treatment

Opiates are the most commonly used drugs for moderate and severe pain, but the correlation between the analgesic drug plasma concentration and validated pain scores is poor (Suri et al. 1997; Franck et al. 2000; Carbajal et al. 2005).

Morphine

Morphine activates μ-, κ- and δ-receptors. Both the analgesic action and the respiratory depression that is seen following morphine administration is principally due to μ-receptor effects. Injection of morphine in to the peri-aqueductal grey matter has an analgesic effect, which can be inhibited if the descending inhibitory path is blocked, demonstrating a supraspinal site of morphine action. A spinal role has also been confirmed by microinjection of morphine into the dorsal horn, where it blocks the release of substance P (Yaksh 1997). There are also populations of peripheral opioid receptors, which are known to be up-regulated during acute inflammation, where topical morphine application could be a treatment option (Barr and Rappaport 1999; Nozaki-Taguchi and Yaksh 1999).

In clinical neonatal practice, morphine has been shown to alleviate prolonged pain, reduce behavioural and hormonal stress responses induced by surgery or NICU care and improve ventilator synchrony (Quinn et al. 1992; Anand and Hall 2006). A recent meta-analysis has shown that morphine infusion in newborn infants may increase the number of days on mechanical ventilation, but that the morphine does not increase other clinical outcomes (Bellu et al. 2005).

The role of morphine for treatment of acute procedural pain in newborns is not clear, as term neonates given intravenous morphine had similar pain scores to those that were administered placebo following heel lancing (Carbajal et al. 2005).

In older children, as with adults, there is still some population variability in morphine effects, probably due to pharmacogenetic factors, with μ-receptor single nucleotide polymorphism accounting for some patients requiring higher opioid doses because of decreased potency of morphine and M6G at their receptors (Anderson and Palmer 2006).

Metabolism of morphine

Morphine is mainly metabolised by the hepatic enzyme uridine 5'-diphosphate glucuronosyl transferase-2B7 (UGT-2B7) to morphine 3-glucuronide (M3G) and morphine 6-glucuronide (M6G). M6G is a potent active metabolite, whereas M3G is inactive and even acting as an antagonist at high concentrations (Harrop 2007). Very preterm infants, born before 32 weeks of gestation excrete significant amounts of morphine unchanged in the urine as late as 24 hours after a single dose (Bhat et al. 1992). In acutely ill preterm infants the amount of metabolised morphine, the rate of renal excretion of morphine, and its metabolites exhibit wide variability. The overall trend is for the low neonatal clearance to increase with gestational age (Saarenmaa et al. 2000b). The hepatic metabolism and renal clearance may not account for all the age-related differences in morphine response. Data from neonatal rat dorsal root ganglion (DRG) cultures have demonstrated significantly more immature neurons that express functional μ-opioid receptors as compared to adult tissue. These μ-receptors were shown to be functional, subjected to postnatal development and probably account for the lower opioid requirements of preterm infants (Nandi et al. 2004).

The metabolism of opioids is immature in newborn infants. The volumes of distribution, drug clearances, side-effects and drug efficacy all differ in newborns as compared to older children and adults (Simons and Tibboel 2006). Systems for drug clearance and metabolic pathways begin to develop during foetal and early postnatal period, reaching adult levels after months and years (Bhat et al. 1992; Hartley et al. 1993; Barrett et al. 1996; Scott et al. 1999; Saarenmaa et al. 2000a; Bouwmeester et al. 2004).

Side- effects from opioid treatment

The arguments against treatment of neonatal pain with opioids includes uncertainty about opioid side effects, increased risk at higher doses and frequency, particularly in the preterm neonates since adverse effects also include hypotension (Hall et al. 2005), respiratory depression, urinary retention, nausea/vomiting, constipation, increased risk of necrotizing enterocolitis, and others are reported in the newborn infants (Anand et al. 2004). Very little is known about side effects from repeated opioid administration, or specific safety issues of opioids when treating critically ill, neurologically impaired, congenitally malformed, or other special populations of neonates (Simons and Anand 2006).

4 AIM

The overall objective of this thesis was to investigate factors alerting pain responsiveness in the newborn infant from a physiological and developmental perspective, and to evaluate effects of opioid analgesia in the mechanically ventilated preterm infant.

The specific aims of the studies included are:

- To assess effects of pre-emptive analgesia with morphine in preterm infants following medical care at the NICU within the NEOPAIN project, (Papers I, II and IV).
    - To investigate short-term outcomes with regard to the incidence of severe IVH grade III and IV, PVL and neonatal death, (Paper I).
    - To compare pulmonary outcomes with relation to morphine treatment, (Paper II).
    - To examine the ability of neonatal staff to estimate analgesia treatment in preterm infants, (Paper IV).
- To record cortical activation in preterm infants following tactile and painful stimulation sequentially, and to identify the factors modifying neonatal responses, (Paper III).
- To measure the responses following graded painful stimulation in the preterm and term newborn infant, (Paper III and V).
- To study developmental changes in the neonatal pain response during the first hours following birth in relation to the type of delivery, (Paper V).

5 METHODS

5.1 STUDY DESIGN

Study I – NEOPAIN Trial, primary outcome

The aim of the NEOPAIN trial (Neurologic Outcomes and Pre-emptive Analgesia in Neonates) was to investigate if pain treatment with morphine reduces early neurological injury in ventilated preterm infants. The primary outcome was defined as a composite outcome (including neonatal survival and absence of IVH grade III-IV or cystic PVL).

The NEOPAIN study is a multicenter randomized controlled trial comparing pre-emptive morphine infusion with placebo. A total of 898 neonates from 12 American and four European centres were included in the study, (including 73 infants from Sweden, 58 at Karolinska University Hospital and 15 at Örebro at University Hospital).

Inclusion criteria were: birth at 23-32 weeks of gestation, intubation within 72 h after birth, and mechanical ventilation for less than < 8 hours. Exclusion criteria were: major congenital anomalies, birth asphyxia, intrauterine growth retardation, maternal opioid addiction, and neonate's participation in other clinical trials.

The power estimation was based on results from a previous pilot study (Anand et al. 1999a) and hypothesized that morphine analgesia would reduce the composite primary outcome from 25.0% to 17.5%. Sample-size calculations showed that 470 neonates were needed in each group to test this hypothesis with α=0.05 and 80% power, assuming complete follow-up.

Infants were randomized to receive either morphine or placebo (dextrose 5%) in a blinded fashion. To ensure equal numbers in each group, randomization was stratified by the participating NICU and by their gestational age at birth (23-26, 27-29 and 30-32 weeks).

Neonates assigned to the morphine group received a loading dose of morphine 100 μg/kg infused during 1 hour, followed by continuous infusions of 10, 20 or 30 μg/kg/h, depending on the gestational-age group, see table 2.

Table 2. Number of included infants and morphine dosing, *modified from Anand, Bergqvist, et al. Lancet 2004*.

GA WEEKS	PLACEBO GROUP	MORPHINE GROUP	MORPHINE LOADING DOSE	MORPHINE MAINTENANCE DOSE
23-26	174	176	100 μg/kg	10 μg/kg/h
27-29	190	190		20 μg/kg/h
30-32	83	85		30 μg/kg/h

The study was designed for weaning and stopping the study drug, if extubation was expected within 24 hours, no spontaneous respiration was present at $paCO_2$ 5.3-6.7 kPa, rapid deterioration in the clinical condition, and after maximum 14 days of the study drug. Protocols for weaning the study drug were designed, to avoid opioid withdrawal. Guidelines included issues as; additional

administration of analgesia and sedative drugs, dosing and drug interactions (muscle relaxants, opioid antagonists, anticonvulsant therapy), for example, use of midazolam and other sedative or analgesic drugs were not allowed; criteria for weaning and stopping the study drug, with opioid weaning to avoid drug withdrawal symptoms; interactions with other therapeutic interventions as type of environmental and nursing such as enteral nutrition.

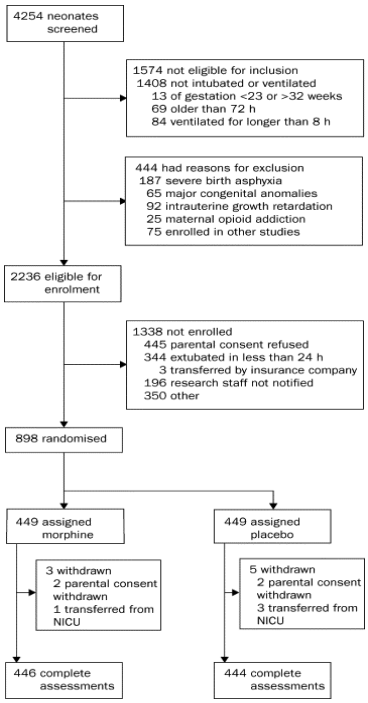

Figure 13. CONSORT diagram showing the inclusion of patients in the NEOPAIN trial, *from Anand, Bergqvist, et al. Lancet 2004.*

In view of ethical concerns related to having a blinded placebo group, intermittent boluses of open-label morphine (AA) were allowed in both groups, on the basis of specific criteria and given as intermittent boluses as decided by the treating physician at each NICU.

Since endotracheal suctioning is one of the most common invasive procedures in ventilated preterm neonates, the response to endotracheal suctioning was assessed with pain scores (PIPP and COMFORT) and HR changes from before to 2 minutes after suctioning. This assessment was done before the start of the study drug infusion, and at 24 h and 72 h during infusion and at 12 h after the end of the infusion.

Data collection included baseline clinical and demographic characteristics: GA, BW, Apgar score, the Clinical Risk Index for Babies (CRIB), presence/absence of respiratory distress syndrome (RDS), air leaks (pneumothorax, pulmonary interstitial emphysema etc) and patent ductus arteriosus (PDA), surfactant treatment, results from repeated cranial ultrasonography, sepsis, bronchopulmonary dysplasia (BPD), neonatal medical index (NMI) duration and type of ventilator support and numbers of days with hospital care.

Study II – NEOPAIN Trial, pulmonary outcomes

The aim of Study II was to evaluate pulmonary outcomes in relation to the study drug (morphine/placebo) in the NEOPAIN trial, secondary analysis. The neonates included in Study II were the same infants as in Study I.

The power calculation was based on an assumption that the incidence of BPD in the target population (23–32 weeks of gestation) was 30%. Three-hundred and seventy infants in each group would allow us to detect a change in BPD of at least 30% with α=0.05 and a power of 0.8.

Data collection included baseline clinical and demographic characteristics, vital signs, and clinical outcomes related to ventilator management.

There were no specific guidelines for extubation in the NEOPAIN protocol; however, most of the participating centres used the following criteria for extubation:

- Peak inspiratory pressure of ≤16 cm H_2O
- Positive end expiratory pressure of ≤5 cm H_2O
- Intermittent mandatory ventilation rate of 15 to 25 breaths per minute
- Fraction of inspired oxygen of ≤0.35

Table 3 Maternal and Infant characteristics in the NEOPAIN study. Numbers are number (percents) unless stated, *modified from Anand, Bergqvist, et al. Lancet 2004.*

	MORPHINE (*N* = 449)	**PLACEBO (*N* = 449)**	***P*-VALUE**
Maternal age, y, mean (SD)	26.8 (6.6)	26.5 (6.3)	0.55
Maternal race, white	273/446 (61.2)	262/448 (58.5)	0.52
Maternal chorioamnionitis	62/446 (13.9)	54 (12.0)	0.41
Maternal antibiotics	276/446 (61.9)	288/447 (64.4)	0.43
Prenatal steroids	364/444 (82.0)	364/445 (81.8)	0.94
Gestational age, w	27.3 (2.3)	27.4 (2.3)	0.80
Birth weight, g	1037 (340)	1054 (354)	0.46
Apgar score at 1 min	5 (3–7)	5 (3–7)	0.66
Apgar score at 5 min	7 (6–8)	7 (6–8)	0.97

Definitions of neonatal and maternal variables are available in the publication, paper II.

Study III – Cortical activation from pain

The primary aim was to investigate whether newborn infants demonstrate cortical activation as a response to pain. The study included 40 vaginally delivered newborn infants who required blood sampling in the NICUs at Karolinska University Hospital (Stockholm, Sweden) and Gaslini Hospital (Genova, Italy).

Inclusion criteria were: birth after 26 weeks of gestational and >24 hours postnatal age. Exclusion criteria were: congenital malformations affecting cerebral circulation or the cardiovascular system; ongoing intubation or mechanical ventilation; administration of analgesic, anaesthetics, and/or sedatives drugs within 24 hours prior to the study.

A total of 40 infants at 28 to 36 weeks of gestational age were included. Their postnatal ages ranged from 25 to 42 hours. The duration of pain stimulus (routine venipuncture) was 48.3 (35 to 60) seconds.

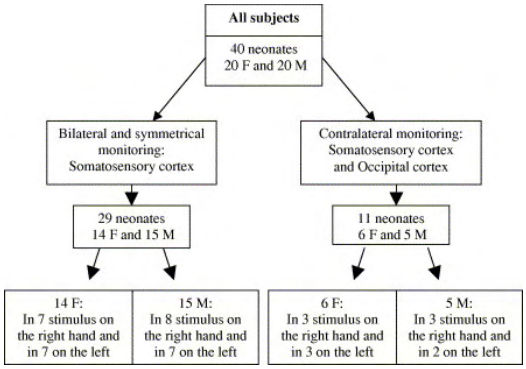

Figure 14. Patient flow diagram, *from Bartocci, Bergqvist, et al. Pain 2006.*

A double-channel NIRS device (NIRO 300, Hamamatsu Photonics, Hamamatsu, Japan) was used to monitor changes in cerebral oxy-hemoglobin [HbO_2], deoxy-hemoglobin [HbH], and total hemoglobin [Hbtot] concentrations. Briefly, the NIRO 300 produces light at four different wavelengths (775, 810, 850, and 910 nm). Each diode has a pulse frequency of approximately 2 kHz that lasts about 100 ns, the average output power is about 1 mW. The emitter and receiver probes constitute one pair of optodes. Through an optical fibre cable, the emitter probe submits near infrared light, which is reflected in brain tissue and detected by the receiver probe (Wyatt et al. 1990).

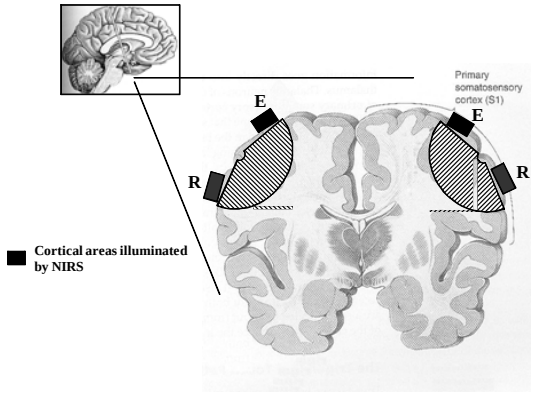

Figure 15. The figure shows the position of the NIRS optodes (E, emitter; R, receiver), viewed with reference to a coronal section of the brain. The dashed areas illustrate cortical regions that are illuminated by the near infrared light, *from Bartocci, Bergqvist, et al. Pain 2006.*

In the preterm newborn brain, with a thinner cortical mantle and lower tissue density (scalp, skull, dura, and brain), the infrared light may penetrate relatively deep, and measure tissue light absorbance within the primary somatosensory cortex and parts of the secondary somatosensory cortex, the insula, and cingulate cortex.

The two pairs of optodes were positioned over the somatosensory cortex symmetrically on each side of the head. Eleven newborns were monitored with the two pairs of optodes placed on the same side of the head: one pair overlying the somatosensory cortex and the other pair overlying the occipital cortex. In these newborns, the tactile and painful stimuli were applied contralaterally to the side where the optodes were placed.

The NIRS measurements were performed in three phases at venous blood sampling: Phase 0 was a 60 seconds baseline; phase 1 included a tactile stimulus, the skin disinfection during 30 seconds and phase 2 included the painful stimulus, i.e. venipuncture, see figure 16.

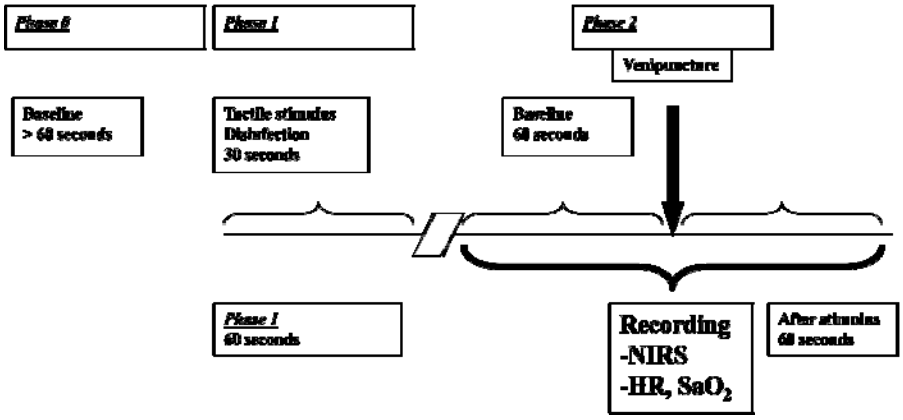

Figure 16. This figure describes the study procedures with the three phases of the study. *Modified figure from Bartocci, Bergqvist, et al. Pain 2006.*

Heart rate, oxygenation and respiration were recorded by a HP monitoring system (Hewlett–Packard, Boeblingen, Germany) simultaneously with the NIRS data. Respiration was observed during the procedure. HR and SaO2 average values were averaged at baseline and at 10, 20, 30, 40, 50, and 60 s after the tactile and painful stimuli, respectively.

Study IV – NEOPAIN Trial, Seeing through the blind!

The aim of this study was to evaluate if neonatal staff can assess whether ventilated preterm infants receive morphine or placebo. The included infants were a subcohort of the NEOPAIN from study I in this thesis.

The neonatal staffs' nurses and physicians were asked to fill in an assessment form when a newborn infant was enrolled in the NEOPAIN trial at the two Swedish centres, Karolinska University Hospital and Örebro University Hospital (Stockholm and Örebro). The staff participation was voluntary and the assessment form was filled in anonymously.

The entire form included five questions:

1. Profession (assistant nurse, registered nurse or physicians)
2. Duration of NICU experience (<1, 1-5 or >5years)
3. Gender (woman or man)
4. Which randomisation group (morphine and placebo) does the neonate belong to?
5. Does the neonate have sufficient analgesia (yes or no)

A total of 516 assessment forms were distributed (Stockholm 371 and Örebro 145), and 360 forms were returned and analyzed. Stockholm collected 288 assessment forms on 43 neonates, and Örebro contributed with 72 forms on nine neonates, see table 4, the described definition of the qualitative and quantitative factors assessed in this study.

Table 4. Factors in the study, including definitions of qualitative and quantitative variables. *Modified from Bergqvist et al. Acta Paediatrica 2007.*

CLINICAL FACTORS	DEFINITION
Qualitative	
Profession	
Education	Physician vs. nurse vs. assistant nurse
Experience at NICU	<1 vs. 1-5 vs. >5 years
Neonates	
Treatment group	Continuous morphine analgesia vs. placebo
Gender	Female vs. male, genitalia on physical examination
GA group	23-26 vs. 27-29 vs. 30-32 weeks
CRIB score	Total score (1-20) from the "Clinical Risk Index for Babies", with 10 as the reference score
Quantitative	
CRIB score	Total score (1-20) from the "Clinical Risk Index for Babies", as continuous variable.
NMI score	Total score from the "Neonatal Medical Index"
Days of ventilation	Number of intubated days with ventilated support by CMV and HFOV
Level of morphine	Plasma level at 24 hours after study drug started (ηg/ml).
Additional analgesia morphine before	Amount of additional morphine (mg/kg) given before the started study drug
Additional analgesia morphine during	Amount of additional morphine (mg/kg) given during study drug

Study V: Mode of delivery affects Pain Responsiveness

The aim of this study was to evaluate if the mode of delivery affected pain responsiveness in term infants during the first hours after birth.

Women admitted to the delivery ward at Karolinska University Hospital with spontaneous vaginally delivery (VD) or planed elective caesarean section (CS) were asked to allow their newborn infant to participate in this trial.

Inclusion criteria were: full-term birth (VD or CS) following an uncomplicated pregnancy, 37 to 42 weeks of gestation, and appropriate weight for gestation (AGA), Apgar score of ≥ 7 at 5 minutes after birth, and normal physical appearance. Exclusion criteria were: premature rupture of membranes, labour lasting longer than 24 hours, infants delivered by vacuum extraction or acute CS, and infants of mothers who required epidural anaesthesia or pudendal nerve block.

For the included subjects, all elective CS were performed under spinal anaesthesia using bupivacaine with the mothers being awake during the surgical procedure, while analgesia during VD was achieved with 50% N_2O. Infants born after VD (N=53) and elective CS (N=23) were similar with respect to gender, birth weight and postnatal age (in minutes), although infants born after elective CS had lower GA, for detailed description see, publication.

Heart rate was recorded with standard electrocardiogram (ECG), with a sampling frequency of 1000 Hz (BIOPAC). The signal was digitized and displayed in real-time using the AcqKnowledge software. The baseline HR in beats per minute (bpm) was evaluated during a 1-minute reference period immediately before the stimulus. Directly following stimulation, the HR was measured at 1-second intervals for a period of 10 seconds; these readings were averaged and then expressed as a percentage of the baseline HR (HR %).

The study procedure included assessments of infants' responses to three standardized stimuli, one painful and two cold stimuli. Our high-intensity pain stimulus was the routine intramuscular injection of vitamin K. After 2 min, the low-intensity stimulus, a cooled metal spoon was applied on the abdominal skin for 10 s. After further 2 min, 10 drops of cold water were dropped at the infant foot. This sequence of stimulation was the same for all infants, and no stimulus was repeated. Infants were randomized to receive these stimuli either within >30 to ≤90 or at >90 to <260 min after birth and were evaluated by blinded observers.

As behavioural response we used facial expression as estimated with the NFCS (Grunau et al. 1990). The NFCS score was evaluated during the first 15 s immediately following each of the three stimuli. Collected data were coded and trained staffs estimated independently each of the seven facial expressions second by second. The analysis of the video tapes was performed by a slow motion with frequent stops and 10% were studied repeatedly to confirm reliability.

Infant's vocalization was recorded by a Digital Audio Tape recorder with a microphone at a distance of 15 cm from the infant mouth. Background sound in the room was monitored and maintained at or below 60 dB and all recordings were calibrated. Vocalizations were analyzed for both acoustical and perceptual features with a computerized voice analysis program (Swell). Vocalization occurring within 30 s from each stimulus was included in this analysis, with a primary focus on the first five expiratory vocalizations and total number of inspiratory vocalizations. Acoustical analysis includes the quantitative and qualitative aspects of infant vocalization.
Acoustical parameters analyzed within the first 30 s from stimuli:

1. Time to response, from start of applied stimuli to start of vocalization.
2. Time of vocalization, total duration of expiratory and inspiratory vocalization.
3. Total time of vocalization, including pause.
4. Sound intensity in decibel (dB).
5. F_0 the strongest of the first five vocalizations.

Perceptual analysis was performed by six listeners (three midwives and three speech therapists) with experience in professional sound judgment. The VISOR program was used for individual training session. The five expiratory phonation's were compressed in the Swell program with the GLUE subprogram to create a listener's test. Each listener received an individual training session with six vocalizations and used the VISOR program in the evaluation (Granqvist and Hammarberg 2003).

Table 5. Six perceptual parameters were selected for the evaluation; the assessments were graded using visual analogue scales (VAS), *Modified from Bergqvist et al. Acta Paediatrica 2007*.

Parameter	Estimation	Scale
Type of vocalization	Cry or grumble	Classification
Level of pain	Non to severe	VAS
Pitch	Low to high	VAS
Sound level	Weak to strong	VAS
Instability	Non- to very much	VAS
Press	Non to very much	VAS

The included parameters are used routinely in clinical voice estimation (Hammarberg 2000). The listeners were instructed to classify the infant's vocalization and assign a numeric value on a visual analogue scale (VAS) marked from 0 to 1000, see table 5.

5.2 STATISTICS

Study I – NEOPAIN Trial, primary outcomes

Since the NEOPAIN study was blinded, coding and data analyses were performed at Maryland Medical Research Institute, USA. All analyses were by intention to treat. Group outcomes were compared by χ^2 tests or Fisher's exact tests and homogeneity of the odds ratios across gestational ages was tested by the Breslow-Day test. Logistic regression models were used to predict each outcome. All analyses were done with SAS software (version 8.1), the p-value was set at α=0.05. Results of logistic regression analyses are presented as point estimate odds ratios with two-sided 95% CI. Pain assessments and vital signs were compared between the randomized groups by use of Student´s *t*-tests.

Study II – NEOPAIN Trial, pulmonary outcome

Treatment group demographic features and outcomes were compared with Student's *t*-test, χ^2 tests, or Fisher's exact tests. For data that did not have a strictly normal distribution, the results are presented as median and interquartile ranges, and nonparametric tests (Wilcoxon test and Kruskal-Wallis test) were used for analyses as appropriate. Multiple linear regression models were used to evaluate effects from various clinical variables, days on ventilation support, nCPAP, and oxygen treatment, as well as the length of stay at hospital. The multiple linear regression models are presented as parameter estimates and SEs. Factors associated with the outcomes of pulmonary air leaks, BPD, and BPD or death was analyzed with logistic models. The fit of each logistic model was assessed with the Hosmer-Lemeshow goodness-of-fit test, and the global test that all regression parameters were 0 was tested with the -2 log-likelihood statistic. Results of these analyses are presented as odds ratios with 2-sided 95% confidence intervals. All analyses were performed with SAS statistical software (SAS Institute, Cary, NC), with the critical p-value set at α=0.05.

Study III – Cortical activation from Pain

The repeated-measures ANOVA and Newman–Keuls *post hoc* tests were used for comparisons between P_0, P_1 and P_2 responses for [HbO_2], [HbH], and [Hbtot] respectively. Student's *t*-tests for independent variables were used to compare $[HbO_2]_{diff}$ values between the parietal and occipital cortex (in neonates where both sets of optodes were positioned contralateral to the stimuli), to compare $[HbO_2]_{diff}$ values between the ipsilateral and contralateral side, to evaluate inter-hemispheric differences depending on whether the left or the right hand was stimulated, and to compare the differences between male and female infants. Correlations between GA, BW, PNA, and duration of the venipuncture, and magnitude of the NIRS response were performed by linear regression analyses. Data were analyzed by the program Statistica® , critical p-values at α=0.05.

Study IV – NEOPAIN, Seeing through the blind!

The estimation of the suggested study drug assessment were analysed by one-sample tests of binominal proportions, and proportions between groups with χ^2-test. Effect of education and years of experience were analysed using the logistic regression model with Fisher's scoring. The fit of each logistic model was assessed using the Hosmer–Lemeshow, goodness-of-fit test and the global test that all regression parameters are zero and tested using the 2-log likelihood statistic. Results of logistic regression analysis are presented as p-values and odds ratios (OR) with two-side 95% confidence intervals (CI) to show the effect of each predictor variable on the indicated outcome. The entire analyses were performed using SAS statistical software (Cary, NC) and the critical p-value was set at α=0.05.

Study V – Mode of Delivery affects pain responsiveness
Power was calculated to indicate a 20% difference in response to the stimuli within the two modes of delivery. As the group of patients was small, nonparametric statistics were used. The possible differences between responses to high-intensity (injection) and low-intensity (two cold stimuli) stimuli were analyzed by Kruskal–Wallis analysis of variance.

The Statgraphics Plus software was used to analysis these data. Correlation between change in HR (%) in response to stimuli and postnatal age (in minutes) of infants was analyzed by linear regression. Relationship between the predictors' independent variables (birth weight, gender, mode of delivery and postnatal age in minutes at stimulation) and dependent variable (change in HR, facial expression, and vocalization) were examined in two steps. First, all possible correlations between the variables were analyzed (Spearman's rank correlation coefficient). Variables that were significantly correlated with the predictor ($p<0.05$), were analyzed for independent association using stepwise multiple regression analysis (backward selection).

The perceptual estimation of pain intensity using VAS in relation to qualities of vocalization such as pitch, intensity, instability and pressure were evaluated by linear regression analysis. Correlation between the two NFCS observers and the six perceptual listeners was analyzed to calculate the inter-rater reliability of these methods.

5.3 ETHICS

The ultimate participation of human subjects cannot be avoided in biomedical research, because the conclusions drawn from animal experiments may not apply to human genetics, development, behaviour, or the susceptibility, aetiology, epidemiology, pathophysiology, and management of human diseases.

The potential costs to a human subject are balanced against potential benefit, either to that individual or to society at large. For newborns, there is no possibility for them to conduct their own risk-benefit analysis with regard to participating in research, and thus extra attention must be paid to ensure that this vulnerable population is not exploited. The major issues related to including infants in research focus on the process of consent, assessment of risks and benefits, the use of placebo controls, and the inclusion of healthy subjects (Kauffman 2000).

In the case of analgesic studies, costs to the subject might include:

- *Withholding of analgesia*. This is of concern when placebos are introduced and it would be important that participation in the study would expose the child to no more pain that what is dictated by the clinical standard.
- *Unknown side-effects*. Especially in studies of safety, there must be means available to address or reverse effects of the study medications.
- *Additional burden related to monitoring*. Blood sampling of drug level and pharmacokinetic was and is a key issue for young premature infants.

Consent issues
Because infants are incapable of being involved in the consent process, a surrogate, usually a parent provides permission on their behalf. There is thus a clear assumption that this surrogate is competent to understand the issues and that the surrogate is only operating in the best interests of the child. Issues pertaining to the care of neonates are complicated and presentation of a research

protocol to parents under emotionally stressful circumstances may prevent the clarity of thought and a thorough cost-benefit analysis. Furthermore, when there are inducements for participation in a study, one cannot take for granted that all decisions are made purely on behalf of a child's best interests.

Risk-Benefit Analysis

Risk-benefit analysis has been divided into four major categories: (a) research not involving greater than minimal risk, (b) research involving more than minimal risk with the prospect of direct benefit to the child, (c) research involving more than minimal risk with no direct benefit but likely to yield important generalized knowledge, and (d) research not otherwise approvable that presents an opportunity to understand, prevent, or alleviate a serious problem affecting the welfare of children (Kauffman 1995).

Although some clinicians may advocate that young children should only participate in studies that pose less than minimal risk or studies that provide direct benefit to the participant (Burns 2003), however, the American Academy of Paediatrics (AAP) urges for a broader interpretation of benefits to include those which will advance the welfare of children in general (Kauffman 1995). Further, when evaluating benefits and risks, the benefits to children arising from being included in clinical research versus the risks of not doing the research in infants and children should be considered, in addition to the direct and immediate risks and benefits to individual subjects. The AAP also pointed out that risks may be minimised by limiting the number and type of invasive tests, improving or developing non-invasive and safe methods of monitoring biological effects, and by fully preparing and informing subjects about the proposed study procedures (Kauffman 1995).

A major concern in the context of analgesic trials in children is the additional burden related to monitoring drug levels or diagnostic tests for side effects. Blood sampling in drug level and pharmacokinetic studies is a key limiting factor in very premature infants. Total blood loss should not exceed 2% of the estimated blood volume of a patient with normal red blood cell mass. If one assumes that blood volume is 80 ml/kg of bodyweight, small premature infants may be exposed to the risks of serious depletion, especially if an array of diagnostic blood tests is performed in addition to a research-related blood sample. Novel strategies, such as scavenging the blood drawn for other reasons may become imperative as a way to gather the desired data. Finally, blood and urine sampling itself will not be invasive if established clinical catheters will be used to draw samples.

Drug studies in neonates

Many drugs used to treat children have never been evaluated in children. None of drugs is registered within EU regarding EMEAs guidelines, presented by Agnes Saint Raymond, the head of Sector Scientific advice. The neonatal age represents the period of life when the most profound and rapid physiological changes occur. Thus, the population as a whole is very heterogeneous. Population based approaches for drug evaluation offers the ability to address the effects of covariates and estimate inter-individual variability in pharmacokinetics and dosing (Jacqz-Aigrain and Burtin 1996).

Investigation must try to minimise anaemia and pain from blood draws while obtaining data from neonates who require specific medications for their survival and care. There is also the potential for rapid changes in clinical status of NICU patients that may affect pharmacology of analgesia (clinical diagnosis: multi-organ system illness affecting hepatic blood flow, dehydration, plasma protein levels, nutritional state, drug interactions etc).

Safety issues

Neonatology is replete with examples of harm from therapeutic misadventures due to lack of adequate clinical studies. Before interventions become commonplace, proper evaluation of safety and efficacy are needed. Safety is best assessed in studies where monitoring guidelines are pre-specified and adhered to in the protocol. It is not easy to identify a-priori all infants at risk of adverse effects so all infants must be carefully and thoroughly evaluated to detect risks.

Investigations must include determination of immediate and longer term safety. In order to evaluate safety, there should be a clear understanding of the relationship between pharmacokinetics and pharmacodynamics, such that drugs are studied for a sufficient time period for their pharmacologic effects to be observed. If drugs are to be used repeatedly, then additional determinations of the time-course of effects are needed for defining the optimal dose and dosing interval. Biologically-based outcomes should be evaluated.

Although neonates may have the highest incidence of adverse events in part due to underlying morbidity, this must be weighed against the need to provide analgesia.

Ethical compliance

The ethical issues in these projects have been scrutinized by local and regional research ethics committees, and ensure compliance with:

- Ethical standards of local and regional ethical committees.
- Decisions and requirements of local and regional ethical boards.
- The Amsterdam protocol on animal protection and welfare.
- The World Medical Association with Helsinki Declaration, 2000 and its amendments.
- Universal Declaration of Human Rights, 1948.
- UN Convention on the Rights of the Child, 1989.
- The Convention of the Council of Europe on Human Rights and Biomedicine (Oviedo, April 1997).Universal Declaration on the Human Genome and Human Rights adopted by UNESCO, 1997.
- The CIOMS (Council for International Organizations for Medical Sciences) International Ethical Guidelines for Biomedical Research Involving Human Subjects, 2002.
- The World Health Organization, Guidelines for Good Clinical Practice for Trials on Pharmaceutical Products, 1995.
- The International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use with Guidelines for Good Clinical Practice, 1996.
- The Charter of Fundamental Rights of the EU.
- The EU Clinical Trials Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use.
- Directive 95/46/EC of the European Parliament and of the Council of 24 October 1995 on the protection of individuals with regard to the processing of personal data and on the free movement of such data.
- Council Directive 83/570/EEC of 26 October 1983 amending Directives 65/65/EEC, 75/318/EEC and 75/319/EEC on the approximation laid down by law, regulation or administrative action relating to proprietary medicinal products.

- Convention of the Council of Europe on Human Rights and Biomedicine signed in Oviedo on 4 April 1997.
- Swedish Code of Statutes 2003:460 and 2003:615 on ethical review regarding research involving humans.

Protection of human subjects

We have strictly complied with widely recognised international texts and codes of practice (as listed above). The projects have respected all ethical requirements in objectives and methodology, and there are no known negative ethical implications of the results to be obtained. Only after obtaining written consent from parents, patient's blood samples or other data were collected without causing additional pain. All specimens and research records were coded to protect the identity of patients and to ensure confidentiality. Patients have been recruited in a consecutive manner without regard to race, gender, social class or other considerations. Patient data have been obtained through medical record chart review and interviews, while avoiding any personal identifiers. We have assured confidentiality by using codes for patient identification, and confidentiality laws will be strictly observed when processing human clinical information. No patient identifying information was or will be published, or to be available after the requisite clinical data have been collected or published. Data have been accessible only to members and coordinators of the participating facility and research team, which will include pathological and medical personnel under approved guidelines. The data registers were designed and kept according to applicable legislation. The content of data collection were and will be reported to authorities, from where the parents have possibility to request information. These clinical trials of drugs have been conducted in compliance with the Helsinki Agreement and only after approval by the local ethics committee at each site.

6 RESULTS

6.1 STUDY I – NEOPAIN TRIAL, PRIMARY OUTCOMES

Primary outcome

When data from all infants were analysed, there were no differences in the composite outcome or its components (neonatal death, severe IVH, or PVL) between the two randomised groups, see table 6. However, at 27 to 29 gestational weeks, the proportion of infants with severe IVH was higher in the morphine group than in the placebo group, and the difference in the frequency of the composite outcome almost reached statistical significance. On the other hand, there were no significant differences in neonatal death, severe IVH and PVL or the composite outcome at 23 to 26 and 30 to 32 gestational weeks.

Table 6. Primary outcomes for all included neonates in the NEOPAIN trial, *Modified from Anand, Bergqvist et al. Lancet 2004.*

OUTCOME	MORPHINE (%)	PLACEBO (%)	*P*-VALUE
Overall			
Severe IVH	13	11	0.24
PVL	7	9	0.35
Death	13	11	0.25
Composite	27	26	0.58
23 to 26 weeks			
Severe IVH	20	20	0.95
PVL	6	10	0.26
Death	26	24	0.58
Composite	40	42	0.70
26 to 29 weeks			
Severe IVH	12	6	**0.04**
PVL	9	7	0.44
Death	5	3	0.31
Composite	23	15	0.053
30 to 32 weeks			
Severe IVH	3	2	0.99
PVL	4	13	0.07
Death	2	-	-
Composite	10	15	0.31

Table 7. Factors associated with the primary outcome and its component (all infants), *Modified from Anand, Bergqvist et al. Lancet 2004.*

	RELATED TO	NOT RELATED TO
Neonatal death	Gestational age	Treatment group
	CRIB score	
Severe IVH	Gestational age	Treatment group
	CRIB score	
	Maternal race	
	Lack of antenatal steroids	
PVL	Maternal chorioamnionitis	Treatment group
		Gestational age
Composite outcome	Gestational age	Treatment group
	Male sex	
	Lack of antenatal steroids	
	Severity of illness - NMI	
	Maternal chorioamnionitis	

PIPP

The PIPP score response to endotracheal suctioning was significantly lower in the morphine group than in the placebo group at 24 h after initiation of study drug. The mean (SD) PIPP was 8.00 (2.69) in the morphine group versus 8.77 (3.18).

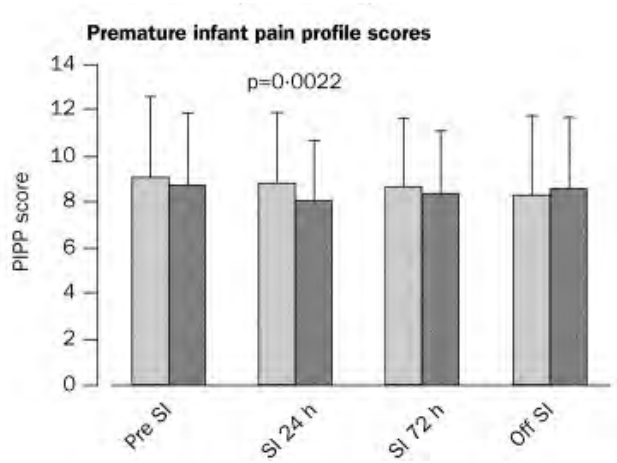

Figure 17. The table shows mean (SD) changes in PIPP during endotracheal suction in neonates receiving morphine (darker bars) vs. placebo (light grey bars), *from Anand, Bergqvist, et al. Lancet 2004.*

Hypotension

Before start of the study drug infusion, arterial hypotension was present in 86/449 infants in the placebo group and in 103/449 infants in the morphine group (20.0% vs. 23.8%, non-significant difference). Hypotension was significantly more common in the morphine group than in the placebo group, after the loading dose and at 24 h of study infusion, see figure 18. After the loading dose, 26 infants in the morphine group and 13 in placebo group developed hypotension.

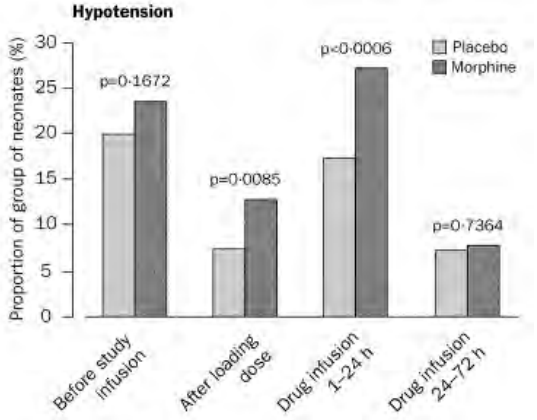

Figure 18. Incidence of clinically hypotension in relation to study drug infusion, *from Anand, Bergqvist, et al. Lancet 2004*.

Other side effects

Neonates in the morphine group had a longer duration of mechanical ventilation but required similar duration of oxygen therapy. Neonates in the morphine group took longer time than the placebo group to tolerate full-volume nasogastric feeds but achieved full volume oral feeds at the same time period (see Figure??). The need for bladder catheterisation (placebo group 22, morphine group 35) was not significantly different between the two groups (p=0.084).

Open label morphine

The decision to use open-label morphine was based on the clinical judgment by the attending physician in the NICU. The open-label morphine analgesia was given to fewer neonates in the morphine group than in the placebo group, 202 (45.3%) vs. 242 (54.6%), p<0.006.

Among the neonates who did not receive open-label morphine, the presence of severe IVH (9 vs. 3 %, p=0.02) and composite outcome (24 vs. 15%, p=0.03) was more frequent in the morphine group. Again it was infants at 27 to 29 gestational weeks who were allocated to the morphine group who had a higher proportion of the composite outcome (20 vs. 9 %, p=0.03).

Table 8. Factors associated with primary outcomes among neonates who did not receive additional analgesia, p-value, *Modified from Anand, Bergqvist et al. Lancet 2004.*

OUTCOMES	COMPOSITE	DEATH	SEVERE IVH	PVL
Treatment group	0.09	0.53	0.04	0.056
Gestational age	<0.001	<0.001	0.002	0.18
CRIB score	0.02	0.00	-	-
Sex (M vs. F)	0.059	-	-	-
Maternal antibiotics	-	0.02	-	-
Maternal chorioamnionitis	-	-	-	0.002

Number of painful and stressful procedures

In the NEOPAIN trial, data were collected from 12 infants in Stockholm in whom all painful and potentially stressful procedures were registered, including all skin breaking procedures such as catheters (arterial, venous, umbilical cord and bladder), different punctures, intubation and procedures like x-rays and diaper changes etc.

Figure 19. Number of painful and stressful procedures during the first 8 weeks of life, in an infant born at 25 gestational weeks, who was included in the NEOPAIN study. Altogether 701 procedures were performed in this infant during 52 days of NICU stay, *from Hébert-Wegmann, Bergqvist et al., unpublished data.*

6.2 STUDY II – NEOPAIN TRIAL, PULMONARY OUTCOME

The frequency of RDS and severity of illness (CRIB scores) was similar in the two groups (morphine and placebo). Infants in the morphine group needed mechanical ventilation on average one day (two days when early deaths were excluded) longer than infants in the placebo group. There were no significant differences of other respiratory variables between the two groups.

To evaluate the effect of open-label morphine, data were analyzed in each group (morphine and placebo) by comparing infants who did versus did not receive open label morphine. Infants who received open label morphine (AA) had lower GA, BW, and Apgar scores at 1 and 5 minutes. These infants died more often and were also more ill with regards to CRIB score and the incidence of RDS, PDA, air leaks, chest tubes and BPD increased. Furthermore, the open label morphine group needed longer durations of mechanical ventilation, oxygen therapy and hospital treatment.

To examine whether morphine therapy contributed to the respiratory outcomes outlined above, we adjusted for BW, CRIB scores (which include GA in the composite scores), maternal chorioamnionitis, RDS requiring surfactant, and PDA in our logistic regression models, using placebo/no label morphine as the reference group. Pre-emptive morphine infusions did not alter these outcomes, but the use of open label morphine (AA) continued to be a strong independent predictor of worse respiratory outcomes even after adjustment for the aforementioned factors, see table 9.

Table 9, Effects of morphine open-label (AA) on respiratory outcomes, after adjustment for BW, CRIB scores, maternal chorioamnionitis, RDS requiring surfactant, and PDA, *Modified from Bhandari, Bergqvist et al., Pediatrics 2005.*

OUTCOMES	PLACEBO GROUP AA*	MORPHINE GROUP AA*
Air leaks, OR	3.43, $p<0.01$	4.28, $p<0.01$
PPV, d	NS	6.15, $p<0.01$
PPV, d (excluding deaths)	NS	6.67, $p<0.01$
CV, d	NS	3.17, $p=0.06$
CV, d (excluding deaths)	NS	3.66, $p=0.04$
HFV, d	2.62, $p<0.01$	3.09, $p<0.01$
HFV, d (excluding deaths)	2.62, $p<0.01$	3.09, $p<0.01$
nCPAP, d	3.22, $p=0.02$	2.85, $p=0.04$
Oxygen, d	7.16, $p=0.01$	8.12, $p<0.01$
LOS	NS	5.43, $p=0.08$
BPD, OR	NS	NS
BPD and/or death, OR	NS	NS

OR, odds ratio; NS, not significant ($p>0.05$); reference group placebo/no AA

Column Morphine/No AA was NS in all variables and not shown

* Placebo group/no AA was used as the reference group

6.3 STUDY III - CORTICAL ACTIVATION RESULTING FROM PAIN

Heart rate and oxygen saturation

Tactile stimulation did not change HR or SaO_2. However, following pain, the mean (SD) HR increased significantly from baseline to 10 and 20 seconds after venipuncture, whereas the mean (SD) oxygenation saturation (%) decreased significantly during 10 to 40 seconds following venipuncture, see figures 20a and 20b.

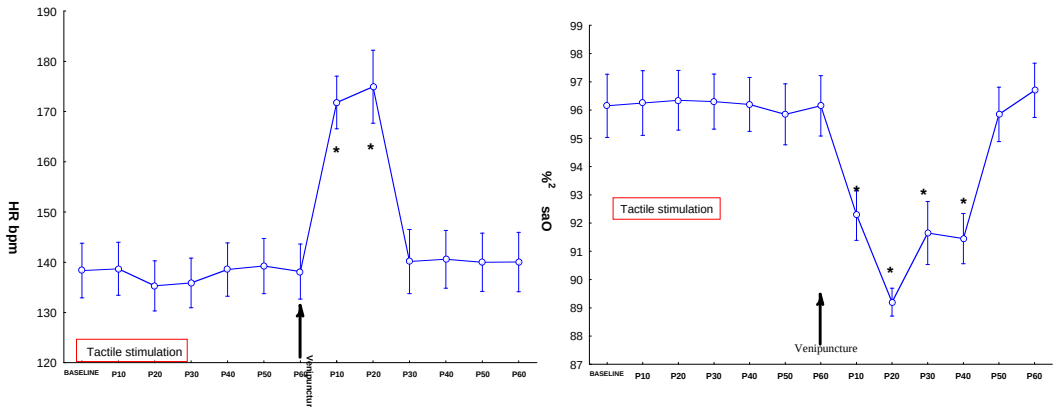

Figure 20a and 20b HR and oxygen saturation response to venipuncture, mean (SD), *from Bartocci, Bergqvist et al., PAIN 2006.*

Cerebral oxygenation

Tactile stimulation resulted in a small increase in $[HbO_2]$ concentrations in both cerebral hemispheres, which was further increased following painful stimulation.

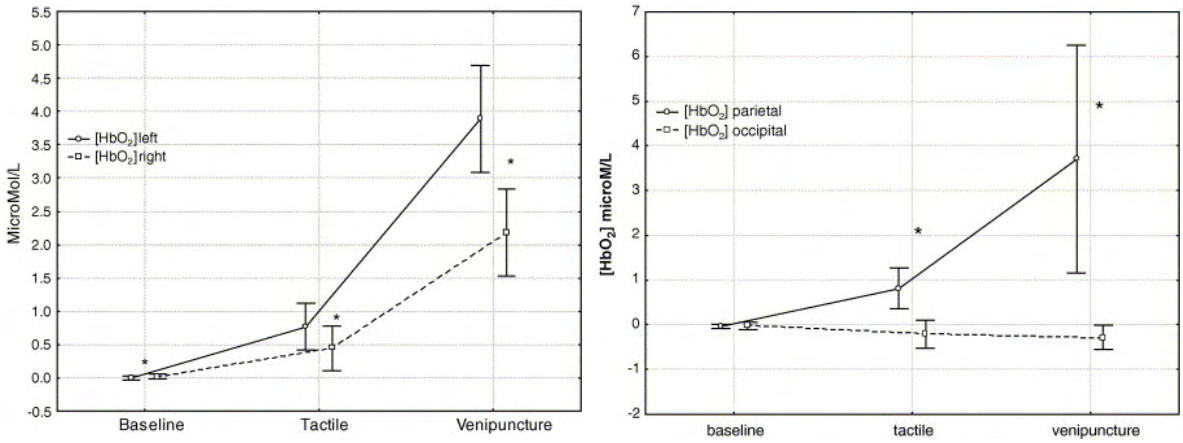

Figure 21. Response in cerebral oxygenation $[HbO_2]$ in the left and right hemispheres (left figure). Simultaneous recordings over the somatosensory and occipital cortex showed significant changes after tactile and pain stimulation, but only over the parietal area not over the occipital area, implying the functional specificity of this response, mean CI, *from Bartocci, Bergqvist et al., PAIN 2006.*

The response to pain was greater in the male than in female preterm neonates, see figure 22.

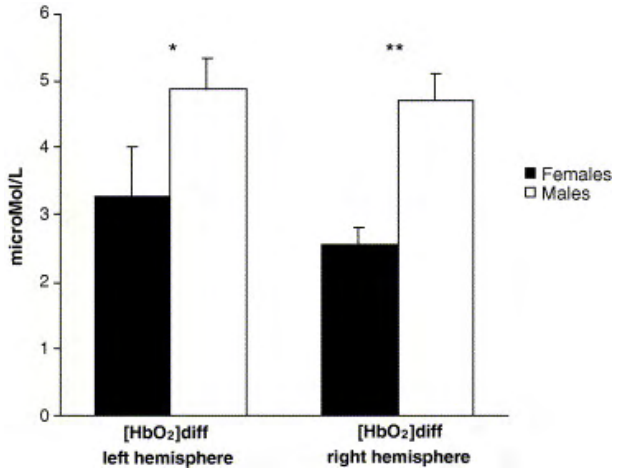

Figure 22. Cortical response [HbO_2] between the female (black columns) and male (white columns) neonates following venipuncture, males more pronounced, mean (SD), *from Bartocci, Bergqvist et al., PAIN 2006*.

The [HbO_2] increases after pain were positively correlated with postnatal age (r=0.75 left and r=0.67 right hemisphere), and negatively correlated with the GA (r=−0.53 left and r=−0.42 right hemisphere.

6.4 STUDY IV – NEOPAIN, SEEING THROUGH THE BLIND!

The ability of the professional neonatal staff to assess pain relive was tested in this study. We compared how the staff rated pain in mechanical ventilated preterm infants who received morphine or placebo in a double blinded design, with the same infants as in Paper I.

Fifty two infants were assessed, 27 were randomized to the morphine group and 25 to the placebo group. There were no differences between the infants in these two groups, see table S2 in the publication. In total, physicians completed 99 forms, the nurses completed 179, and the assistant nurses 82 forms. The proportions of correct answers were 213 compared to 147, out of 360. Thus, 59% of all answers correctly identified the assignment group ($p<0.001$).

Table 10. The rates of correct answers were comparable between the groups of all neonatal staff, *modified from Bergqvist et. al., Acta Paediatrica 2007.*

EDUCATION	**PERCENTAGE OF CORRECT ANSWERS**	***P*-VALUE**
Physicians (N=62)	63%	
Nurses (N=107)	60%	p=0.60
Assistant nurses (N=44)	54%	
NICU Experience		
<1 year (N=50)	62%	
1–5 years (N=64)	65%	p=0.28
>5 years (N=99)	55%	

Increasing amounts of additional open label morphine before and during the study drug infusion reduced the staff's ability to correctly assess treatment group both before and during the study drug.

Clinical factors

Clinical factors that affected the staff´s ability to predict a correct answer:

1. CRIB score had an Odds Ratio (OR) of 0.86 in predicting agreement with the Study Drug. Since "agreement" is the outcome being modelled, a baby with CRIB score 1 unit greater than the reference baby (with CRIB score=10), the probability of agreement with the Study Drug is reduced by 14% (0.86 vs. 1.0). In other words, the higher the CRIB score, the lower is the likelihood for "correct" identification of Study Drug. This is clinically evident, since patients who are sicker will be less likely to show the clinical signs of pain vs. analgesia. Patients who are very sick hardly show any signs of pain, e.g., babies with NEC.
2. Gender does not have a significant effect on correct identification of Study Drug($p=0.056$, OR=0.58, CI=0.34–1.01), although there was a strong trend towards more frequent correct responses for male infants. This is consistent with the NIRS data, where male neonates showing greater cortical hemodynamic responses to acute pain as well.
3. Other Factors: There were no effects on the ability of staff to assess the correct randomized group by other clinical factors Neonatal Medical Index (NMI), days on ventilatory support (CMV and HFOV), or plasma morphine levels.

6.5 STUDY V – MODE OF DELIVERY AFFECTS PAIN RESPONSIVNESS

Heart rate

The baseline HR was similar in both groups, VD and CS 135.5 (65.0) vs. 138.3 (50.8) bpm. The HR changes were larger after high intensity acute pain (vitamin K injection) as compared to the two low-intensity thermal stimuli (cold). The HR changes in response to high- and low-intensity stimuli were affected predominantly by the postnatal age (min) and the mode of delivery, see table 11.

Table 11. Mode of delivery and postnatal age, *modified from Bergqvist, et al. J Perinatol 2008*.

Dependent variable	Independent variable	*p*-value	Explained variance (%)
HR%: pain	PN age (min)	0.001	14.2
	Mode of delivery	0.03	4.2
HR%: cold	PN age (min)	0.000	11.5
HMS: pain	Mode of delivery	0.000	36.6
HMS: cold	Mode of delivery	0.000	12.5
Mean F_0: pain	Mode of delivery	0.04	7.3
Mean F_0: cold	Mode of delivery	0.004	13.9
Phonation time (%): pain	Mode of delivery	0.002	21.6
Phonation time (%): cold	Mode of delivery	NS	

During the first 4 h after birth, HR response to both stimuli increased progressively by postnatal age disregarding the mode of delivery (all infants).

Following VD, the HR response to both high and low-intensity stimuli increased progressively by postnatal age whereas no such change was observed in the CS group, see figure 23.

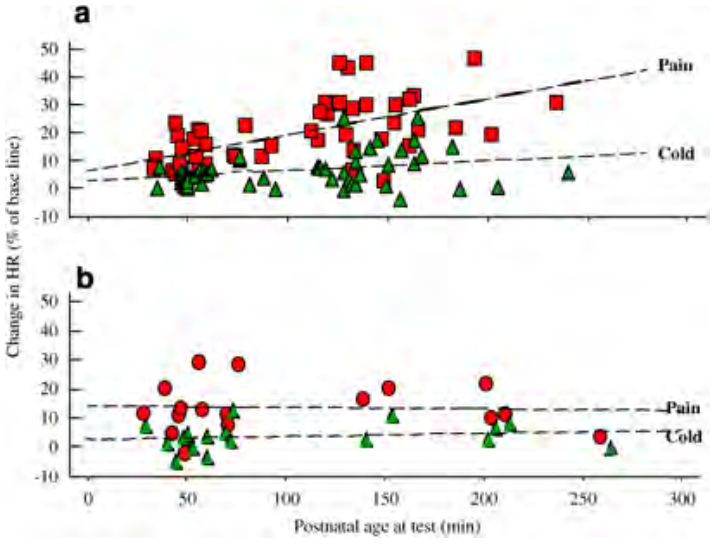

Figure 23. The HR response to high- and low-intensity stimuli with postnatal age in VD infants with a progressive increase of postnatal age (a), no such change was observed in CS infants (b).

Behavioural response

The facial expressions were stronger during acute pain (vitamin K injection) as compared to the two thermal stimuli (cold).

The facial expression, horizontal mouth stretch (HMS) was specifically affected by the mode of delivery whereas the other facial expressions were not. The HMS expression was less pronounced in VD than in CS infants in response to both higher and lower intensity stimuli.

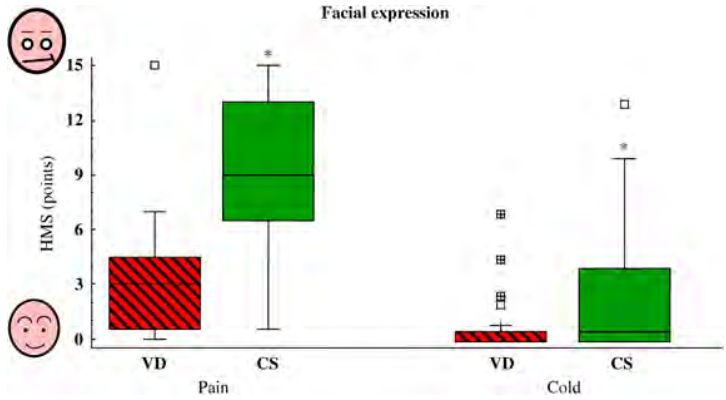

Figure 24. Mode of delivery affects the HMS facial expression to higher and lower intensity pain stimuli with a weaker response in the VD infants than CS infants with, *modified from Bergqvist, et al. J Perinatol 2008*

Vocalization, acoustical and perceptual analysis

It is remarkable that no vocalizations occurred in 10 of 53 VD infants and 1 of 23 CS infants following high-intensity painful stimuli (p=0.09, χ^2-test), these infants could not be included in the analysis of vocalization. In the remaining infants, vocalizations were stronger during the vitamin K injection as compared to the two cold interventions.

The high and low intensity stimuli were affected by the mode of delivery with mean F_0 response, lower frequency band and longer vocalization latency in VD infants.

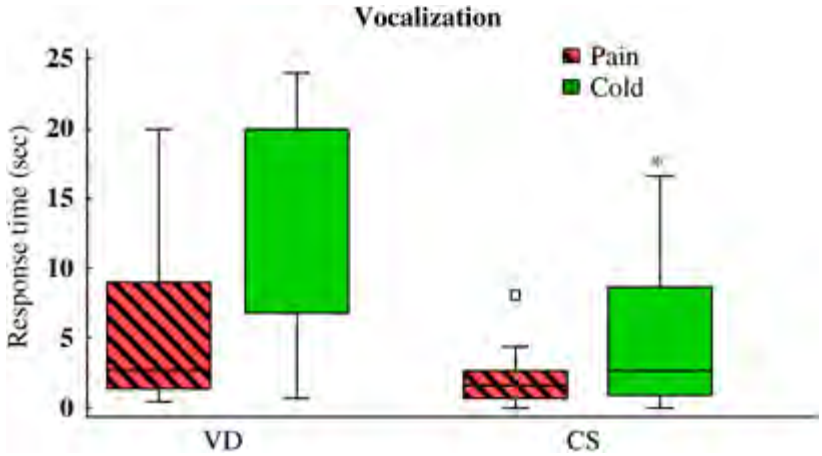

Figure 25. Mode of delivery affects response time to high- and low-intensity pain stimuli with longer time to start of vocalization in VD infants than CS, *modified from Bergqvist, et al. J Perinatol 2008*

7 DISCUSSION

7.1 FACTORS MODULATING NEONATAL PAIN RESPONSIVNESS

The capability of the newborn infant to feel and remember pain has been underestimated for a very long time, leading to unnecessary painful experiences in this population. Infants and neonates are even more sensitive to pain due to the immaturity of endogenous modulation. Despite the newborn infants' limited ability to communicate their pain, they should have the same human right to be alleviated from pain.

A scientific rationale for this thesis are illustrated in the figure ??showing the pathophysiology by which the physiological and behavioural responses of neonates exposed to acute and repetitive painful stimuli may lead to development of IVH and PVL in the preterm infant. Why do some infants develop severe neurologic injury, whereas others with similar clinical characteristics appear to do well? Does repetitive pain play a role? Does hyperalgesia produced by acute pain contribute to this vulnerability? Can pre-emptive morphine prevent some of these complications?

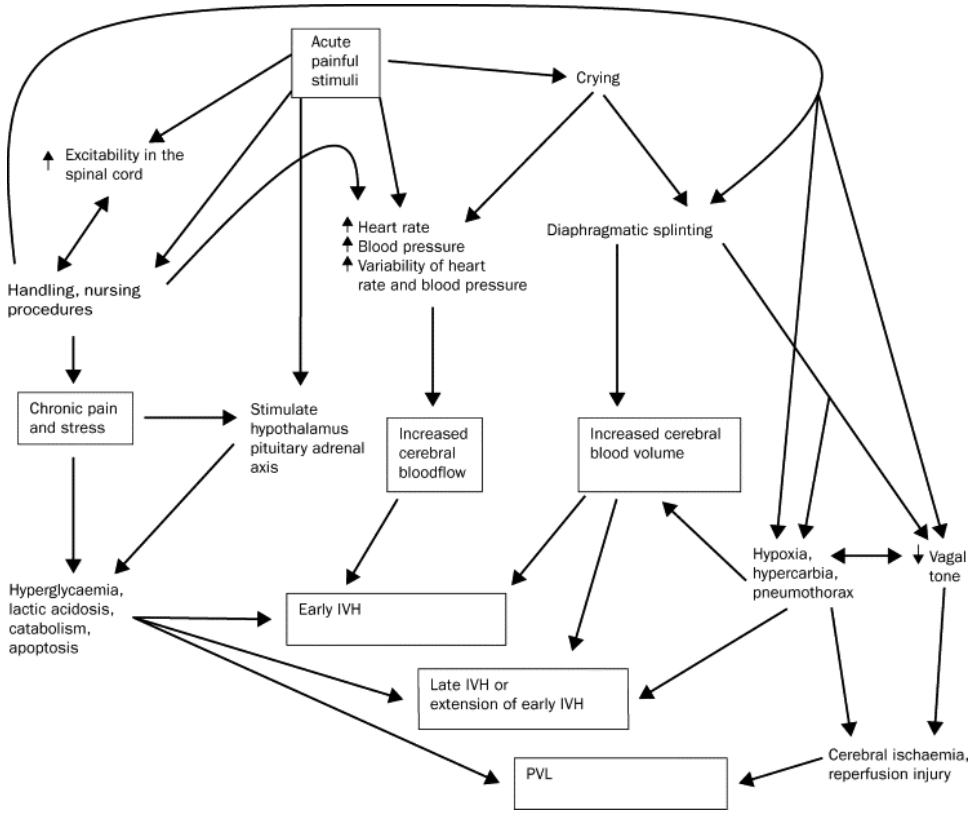

Figure 26 Pathophysiology of development to IVH and PVL, *from Anand, Bergqvist, et al. Lancet 2004*.

In the NEOPAIN Multicenter Trial (Paper I-II, IV), pre-emptive morphine infusions did not reduce the frequency of neonatal death, severe IVH and PVL in mechanically ventilated preterm infants. In the subgroup 27 to 29 weeks of GA, the incidence of severe IVH was higher in the morphine group. Due to ethical reasons, the protocol was designed with intermittent extra bolus doses of morphine based on clinical judgement at each individual NICU. An increased incidence of

the composite primary outcome also occurred in those who received intermittent bolus doses of open-label morphine.
Primary outcome in the two subgroups that did not receive any extra morphine doses showed an increase in the incidence of severe IVH and composite outcome (all infants), infants at GA 23 to 26 weeks showed an increased incidence of severe IVH, and at GA 27 to 29 weeks showed an increased incidence of the composite outcome. Several other factors were related to primary outcome, as GA, CRIB score, maternal antibiotics and chorioamionitis, has been previously documented (Volpe 2001). The logistic regression analyses showed that none of the poor neurologic outcomes were related to the randomized treatment group (pre-emptive morphine analgesia), but in the subgroup analyses the incidence of severe IVH was correlated to treatment group. Hypotension occurred more frequently in the morphine group at the end of the loading dose and at 24 hours after start of the infusion drug. Reduced noradrenaline levels following morphine therapy in preterm neonates may explain this degree of hemodynamic instability (Quinn et al. 1992). Accumulation of the study drug in the group of 27 to 29 gestational weeks might occur, because of hepatic or renal dysfunction and the use of higher infusion rates (20 μg/kg/hr) in this group.

The NOPAIN trial (Anand et al. 1999a) the pilot trial prior to the large NEOPAIN trial, that is included in this thesis. It showed that pre-emptive analgesia, may reduce the incidence of poor neurologic outcomes in preterm neonates who require mechanical ventilation support, but with a limitation in the sample size.

The design was a loading dose of 100 μg/kg followed with 10, 20, or 30 μg/kg/hour for GA 23 to 26, 27 to 29, and 30 to 32 weeks, respectively. Maybe this dose was too high? Simon et al 2003, had similar design with 150 neonates up to full term GA with the same loading dose but no increase in the infusion rate with GA, being standardized at 10 μg/kg/hour for all neonates. In this study, pre-emptive morphine analgesia actually decreased the incidence of IVH (23 vs. 40%, p=0.04) versus the placebo control group.

The pre-emptive morphine infusions did not improve short-term pulmonary outcomes. Additional morphine aggravated respiratory outcomes in preterm neonates with RDS. Dyke et al reported that morphine therapy improved mechanical synchrony and reduced HR and respiratory rate, without altering blood pressure, but also decreased the duration of oxygen therapy (Dyke et al. 1995). A Cochrane review by Bellu, 2008 concluded that there was no evidence for routine use of opioids in mechanical ventilated newborn infants (Bellu et al. 2008). We describe that pain expression during and after the loading dose decreases in the morphine group, with 1 PIPP score. Pain is central among the clinical outcomes that are selected for evaluation of opioid effects.

Our conclusion is that continues infusions of morphine do not per se increase the vulnerability of ventilated preterm infants, in their risk for early neurological injury. Risk factors worsening neurologic outcomes may include: systemic hypotension before starting a morphine infusion, the need for repeated intravenous boluses, as well as increasing infusion rates in preterm neonates. The final recommendation with maximal effect and minimal side effect is an issue that still not is solved (Levene 2005). We still have the dilemma to balance the importance of relieving pain and side effects by giving analgesia. Our future plans to explore the opioid treatment with an individualized dosing strategy within the NeoOpioid Consortium that aims to improve analgesia in the newborn population is described further in this thesis.

7.2 GENDER ASPECTS IN NEONATAL PAIN

Greater cortical activation was observed in male preterm neonates, in both cerebral hemispheres, contralateral and ipsilateral to the venipuncture, implying an increased neuronal activation caused by acute pain in the underlying brain regions (Paper III). This may result from surround-activation in the cortex and other areas activated to expand the signal, whereas other neonates (e.g. females) may develop surround-inhibition to localize the signal in these areas (Derdikman et al. 2003). Neurotransmitter release, use of metabolic energy, and activated or inhibited. Although neonatal NIRS response are modestly correlated with their pain assessments using PIPP scores, the greatest correlation occurs with facial expressions (Slater et al. 2008).

In a previous study, gender differences occurred in pain expressions within preterm and term newborn infants, studied PN day 1 to 5, who required a capillary puncture. The pain scales NFCS and NIPS were evaluated at bedside prior, during, 1, 3, and 5 min after heel lancing. The NFCS score profile in female neonates of all gestational ages expressed more facial features of pain than male infants (p=0.025) (Guinsburg et al. 2000). It is possible that female neonates have greater facial expressiveness, whereas male neonates show greater physiological instability in response to pain. Our data strongly support the need for further studies, particularly because of the potential to explain long-term effects of neonatal pain

Higher pain sensitivity occurred in ex-preterm girls in terms of tender points (Buskila et al. 2003), as compared to ex-preterm boys. We postulate that innate mechanisms of cell survival and cell differentiation in the developing preterm brains of boys and girls may be related to modelling of the pain system through adverse experiences in early life (Anand and Scalzo 2000b; Du et al. 2004).

7.3 FREQUENCY OF CLINICAL PROCEDURES IN THE NICU

The lack of central inhibition in the immature subject leads to exaggerated and generalized responses to all sensory inputs, affecting low as well as high-threshold stimuli. Specific pain responses may require convergent afferent inputs building up over time to become clinically apparent in the ex-preterm infants.

In a nested study within the NEOPAIN trail, we calculated number of pain and stressful procedures during the NICU stay. It was surprising that a small neonate born at GA week 25, was exposed to 700 procedures during 52 days NICU care.

The nature and numbers of pain of invasive procedures is described by others, Baker at al describe over 3 000 procedures in 74% of the infants below 31 weeks in the NICU (Barker and Rutter 1995b). More recently, Carbajal et al. found that each neonate experiences a median of 16 (range: 0-62) procedures per day of NICU stay. Of these, 79.2% procedures are performed without specific analgesia. Greater prematurity, type of procedure, parental presence, surgery, and other factors were associated with greater use of procedural analgesia, whereas mechanical ventilation and nonspecific analgesia were associated with less use of procedural analgesia (Carbajal et al. 2008). It is evident that this degree of exposure to acute pain may be associated with long-term effects.

When evaluating 19 pain indicators including both physiology and behavioural parameters. There were no consistent differences in the factor structures when contextual factors were explored (Stevens et al. 2007).

7.4 LONG-TERM EFFECTS OF UNTREATED PAIN

Exposure to repetitive neonatal pain may cause permanent or long-term changes because of the developmental plasticity of the immature brain. In an animal study resembling NICU care, repeated daily pain stimuli; describe decreased pain thresholds during development (Anand et al. 1999b). Increased plasticity of the neonatal brain may allow these and other changes in brain development to increase their vulnerability to stress disorders and anxiety-mediated adult behaviour (Anand and Scalzo 2000a; Bhutta et al. 2001).

There are strong indications that chronic pain may affect the wiring of the neuronal networks possibly leading to attention deficit disorders (Bhutta and Anand 2002; Bhutta et al. 2002; Nagy et al. 2003). In the newborn infants, functional magnetic imaging (fMRI) was used to study the resting-state networks driven by spontaneous signal fluctuations (Fransson et al. 2007). How does early pain affects these spontaneous activities with cerebral pain processing following NICU care?

7.5 MODE OF DELIVERY

Infants born after spontaneous VD showed a dampened behavioural (facial expression and vocalization) response to both pain and cold stimuli as compared to infants born by CS. Physiological responses (HR%) in VD infants were dampened just after birth, but increased progressively during the first hours after birth. This pattern was not evident among CS infants.

The objective assessment of pain perception based on physiological criteria lacks specificity for newborn infant (Stevens and Franck 2001). An increase in HR is, however, one of the most frequently reported physiological sign of acute pain or stress. We monitored such increase in response to two types of stimuli, which affect the same nerves fibres (c- and Aδ-fibre) but with different intensity. Strong correlation between HR increases and tissue damage following circumcision, heel lance or immunization are well documented in infant, (Franck 1986; Stevens et al. 1994; Johnston and Stevens 1996; Stevens et al. 2000) although no previous studies have examined these responses just after birth, or their progression in the hours following delivery, or the mode of delivery affects the strength of the response.

The dampened behavioural and physiological responses to pain and cold stimuli in our VD infant population, especially within the initial hours after birth, suggest that newborn infants may remain in a state of foetal inhibition for a variable period of time immediately after birth. To further investigate this phenomenon, we randomized infants to receive pain and cold stimuli directly after birth (>30 to ≤90 min after delivery) or later (>90 to <120 min) in a more sleepy state.

When fentanyl analgesia was administered to the foetus during invasive procedure at GA of 20 to 35 week, an attenuated stress response to painful stimuli was found, indicating that the fetus can react to pain before birth (Fisk et al. 2001).

Thus our findings suggest that VD *per se* triggers the activation of an analgesic mechanism. Very high levels of CA are released following VD (Faxelius et al. 1983; Lagercrantz and Slotkin 1986). The associated arousal is mediated by this sympathoadrenal activation is probably associated with

increased noradrenergic activation of the whole brain from the locus coeruleus (Lagercrantz 1996). Activation of adrenergic α_2-receptor through the released noradrenaline may have strong analgesic effects like clonidine (Kamibayashi and Maze 2000). VD neonate also exhibits significantly higher plasma levels of β-endorphin than infant born by CS, which may further promote this difference (Facchinetti et al. 1986; Raisanen et al. 1986; Bacigalupo et al. 1987). The relative low baseline HR in the VD infants with gradual increase in HR after VD in response to pain and cold stimulus during the first 4 h after birth may also be due this endogen mechanism of stress-induced analgesia (Mogil et al. 1996). The rapid decline in circulating levels of β-endorphin during the initial 2 h following VD is in agreement with our findings.

CS mothers in the present study received spinal anaesthesia with Bupivacaine, whereas VD mothers used N_2O during uterine contractions in the first and second stage of labor. Bupivacaine given spinally remains inaccessible to the paravertebral venous plexus and is unlikely to cross the placenta to produce systemic effect in the foetus or neonate. Such effect of Bupivacaine would have resulted in a lower baseline HR in the CS than in VD infants, but no such difference actually occurred. As VD mother rapidly eliminate N_2O through the lungs, it seems unlikely that N_2O could affect their newborn infant studied at ≥30 min after delivery (Stenqvist 2000).

7.6 CORTICAL ACTIVATION

Neuronal activation within cortical areas is a network with regional changes in cerebral flow (r-CBV), thus reflected in the [HbO_2] changes measured in preterm newborn infants. Both tactile and painful stimuli elicited bilateral increases in cortical [HbO_2] concentrations, implying increases in regional cerebral blood flow that resulted from changes in metabolic activity within the somatosensory cortical areas. Slater et al confirmed that similar cortical activation correlates with the behavioural responses to pain, with their overall PIPP score and particularly well with their facial expressions (Slater et al. 2008). However, some infants without any facial expressions and low PIPP scores were still noted to have cortical activation of the somatosensory areas. Further research to elucidate the neurophysiological correlates of these findings may explain the discrepancy between cortical activation responses and neonatal behavioural responses to acute pain.

8 CONCLUSIONS

- We have demonstrated that pre-emptive morphine analgesia in very preterm infants does not reduce the primary neurological outcomes described as death, severe IVH or PVL. However, extra intermittent doses of morphine by clinical judgements were associated with an increased incidence of these outcomes.

- Infants receiving the extra intermittent doses of morphine in the NEOPAIN trail had a lower gestational age, higher severity of illness score (CRIB), greater use of maternal antibiotics and more frequent signs of chorioamnionitis. Consequently, these sicker and smaller infants were at increased risk for early neurological injury already before opioid administration, which usually presents clinically as increased irritability. Greater irritability and agitation in ventilated preterm neonates may have been estimated as expression of pain and treated with morphine analgesia.

- The pre-emptive morphine infusions did not improve short-term pulmonary outcomes, but additional morphine doses appeared to worsen the respiratory outcomes in preterm infants with RDS. Again, the caveat of greater severity of illness applies to these outcomes as well.

- Prolonged pain in ventilated preterm neonates is extremely difficult to identify with any degree of accuracy. It is not surprising; therefore, that neither experience nor education improves the ability of professional NICU staff to estimate analgesia in preterm infants. Because of these difficulties in clinical assessments, we investigated other methods that may provide a clearer picture of pain responses in the immature brain.

- Near infra-red spectroscopy documented a functionally specific somatosensory cortical activation following pain and tactile stimuli in preterm infants. We documented bilateral responses after a unilateral stimulus, and these responses were accentuated in male infants, at lower gestational ages, and at higher postnatal ages. A graded response in the somatosensory cortex supports the possibility of conscious sensory perception in preterm neonates.

- Vaginally delivered term infants demonstrated less responsiveness to pain with facial expression, cry, and heart rate changes, following injection of vitamin K as compared with infants born by caesarean section. Pain and stress reactivity show dampened and gradually increasing pain response in the first hours following birth suggesting that there is a foetal inhibition activated during vaginal delivery, but this does not occur following caesarean section delivery.

Recommendation from our results

- Reduce the numbers of painful or distressing procedures as much as possible in newborn infants.

- Always assess newborn infants with validated pain assessment tools for all painful or stressful procedures. Additionally, use pain scales for estimation of chronic pain in all preterm and newborn infants needing intensive care treatment.

- Register all pain and stressful interventions.

- Continue further studies to investigate the safety and efficacy of other analgesics including opioid in ventilated neonates and develop more reliable methods to assess prolonged or chronic neonatal pain.

- The challenge is to find the best ways to treat neonatal pain, with maximal analgesia and minimal side-effects from the short- and long-term perspective.

9 FUTURE DIRECTIONS

My experience during these years have been educating in so many perspectives performing the practical recruitment for the patients in both NOPAIN and NEOPAIN studies. Have participated in meetings, discussions for design development to hypothesis, project plans, coordinated both National and International parts.

Although our understanding of neonatal pain has increased during the last years, there are a number of remaining problems particularly with regard to alleviating drug treatment. To develop consensus how to prevent and treat neonatal pain I have coordinated together with a team co-workers in the design of an EU project to get a stream of all positive knowledge to improve our analgesia in the newborn infant.

The overall aim of this EU project is to assess effects and safety of opioid treatment in very preterm infants in relation to pharmacokinetics and genetic predisposition. Furthermore, a major goal is to develop a Paediatric Use Marketing Authorisation (PUMA) for safe administration of opioid analgesic treatment in newborn infant. Both morphine and fentanyl are off-labelled (EMEA) drugs. Several studies have shown that opioid treatment in neonates reduce pain and stress responses. However, these drugs also have side-effects related to plasma concentrations of the drugs and their metabolites. The new approach in the current project is to obtain a more personalized drug therapy to the newborn infant, and to consider individual differences in pharmacokinetics and genetics, as well as gender effects. We will perform a multinational European survey of practices regarding sedation and analgesia in neonatal units. Clinical multicenter trial evaluating safety aspects of morphine and fentanyl will be performed. The responses (evaluated with validated pain scales, stress hormones, clinical variables, near infrared spectroscopy and aEEG) will be related to pharmacokinetics and genetic predisposition. There are strong indications that chronic pain may affect the wiring of the neuronal networks possibly leading to attention deficit disorders. In a few infants, functional magnetic imaging (fMRI) will be used to study cerebral pain processing, complementary to NIRS and aEEG measurements. A "child friendly" formulation adapted for use in neonates will be developed with by a SME partner in the project. The results will be disseminated by scientific and popular articles, the web, videos and special pain courses for physicians and nurses etc. The project will lead to a considerable improvement of the strategy to alleviate neonatal pain. It will also generate new information about brain development, pharmacokinetics and genetics.

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