Early Pain, Beware the Brain!

Long-term effects of neonatal pain experiences

Vroege pijn, kijk uit voor de hersenen!
Lange-termijn effecten van neontale pijn ervaringen

Proefschrift

Renata Henrica Johanna Adriana Schouw

CIP gegevens

ISBN: 90-8559-211-9

Printed by: Optima Grafische Communicatie, Rotterdam
Lay-out: Renata Schouw & Jurgen van Oorschot, Heijningen
Photo cover: ESA and Justyn R. Maund (University of Cambridge)

New observations with the Hubble Space Telescope allow a look into a supernova explosion under development. In this artist's view the red supergiant supernova progenitor star (left) is exploding after having transferred about 10 solar masses of hydrogen gas to the blue companion star (right). This interaction process happened over about 250 years and affected the supernova explosion to such an extent that SN 1993J was later known as one of the most peculiar supernovae ever seen.

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Ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam,
op gezag van de rector magnificus
Prof. dr. S.W.J. Lamberts
en volgens het besluit van het College voor Promoties

De openbareverdediging zal plaatsvinden op
woensdag 27 september 2006 om 13:45

door

Renata Henrica Johanna Adriana Schouw

geboren te Roosendaal en NIspen

Promotiecommissie:

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Chapter 1

General Introduction

General introduction to the pain system

1.1 Introduction

Up to the 1980's pain in infants was a neglected issue in medical practice. It was generally believed that inadequate myelinisation of nerve fibers, prevented newborns from feeling pain. Yet there were doubts about the veracity of this assumption. In 1987, Anand and co-workers 'solved' this controversy: they reported that administration of analgesics significantly reduced morbidity and mortality following neonatal surgery, suggesting that the systems necessary for pain transmission are intact and functional at birth[1]. Since this landmark publication, our understanding of neonatal pain processing and neurobehavioral development has greatly increased.

These days, the human central nervous system (CNS) is generally acknowledged to be very immature at birth and to show great plasticity in the first year of life[2-4]. Disturbances of the normally occurring activity patterns are likely to distort the development of the CNS. During this critical period, therefore, the CNS is most susceptible to perturbation than at any other time of life [2, 3, 5].

The immaturity of the CNS in humans has been demonstrated most clearly by Andrews and Fitzgerald. They showed that newborns (27 to 32 weeks post conceptional age) who were not given analgesics showed a decreased flexion withdrawal reflex following von Frey hair stimulation, when compared with the intact heel[6]. This reflex threshold was found to increase with gestational age[7]. Moreover, the receptive field for this reflex is considerably larger in preterm neonates and reduces with postconceptional age[6, 7]. Prematurely born neonates (< 30 weeks gestational age) are more sensitive to repeated pain stimuli, resulting in sensitization[6, 8]. This is in contrast with neonates with a postconceptional age of 36 weeks or older, who develop habituation after repeated pain stimuli. Sensitization is, however, not only restricted to the ipsilateral side of wounding but extends to the contralateral side, as suggested by the finding that the threshold to evoke a flexion withdrawal reflex of the non-stimulated opposite foot was also sensitized[6]. The same is true for reflex thresholds on the leg, since there was an increase in threshold progressing up the leg from the injured foot to the knee[6].

1.2 Development of the pain system

Morphological studies in animals provide more detailed information about the maturation of the CNS. For example, it was found that, the developing rat nervous system perinatally undergoes considerable structural and functional reorganization largely governed by neuronal activity and experience[9]. Human pain system development (table 1) already starts as early as six weeks gestational age. At this time the first synapses between sensory neurons and dorsal horn cells are formed[10]. Further organization of the laminar structure of the dorsal horn cells and their synapses begins in the following weeks. Around the same time, from 8 to 10 weeks gestational age, the fetal neocortex starts to develop[11]. We see the onset of peripheral nervous system development in the 11-weeks-old embryo. At this time sensory neurons reach the skin of the limbs[11].

During the second trimester, the sensory nerves innervate the trunk[11]. In the dorsal horn specific neurotransmitter vesicles appear that are of importance for the release of glutamate, glycine or substance P in response to pain stimuli[12-14]. Further cerebral maturation and differentiation takes place as dopaminergic and noradrenergic fibers penetrate the cortex by 16 weeks gestational age[15, 16]. Around 19-20 weeks gesta-

tion synaptogenesis begins. During synaptogenesis, the dendritic processes undergo extensive division[11, 17] and will reach the substantia gelatinosa (lamina I and II) of the dorsal horn. Dorsal horn pain signaling involves the NMDA receptor. This receptor does not participate in normal transmission. Only during maintained C fiber stimulation, as in case of peripheral inflammation or nerve damage, peptides are released that allow the NMDA receptor to be activated[18]. NMDA receptor dependent amplification results in decreased thresholds, increased amplitude and number of responses, a phenomenon also referred to as wind-up[19].

However, before the NMDA receptor participates in transmission, several events must have taken place. First the peripheral input needs to be of sufficient intensity and duration[19, 20]. Then glutamate is able to bind to the NMDA receptor. For receptor activation glycine, which usually acts as an inhibitory transmitter, binds to the receptor as a co-agonist. After this binding process, the NMDA receptor ion channel is still blocked by a magnesium ion[19, 21]. This channel block can be removed by sufficient repeated membrane depolarization. When the ion channel opens, sodium and calcium flow into the neuron, resulting in an increased excitability[19].

In humans the second trimester is further characterized by an increase in cortical thickness. However, cell density reaches its peak at 20 weeks gestation after which it progressively declines and reaches a stable number at 40 weeks.

In the third and last trimester, nerve tracts associated with nociception are fully myelinated up to the thalamus[11]. The descending inhibitory controls have grown down from the brainstem via the dorsolateral funiculus of the spinal cord to the dorsal horn. These descending tracts are important for the modulation of nociceptive input during life. At birth, however, this inhibition is still immature[6, 22, 23]. Complete skin innervation, by both large A-fibers and small C-fibers, is fully present at birth (36 to 42 weeks)[24]. In rats, the final maturation of these sensory nerves and end-organs (e.g. Merkel cells and Meissner's corpuscles) takes place during the first postnatal weeks[25].

During the first months of life, there appears to be an overexpression of NMDA receptors. Apart from its function as a signalling mechanism, the NMDA receptor is also crucial for the development of normal connections in the neonatal nervous system. During this distinct period, receptor excitation is great in amplitude and the channel is less sensitive to magnesium ions and therefore more easily activated. These characteristics account for the higher pain sensitivity observed for neonates in comparison with older children and adults[19.]

Subsequently, the sensitivity of neurons to nerve growth factor (NGF) declines during maturation. As a consequence, a significant number of axons die postnatally. This regressive event occurs early in life, which is important for normal neuronal development [26 27]. This process of normal neuronal cell death is also referred to as 'fine-tuning' of neuronal circuits[28].

1.3 Susceptibility to perturbations

Several animal studies found that neonatal inflammation and tissue damage may affect the maturation of the central nervous system and consequently increase pain sensitivity on the long-term (Table 2). Studies suggest that a rat at birth is comparable to a preterm infant, and that rat postnatal day 7 corresponds with a human postconceptional age 40 weeks. At postnatal day 14 a rat is comparable to a one-year-old and at three weeks to an adult human.

Table 1 Development of the human pain system

Age	*Development*
6 Weeks postconceptional age	* Dorsal horn cells start to develop * First synapses between sensory neurons and dorsal horn cells are formed
6 to 13 weeks	* Organization of the laminar structure of the dorsal horn and synapses
8 to 10 weeks	* Development fetal neocortex
11 weeks	* Sensory neurons reach the skin of the limbs
11 to 20 weeks	* Sensory innervation of the trunk
13 weeks	* First neurotransmitter vesicle appears
16 weeks	* dopaminergic and adrenergic fibers penetrate the cortex
19 to 20 weeks	* Synaptogenesis in the cortex begins * Extensive division of dendritic processes
20 weeks	* Sensory innervation of mucosal surfaces * Peak cortical density is reached followed by a steadily decline
30 weeks	* Dorsal horn completely developed and myelinated up to the thalamus
Birth	* Complete skin innervation by A and C fibers * A stable cortical cell number is reached
1 week postnatally	* C fibers start to evoke electrical activity
Following postnatal weeks	* Final maturation of sensory nerves and end-organs * Decreased sensitivity to NGF and therefore death of axons (fine-tuning)

1.3.1 Inflammatory pain exposure

Several animal studies have evaluated the consequences of neonatal inflammation. Inflammation can be induced by a variety of agents, i.e. Complete Freund's Adjuvans (CFA), formalin, bee venom, carageenan and capsaicin. These result in specific nocifensive behavior, e.g. inflamed limb guarding, limb flexion, squirming and rhythmic mouth opening[29, 30].

Besides these short-term effects, some of these inflammatory agents may induce dose-dependent long-term sequelae. For example, 5µl-25µl carageenan injected at neonatal age[4, 31] will result in increased primary afferent terminal fields on the short term, but not alter mechanical and heat pain thresholds at adult age[4, 31]. Nevertheless,

the behavioral response to acute pain does appear to be diminished as there are less stress-related neuro-endocrine markers detectable (CRF, vasopressin, ACTH)[32]. The same is true for both the doses 5 µl and 25 µl CFA[2, 4]. No alterations in mechanical or heat pain sensitivity could be found in adulthood. However, reinflammation induced by injecting 25 µl CFA, seemed to produce hypersensitivity in rats neonatally exposed to 25 µl CFA[2, 4]. Histological examination of the 25µl CFA group, revealed permanent expansion of the thermal receptive field[4]. Moreover, CFA was found to increase the number of primary afferents in the substantia gelatinosa of the dorsal horn and even to extend several spinal levels caudally ipsi- and contralateral[2]. Regrettably, no data concerning peripheral alterations are available.

1.3.2 Tissue damage

As a consequence of wounding the skin shortly after birth, A-fibers and C-fibers develop ectopic activity which may persist for many weeks[21]. This will lead to enhanced pain sensitivity on the short term, both at the site of injury (primary hyperalgesia) and the surrounding skin (secondary hyperalgesia)[33]. Long-term findings are controversial. Neonatal surgery, followed by an analgesic dose of morphine, lead to decreased pain behavior in mice[34]. Decreased pain thresholds, however, were reported in rats formerly exposed to neonatal pain[33, 35]. A possible explanation for this hypersensitivity may be the occurrence of neuroanatomical alterations. Animal data show that wounding the skin results in peripheral nerve death, leaving an area of deafferentiation. Nevertheless, five days later however, a peripheral sprouting response is observed, overcompensating the damage, and resulting in hyperinnervation[31, 36]. Both myelinated A-fibers and unmyelinated C-fibres contribute to this sprouting response[29]. Besides local collaterals, also sensory fibres drawn from deeper tissue and non-cutaneous nerve bundles sprout into the deafferentiated area[29].

These neuro-anatomical alterations cause the peripheral nervous system to become permanently distorted when sprouting takes place the first week after birth (in rats). Low threshold information will then be interpreted as nociceptive. However, wounding induced in the second week of life or thereafter, elicits a reversible sprouting response that not leads to altered sensitivity on the long term[2]. There thus seems to be a window of susceptibility during which alterations will persists and leading to permanently altered sensitivity. Unfortunately, there are no data concerning spinal and supraspinal alterations following the infliction of skin wounds.

1.3.3 Visceral pain exposure

Neonatal patients not only suffer from pain due to tissue damage associated with high technological ICU environment[37] and surgery, but may also suffer from visceral pain. An animal model for visceral pain was created, by exposing neonatal rats to mechanical (colorectal distension) or chemical (intracolonic injection of mustard oil) irritants for variable times and at different ages. Visceral nociceptors are susceptible to sensitization, resulting in sensory hypersensitivity on the short-term[38]. Long-term behavioral responses can be assessed by measuring the abdominal withdrawal reflex (AWR). This is an involuntary motor reflex similar to the visceromotor reflex. Colonic irritation in postnatal rat pups appeared to result in chronic hypersensitivity at adult age, as manifested by a significantly increased AWR[39]. Moreover, neonatal visceral irritation may also lead to higher prevalence of functional intestinal disorders in adulthood[40]. As chronic visceral

hypersensitivity is also associated with both peripheral sensitization and central sensitization, it is thus likely that visceral pain and irritation at neonatal age leads to abnormal visceral pain processing later in life[39, 41]. However, only few data are available concerning neuro-anatomical alterations following neonatal visceral pain exposure.

Table 2 Animal studies

Type of stimulation	*Results*
Inflammatory (e.a. CFA, carageenan)	- C-fos expression - Increase in primary afferents dorsal horn, extending caudally to the level of pain exposure - Long-term effects are related to dose and type of inflammatory stimulus, only 25µl CFA causes long-term alterations
Tissue damage	- Peripheral and spinal nerve death, predominantly the developing neurons - Sprouting response after 5 days resulting in peripheral hyperinnervation - Ectopic activity A and C-fibers
Visceral pain	- Neonatal colonic irritation results in chronic hypersensitivity in adults, evaluated by the withdrawal reflex

1.4 Human studies

While providing unique opportunites to investigate basic mechanisms concerning pain threshold alterations, animal models can never match the complexity of the human condition[42]. An important problem is the near impossibility of generalizing findings to humans, which becomes apparent from various factors. First, there appear to be interspecies differences concerning pain sensitivity[43, 44]. Second, animals are kept in well-controlled environments with less variability than a NICU[42,] and it is stimulus deprivation within the first period of life that itself may affect CNS[45]. Third animal mothers have been found to act differently on their pups that were exposed to pain, in comparison with human mothers. This is a factor which we cannot control for[3].

Until now only few studies have evaluated the prolonged effects of neonatal pain exposure in humans, and results are conflicting (Table 3). These studies are discussed below.

As mentioned in the introduction, preterm babies are thought to be more vulnerable to adverse stimuli, such as pain, than are term born babies. Repeated stimulation with Von Frey hairs in infants of less an 35 weeks gestational age, resulted in sensitization, as manifested by increased amplitude and number of responses together with a drop in threshold[6, 8]. The sensitization is considered to be a manifestation of the immaturity of spinal or supraspinal modulation[46]. Oberlander was unable to demonstrate any long-term alterations in biobehavioral responses due to neonatal pain exposure during finger lance at 4 months[47]. On the contrary, premature neonates demonstrated increased cortisol stress responses in new situations at 8 months, and increased somatization at the age of 3 years; they also reported higher intensity of medical pain at 10 years and dimi-

nished quality of life during puberty (12 to 16 years)[48-51]. Pain exposure thus not only alters the development of the pain system, but effects long-term social-emotional functioning as well. To date, only one study reported alterations in pain sensitivity up to 18 years following former preterm infants' NICU admission[14]. The former preterm infants appeared to have more tender points and reported lower pain thresholds assessed by a dolorimeter, when compared with healthy full term born controls[14]. Regrettably, possible differences in long-term pain sensitivity between premature- and term infants both neonatally exposed to pain have not yet been explored.

In contrast with preterm infants, term infants develop habituation to repeated stimulation with Von Frey hairs shortly after birth, as manifested by a decreased number of responses and no lowering in pain thresholds[6, 8]. During postnatal maturation, the initial threshold increases gradually[6, 8]. However, following injury the spinal cord excitability increases. During this period, single stimuli can cause long-lasting after discharges in dorsal horn cells. This was observed by Andrews, in the shape of increased pain sensitivity at the wound and contralateral side 24 hours after surgery in infants of 36 to 89 weeks of age[52]. Taddio found abnormally increased pain sensitivity during routine vaccination at ages 4 and 6 months in term born infants who as newborns had been exposed to circumcision[53]. This type of hypersensitivity might normalize during early childhood, seeing that as Peters et al could not demonstrate altered behavioral and physiological pain responses during routine vaccination at 14 months and 4 years in term born children who had undergone surgery as neonates. These latter findings suggest that either the long-term effects recover when children grow older or that these long-term effects are local and not generalized. Peters and colleagues made this assumption as they induced vaccination in a completely different dermatome than that of tissue damage. Long-term pain threshold development during childhood (>4 years) is a study issue that deserves our full attention. Nowadays, adequate analgesics are administered in order to prevent long-term alterations in pain sensitivity[54]. Some children, however, are exposed to high and prolonged dosages of morphine for sedation purposes in the absence of pain, for example during ECMO treatment. The effects of morphine administration in the absence of pain have been evaluated in an experimental setting[55], but there are no human studies evaluating the effect of morphine on long-term pain sensitivity.

1.5 Scope of this thesis

Only as late as two decades ago, physicians have become aware that newborns are capable of feeling pain. Since then, several studies have been performed evaluating the effects of early pain exposure. Up to now, there are no reports demonstrating long-term alterations in pain thresholds and pain tolerance six to ten years after neonatal pain exposure. Moreover, there are indications that exposure to excessive amounts of morphine might alter the central nervous system. Therefore, the studies in this thesis aim to improve the knowledge on long-term consequences of both early pain and morphine exposure.

Firstly, we analyzed the most probable location (dermatome) of pain threshold alterations (**Chapter 2**).

Tabel 3

	Age	*Assessment method*	*Groups*	*Results*
Fitzgerald 1989	27 to 32 PCA	Von Frey hair following heel lance	* no analgesics * EMLA	Decreased flexion withdrawal reflex at the heel compared to the EMLA group
Andrews 1994	27,5 to 42,5 weeks PCA	Repeated Von Frey hair stimulation	* Preterm (27,5-35) * Term (36-42,5)	Decreased threshold flexion withdrawal reflex in preterms. Habituation in term neonates
Andrews 1999	28 to 42 weeks PCA	Von Frey hair stimulation	68 neonates	- Flexion withdrawal reflex increases with age - the reflex decreases at wounding the skin and after repeated stimulation
Andrews 2002	36 to 89 weeks 24h after surgery	ASR Von Frey hairs	* 21 patients * 4 controls	Wound and contralateral side show increased pain sensitivity
Oberlander 2000	4 months	Fingerlance	* ELBW * Control	No differences in biobehavioral pain response
Taddio 1997	4 months 6 months	Vaccination	* Circumcision EMLA placebo * Control	Increased pain sensitivity in circumcision group

	Age	*Assessment method*	*Groups*	*Results*
Grunau 2004	8 months	Cortisol stress response	* Preterm * Control	Preterms react with increased cortisol stress response to the introduction of a new toy
Grunau 1994	3 years	Personality Inventory for Children	* Extremely low birth weight (ELBW) neonates * Healthy term controls	ELBW neonates showed higher somatization
Peters 2003	14 months 4 years	Vaccination	* Surgery < 3 months of age * Control	No differences in behavioral and physiological pain response
Grunau 1998	8 to 10 years	Pediatric Pain Inventory (PPI)	* ELBW * Control	ELBW children reported increased intensity of medical pain events
Saigal 1996	12 to 16 years	Quality of Life questionnaire	* ELBW * Control	ELBW children reported diminished quality of life compared to controls
Buskila 2003	12 to 18 years	Dolorimeter	* Preterm * Control	Former preterm infants had more tender points and reported decreased pain thresholds

To investigate long-term alterations in pain sensitivity, we assessed thermal detection and pain thresholds using a Termal Sensory Analyser (TSA) device. Thresholds were assessed in three different groups, i.e. children who had undergone abdominal or thoracic surgery within the first three months of life (**Chapter 3**), children who had undergone artificial ventilation during the first weeks of life without being given morphine (**Chapter 4**) and children who had undergone Extra Corporeal Membrane Oxygenation (ECMO) during which they had received excessive amounts of analgesics (**Chapter 5**).

Pain is a complex experience, consisting of a sensory discriminative component and affective emotional component. The first can be assessed by evaluating pain thresholds. However, as neuroanatomical alterations following pain exposure are suspected to extend to central levels, the affective emotional component of pain may show alterations, too. This is why we assessed the suprapain thresholds as well (**chapter 3, 4 and 5**).

In **Chapter 6** we further studied the affective emotional component by evaluating differences in pain perception and coping among the three groups of patients described in chapters 3 to 5, in contrast to healthy controls by administering the Pain Coping Questionnaire (PCQ) and the Pediatric Pain Inventory (PPI).

Furthermore, we simultaneously evaluated the occurrence of chronic pain and somatization in the previously described groups in contrast to healthy controls by administering the structured pain questionnaire and the children's somatization inventory (**Chapter 7**).

The final chapter, incorporates the results of our studies into the discussion of the main research questions concerning long-term alterations in pain thresholds and pain tolerance (**chapter 8**). Furthermore, suggestions for future studies are given.

1.6 Supplement

1.6.1 The flexion withdrawal reflex

An easy and frequently used method to evaluate pain sensitivity in neonates is the nociceptive withdrawal reflex. This reflex was first described by Sherrington in 1910, as a classic nociceptive reflex resulting in withdrawal of the limb from a noxious stimulus[56]. It can be used as an index of spinal cord excitability, as the reflex amplitude was found to be linearly correlated with the perception of pain[6-8]. Myelinated A-fibers and unmyelinated C-fibers convey the reflex impulses[57]. In experimental settings, this reflex can be elicited with Von Frey hairs, a single nylon monofilament inserted into a Perspex rod[7]. The Von Frey hair filament is pressed onto specific areas of the body with enough pressure to make the filament bend[58].

1.6.2 Quantitative Sensory Testing (QST)

Quantitative Sensory Testing (QST) determines differences in thermal thresholds, using the Medoc Thermal Sensory Analyser (TSA) II (Medoc, Ltd. Advanced Medical Systems, Ramat Yishai, Israel®). This is a precise, computer-controlled device capable of generating and recording a response to highly repeatable thermal stimuli, i.e., warmth, cold, heat-induced pain or cold-induced pain at a minimum and maximum temperature of –9.9 and 50C, respectively. Testing is based on the "method of limits". Starting from a baseline temperature of 32C warmth or cold is steadily, linearly increased. As soon as the subjects perceive the stimulus (i.e. warmth or cold), they press a button, after which the

stimulus directly reverses to baseline, i.e. temperature is set back to 32C. The temperature at which the button was pressed is the detection threshold. In a following test, subjects are asked to press the button not until the moment when warmth or cold becomes painful. The temperatures at these moments are their pain thresholds. If subjects do not press the button before –9.9 or 50C the test is automatically terminated, returning to baseline temperature. The computer calculates the difference between the baseline and the signaled peak temperature. With this test, both the A delta (first pain) and C-fibers (second pain) are activated.

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Chapter 2

Does neonatal surgery lead to increased pain sensitivity in later childhood?

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Pain 2005; 114: 444-454

Abstract

Does pain or tissue damage in early life lead to hyperalgesia persisting into childhood? We performed a cross-sectional study in 164 infants to investigate whether major surgery within the first 3 months of life increases pain sensitivity to subsequent surgery and to elucidate whether subsequent surgery in the same dermatome or in a different dermatome leads to differences in pain sensitivity. All infants received standard intraoperative and postoperative pain management, with rescue analgesia guided by a treatment algorithm. Differences in pain sensitivity during surgery were assessed by the intraoperative fentanyl intake and by (nor)epinephrine plasma concentrations. Differences in postoperative pain sensitivity were assessed by the observational pain measures COMFORT and VAS, and by morphine intake and (nor)epinephrine plasma concentrations. Infants previously operated upon in the same dermatome needed more intraoperative fentanyl, had higher COMFORT and VAS scores, had greater (nor)epinephrine plasma concentrations, and needed also more morphine than did infants with no prior surgery. In contrast, infants who previously underwent surgery in another dermatome had only significant higher postoperative analgesic requirements and norepinephrine plasma concentrations in comparison with infants with no prior surgery. These preliminary differences may indicate the occurrence of spinal and supraspinal changes following neonatal surgery. We conclude that the long-term consequences of surgery in early infancy are greater in areas of prior tissue damage and that these effects may portend limited clinical but important neurobiological differences.

1. Introduction

Multiple lines of evidence suggest that exposure to acute pain during the neonatal period leads to a prolonged hypersensitivity extending beyond the period associated with complete healing of the initial tissue injury[2-4-8,9]. Confirmatory animal experiments illustrate that exposure to neonatal pain is developmentally inappropriate. It disturbs normal development of the nociceptive neural circuits, causing structural and functional neuroanatomical changes, both peripheral[10, 11,12] and at the level of the spinal cord[13], but may not alter responsiveness of the HPA axis[14,15]. Depending on the degree and duration of inflammation, anatomical changes at the level of the spinal cord were not restricted to the dermatome of neonatal pain exposure, but extended to other segments of the spinal cord[16,13 ,15]. Similar neuroanatomical changes cannot be ruled out in newborn infants exposed to early pain or tissue damage, but have never been demonstrated in humans.

Whether administration of analgesics prevents neonates from developing these long-term sequelae is not clear. In extremely low birth weight infants, early morphine treatment affects their pain response to subsequent pain exposure at the age of 4 months [6]. Grunau[17] suggested that morphine therapy ameliorates the effects of early repetitive pain in preterm neonates, whereas Taddio[4] found 4–6 month-old term infants, who had undergone unanesthetized circumcision, responded more intensely to immunization in comparison with matched, but uncircumcised peers. Recently, we found that 14 and 45-month-old children who had been operated within the first three months of life following pre-emptive analgesia responded similarly to immunization pain as their non-operated age-matched controls[18.] In contrast to animal data[19], Andrews et al.[8] found that surgery under caudal or epidural analgesia did not prevent human infants from developing hyperalgesia. The area of incision, and to a lesser extent the unaffected contralateral side of the body, demonstrated greater tenderness to mechanical stimuli 3 months after surgery in comparison with matched control infants.

In summary, it is disputable whether adequate analgesia prevents the development of long-term alterations in pain sensitivity and whether this pain sensitivity is restricted to the dermatome of tissue injury (spinal changes) or whether is generalized all over the body (supraspinal changes)20. This study was designed to assess whether major surgery during early infancy increases pain sensitivity to subsequent surgery; and to elucidate whether the subsequent surgery in the same dermatome or in a different dermatome leads to differences in pain sensitivity.

2. Materials and methods

2.1. Study design

We performed a cross-sectional follow-up study of subjects previously enrolled in a randomized clinical trial (RCT) described elsewhere[21-23]. In brief, this RCT included 0–3-year-old infants (N=202) admitted to the pediatric surgical intensive care unit (ICU) after major non-cardiac thoracic or abdominal surgery. Patients were excluded from the RCT if surgery was carried out at <36 weeks post-conceptional age, if they had received analgesic or sedative drugs <6 h before surgery, if they had received neuromuscular blockade, and/or suffered from hepatic, renal or neurological disorders or altered muscle tone.

In 1995, the anesthetic and postoperative analgesic management was altered and standardized according to the protocols described below (Sections 2.3.1 and 2.3.2). Infants were excluded from this cross-sectional study (1) if they had undergone more than one major surgical procedure during their life (n=16), (2) if their first surgical procedure was carried out before 1995 (n=13), or 24 if their first surgical procedure was carried out at >14 weeks of corrected postnatal age (n=9).

A total of 164 infants were allocated to one of three groups based on their previous medical history. Infants undergoing surgery for the first time were considered as controls (group I) and compared with infants undergoing their second surgical operation but not within the same dermatome as their first operation (group II), and those undergoing a second surgical operation in the same dermatome as their first surgery (group III).

2.2. Measures

2.2.1. Intraoperative pain sensitivity

Intraoperative pain sensitivity was assessed by total fentanyl dose required during surgery (μg kg−1 h−1) and by epinephrine and norepinephrine plasma concentrations, measured by HPLC using fluorimetric detection. Arterial blood samples for measuring epinephrine and norepinephrine plasma concentrations were taken after induction of anesthesia, at the end of surgery and at 6, 12 and 24 h after surgery.

2.2.2. Postoperative pain sensitivity

Postoperative pain sensitivity was assessed by: total morphine required during the first 36 h (μg/kg/h) after surgery, postoperative COMFORT 'behavior' scores, visual analogue scale (VAS) scores, and postoperative epinephrine and norepinephrine plasma concentrations.

The COMFORT 'behavior' scale[25] comprises six behavioral items: alertness, calmness, muscle tone, physical movement, facial tension, respiratory response (in ventilated patients) or crying (in non-ventilated patients), scored on a 5-point scale, ranging from 1 to 5, with total scores ranging from 6 to 30. This scale was proven to be reliable and valid for postoperative pain assessment. Inter-observer reliability of trained nurses was assessed by linearly weighted Cohen's kappa and ranged between 0.54 for respiratory response and 0.74 for alertness[25]. The VAS is a horizontal continuous 100 mm line with the anchors of 'no-pain' on the left and 'extreme pain' on the right. The VAS was scored by the nurses at the bedside. Congruent validity of nurses using a VAS has been demonstrated[18,25,26]. COMFORT 'behaviour' and VAS scores were assessed by nurses just after admission to the intensive care unit and every 3 h thereafter until 36 h after surgery.

2.2.3. Complementary measures

The severity of surgical stress (SSS) was assessed with the scoring method developed by Anand and Aynsley-Green[27]. This method scores seven items on various scales: amount of blood loss (score range 0–3); site of surgery (score range 0–2); amount of superficial trauma (score range 1–3); extent of visceral trauma (score range 1–4); duration of surgery (score range 1–5); associated stress factors: (a) hypothermia (score range 0–3), (b) infection (score range 0–3), and (c) prematurity (score range 1–2); cardiac surgery

(score range 2–4). As the last two items were not applicable in this study, the total score could range from 3 to 24. High SSS scores have been associated with greater hormonal-metabolic changes [27-29,30], higher postoperative morphine requirements[31], longer hospital stay and other postoperative outcomes[27, 32]. The SSS-score was determined by the anaesthetist and surgeon after surgery.

2.3. Procedures

Infants in this study received standard anesthetic and postoperative management by well-described protocols [21, 22, 23].

2.3.1. Anesthetic management

Anesthesia was induced with thiopentone 3–5 mg/kg or by inhalation of halothane in oxygen. Endotracheal intubation was facilitated with fentanyl 5 μg/kg and with atracurium 0.5–1 mg/kg or suxamethonium 2 mg/kg. For anesthetic maintenance the end-tidal isoflurane concentrations were set at 0.9% for neonates and children aged 1–3 years, and at 1.0% for infants aged 1–12 months. When a nitrous oxide/oxygen mixture was used, end-tidal isoflurane concentrations were set at 0.5 and 0.6%, respectively. If necessary, these end-tidal isoflurane concentrations were adjusted for the child's clinical condition. A peripheral artery was cannulated and the measured mean arterial blood pressure (MAP) and heart rate [14] were used as preoperative baseline data. After the first arterial blood sample (baseline), patients received a second dose of fentanyl 5 μg/kg before surgical incision. Additional doses of fentanyl 2 μg/kg were administered when MAP and/or HR were ≥15% above baseline value. Perioperative fluids were standardized to maintain glucose infusion rates between 4 and 6 mg/kg/min. Body temperature was kept within normal ranges.

2.3.2. Postoperative analgesia

At the end of surgery, a loading dose of morphine (100 μg/kg) was given intravenously (i.v.) followed by a morphine infusion of 10 μg/kg/h or bolus doses of morphine 30 μg/kg every 3 h. Rescue analgesia was given by nurse controlled analgesia, i.e. if the nurse judged the infant to be in pain, defined as a VAS score ≥4. During the first hour after surgery, one-third of the loading dose of morphine could be repeated every 15 min, and thereafter morphine 5 μg/kg could be given every 10 min if required. No other analgesic or sedative drugs were used.

2.4. Data analysis

2.4.1. Selection of statistical technique

Multiple regression analyses were carried out to analyze the effects of early surgery on the pain sensitivity for the following outcome variables: the fentanyl doses required, epinephrine and norepinephrine plasma concentrations during surgery, as well as postoperative COMFORT and VAS scores, and epinephrine and norepinephrine plasma concentrations. These outcome variables, except fentanyl dose, were averaged for each patient according to the summary measurement approach[33]. Log (natural log) or square root transformations were carried out to achieve normal distributions for outcome variables with skewed distributions (all outcomes except total fentanyl dose).

Postoperative morphine requirement, in contrast, was analyzed using ordinal regression analysis, because this outcome variable was highly skewed and could not be transformed to normality. Ordinal regression analysis technique is generally used for categorical data and, therefore, is highly suitable for outcome data that are not normally distributed. This technique does not evaluate relationships between the outcome variable and independent variables but rather computes cumulative probabilities for each of the categories of outcome variables. Since the postoperative morphine doses were measured on a ratio scale, this outcome variable was categorized into four groups, i.e. ≤10.0, 10.0–11.0, 11.0–12.0, and >12.0 μg/kg/h. To get the most optimal model, first, $\chi 2$-tests were used to select the negative log–log link function ($\chi 2$ 49.49; df 7; $P<0.01$). Second, Pearson's $\chi 2$-test statistic ($P=0.62$) showed that the model predictions were consistent with the observed data. Third, the test of parallel lines showed that the ordinal regression model's location parameters were equivalent ($P=0.22$) across all levels of the dependent variable, i.e. the postoperative morphine requirements.

2.4.2. Independent variables

Group assignments were entered in all analyses as dummy variables for infants previously operated in the same dermatome (group III) and for infants previously operated in another dermatome (group II), whereas the first time surgery group was used as the reference group (group I). Confounding variables such as post-conception age (weeks), gestation period (weeks), SSS-score, and duration of mechanical ventilation (for the postoperative analyses only) were entered in all analyses. COMFORT and VAS scores were adjusted for postoperative morphine intake; intra- and postoperative epinephrine and norepinephrine plasma concentrations were adjusted for the epinephrine and norepinephrine plasma concentrations before surgery. Because the intra- and postoperative epinephrine values increased during the first year of life but decreased thereafter, we added an extra confounding variable (age×age) to the intra- and postoperative epinephrine models. Morphine analgesia was adjusted for intra-operative fentanyl intake.

To reduce the risk of collinearity, all continuous confounding variables were centered, i.e. we subtracted the mean of the scores from each individual score[34]. The mean post-conception age was 58 weeks, the mean duration of gestation was 38 weeks, mean SSS-score was 10, mean baseline epinephrine and norepinephrine plasma concentrations were 60 and 380 nmol/L, respectively, the mean operative fentanyl requirement was 5.9 μg/kg/h, and the mean postoperative morphine requirement was 12.75 μg/kg/h.

2.4.3. Assumptions

When inspecting the residual plots of the COMFORT and VAS models, we found a parabolic pattern in the partial regression plots of morphine intake. We tried to solve this by adding a quadratic term (i.e. morphine intake×morphine intake) in the models, but this induced severe collinearity. For this reason, linear and quadratic values of morphine intake (i.e. first and second order orthogonal polynomial values) were used instead of the original morphine variable, according to the method described by Kirk[35].

Each model was tested for first-order interaction effects in two ways. First, using the F-test statistic, we evaluated whether the model with the significant interaction terms added was significantly better than the model without the interaction terms (see Table 5 legend)[34]. Second, we checked whether the P-value of each interaction term was ≤0.10. Interaction terms with P≤0.10 remained in the model, even if the model was not significantly better than the model without this interaction term.

2.4.4. Differences between the three groups

To evaluate whether the groups differed clinically from each other, the regression equations were used to calculate the population values. This method gives, with a high level of precision, valid estimations of differences between the groups at a population level adjusted for the other 'medical' confounding variables.

3. Results

3.1. Clinical data

A total of 164 children were included; 129 of them were stratified to group I, 13 to group II and 22 to group III. The indications for surgery varied within and between groups (see Table 1), such that infants in group I mostly underwent thoracic (19%), high abdominal (37%) and low abdominal (36%) operations, in group II predominantly thoracic (38%), high abdominal (23%) and low abdominal (31%) operations, and in group III mostly lower abdominal surgery (90%). The gestational periods of the three groups are given in Fig. 1. Gestation was the shortest in the group III infants and the longest in the group I infants, whereas the post-conceptional age at the time of this study was lowest in the group I infants. The same holds true for postnatal age at the time of this study (see Table 2). SSS-scores did not differ between the three groups. Groups II and III did not differ in their age at first operation, duration of hospital or ICU stay, or duration between the first and second operations (P=0.32). Infants of group I had shorter stays in the hospital (P<0.01) than group II and III infants. In comparison with group III infants, group II infants had also shorter stays in the hospital (P=0.05) but not in the ICU (P=0.14). Former preterm neonates with previous severe bowel disease (NEC, invagination, or malrotation) more commonly belonged to group III (55%) than group II (31%), but this difference did not reach statistical significance (P=0.17). Prior morphine intake did not differ between infants of groups II and III (P=0.13).

3.2. Altered pain sensitivity

3.2.1. Operative period

Fentanyl requirements during surgery (multiple regression analysis: F-value 3.83; df 5) were significantly higher in infants of group III than in group I (P=0.03), but no differences occurred between groups I and II (P=0.70; see Table 3). Plasma epinephrine (F-value 8.52; df 7) and norepinephrine concentrations (F-value 6.34; df 6) at the end of surgery were higher in infants of group III (P<0.01 and P=0.05, respectively) than in group I, but did not differ between infants of groups I and II (P=0.99 and 0.64).

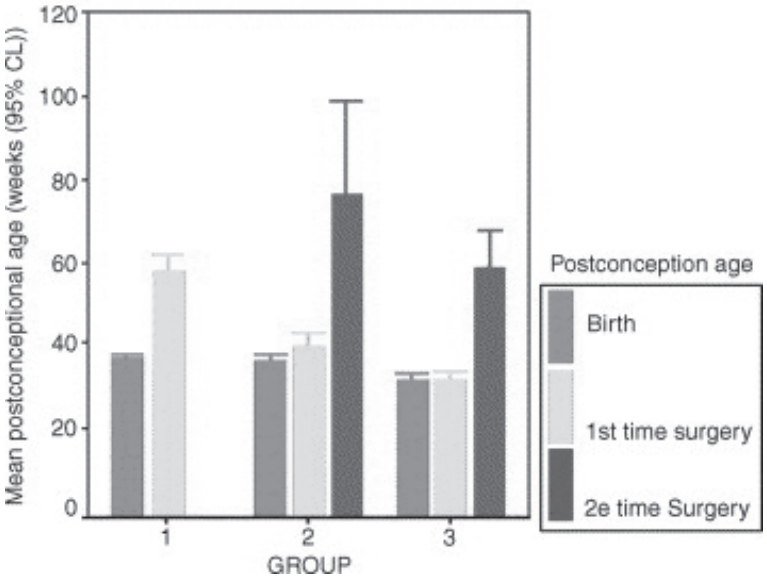
Table 1 Classification of type and location of surgery

Type of surgery	Group		
	1	2	3
Low intestinal surgery	33	2	17
Gastrointestinal surgery	20	3	1
Congenital diafragmatic hernia	19	0	0
Esophageal surgery	13	4	0
Laparotomy/adhesiolysis/resection teratoma	12	0	3
Pulmonary surgery	10	0	0
Hepatic surgery	4	1	2
Urology surgery	6	0	0
Nissen fundoplication	3	2	0
Vascular surgery	2	0	0
Extirpation neuroblastoma	1	0	0
Partial pancreatectomy	1	0	0
Closure gastroschisis	2	0	0
Thoracic plastic surgery	1	0	0
Appendectomy	1	0	0
Herniotomy	0	1	0
Resection of teratoma	1	0	0
Site of surgery			
Abdominal high	48	3	3
Abdominal low	46	4	19
Thoracic	24	5	0
Superficial	9	1	0
Thoracic and high abdominal	2	0	0

3.2.2. Postoperative period

Rescue morphine was given by standard protocol for postoperative analgesia, as described above (Section 2.3.2). Seventy-two infants of group I (56%), nine (69%) of group II, and 16 (73%) of group III required extra morphine analgesia. Multivariate logistic regression analysis showed that these proportions did not differ between the three groups ($\chi 2$ 33.43; df 7). Morphine requirements postoperatively were (median (IQR)) 10.4 (10.0–17.5) in group I, 11.5 (10.0–21.6) in group II, and 11.9 (10.0–21.3) µg/kg/h in group III. Multivariate ordinal regression analyses ($\chi 2$ 49.49; df 7) showed that infants of group II and group III received significantly more morphine analgesia than infants of group I ($P<0.01$ and $P=0.02$, respectively; the overall goodness of fit for this model assessed by Nagelkerke R2 was 0.31).

Figure 1 Background Characteristics: age



3.2.3. Clinical relevance

In comparison with groups I and II (see Table 4), the infants in group III (1) needed 1.6–1.9 µg/kg/h more fentanyl during surgery, (2) had 232–233 nmol/L higher plasma epinephrine concentrations, had 117–149 nmol/L higher plasma norepinephrine concentrations, (4) COMFORT scores were two points higher, (5) VAS scores were 3–7 mm higher, and (6) they had 235–427 nmol/L higher plasma epinephrine concentrations in the first 24 h. Plasma norepinephrine concentrations in infants of group III and II were 108 and 127 nmol/L, respectively, higher than in group I.

3.3. Post hoc analyses

Three post hoc analyses were carried out. The first served to find out whether prior morphine administration during their ICU stay or following previous surgery might explain hypersensitivity (i.e. increased intra-operative fentanyl requirements, higher epinephrine and norepinephrine plasma concentrations, greater morphine requirements, and higher observational pain scores). A second analysis was carried out to find out whether previous adverse hospital events may be related with hypersensitivity. For this purpose total length of stay in the hospital and the ICU were standardized and summed up. The third analysis was carried out to assess whether previous severe bowel diseases (i.e. NEC, invagination, or malrotation) at time of first surgery could be related with subsequent hypersensitivity. To answer these questions, multiple regression analyses were carried out with either prior morphine intake (µg/kg), or total stay in the hospital and ICU, or previous severe bowel disease entered as an extra confounding variable in each analysis. Table 5 shows that none of these models performed significantly better than our previous model. Also none of these extra confounding variables had a P-value of ≤0.10. This means that prior morphine intake, extended hospital stay, or previous severe bowel diseases were not associated with increased intra-operative fentanyl

Table 2 Background characteristics

	Group median (10-90th percentile)				p val
	I n=129	II n=13	III n=22		
Gestation period (weeks)	39 (35-41)	38 (30-41)	35 (27-40)	I vs II I vs III II vs III	0.05 0.00 0.05
Age (postnatal age)	4 (0-63)	21 (4-104)	17 (8-70)	I vs II I vs III II vs III	0.00 0.00 0.15
Sex (boys/girls)	72-57	13/5	13/9	I vs II I vs III II vs III	0.00 0.60 0.26
SSS	9 (6-14)	10 (4-14)	10 (6-13)	I vs II I vs III II vs III	0.49 0.91 0.58
History of child					
Total length of stay in hospital (days)	3 (0-19)	46 (11-91)	63 (31-108)	I vs II I vs III II vs III	0.00 0.00 0.05
Total length of stay in ICU (days)	1 (0-7)	28 (1-63)	32 (3-85)	I vs II I vs III II vs III	0.00 0.00 0.14
Time between first operation and this study (weeks)	-	10 (3-34)	15 (8-70)		
Prior morphine intake µg/kg	-	638 (296-1036)	912 (586-2929)		
Former premature with NEC/invagination/malrotation	-	4	12		

requirements, or plasma epinephrine and norepinephrine concentrations, or their postoperative morphine analgesia and observational pain scores.

4. Discussion

Even with adequate anaesthesia and analgesia, infants who had previously been operated upon in the same dermatome (group III) reacted with greater distress during subsequent surgery than did infants with no prior surgery (group I); or infants who previously underwent surgery in another dermatome (group II). The fact that all children were exposed to similar degrees of surgical stress, but only group III infants required higher fentanyl dosages intra-operatively, displayed greater postoperative distress as reflected by their COMFORT scores, VAS scores, and hormonal stress responses, and had required higher postoperative morphine dosages than the other patients, sug-

Table 3a Final outcome multiple regression analysis

	Intra-operative measures								
	Fentanyl			**LN (epinephrine)**			**LN (norepinephrine)**		
	ß	**Beta**	**Sign**	**ß**	**Beta**	**Sign**	**ß**	**Beta**	
Intercept	5.65		0.00	4.38		0.00	5.89		0.00
Group II	-0.29	-0.03	0.70	$5x10^{-3}$	0.00	0.99	0.09	0.03	0.64
Group III	1.66	0.22	0.03	1.37	0.33	0.00	0.35	0.17	0.05
Age[1]	0.01	0.16	0.04	0.02	0.51	0.00	$-4x10^{-3}$	0.19	0.01
Age x age[1]				$-6x10^{-6}$	-0.42	0.00			
Gestation[1]	0.07	0.09	0.36	0.02	0.04	0.69	$-8x10^{-3}$	-0.04	0.67
SSS total[1]	-0.20	-0.23	0.00	0.05	0.11	0.13	0.02	0.06	0.39
Duration mechanical ventilation[1]									
Baseline (nor)-epinephrine concentration[1]				$2x10^{-3}$	0.24	0.00	$4x10^{-4}$	0.36	0.00
Morphine[2]									
First order[1]									
Second order[1]									
Adjusted R square	0.08			0.24			0.16		

gests that the long-term effects of surgery in early infancy may be related to the area of prior tissue damage.

We found that group II infants had greater postoperative morphine requirements and higher plasma norepinephrine concentrations than those of group I, although their intra-operative fentanyl requirements and plasma epinephrine concentrations were not significantly greater in comparison with infants of group I. In this study we adjusted for gestational age at birth, postnatal age, and for severity of surgical stress. Our post-hoc analyses further showed that prior morphine intake, previous prolonged hospital stay, or previous severe bowel disease did not explain the higher morphine requirements in group III. This conglomeration of findings, within the context of animal experimental data, suggests that the long-term effects of early pain exposure may result from a combination of peripheral/spinal and supraspinal changes. Long-term developmental changes in peripheral/spinal mechanisms of subsequent pain processing are reasonably well-characterized[9, 12, 36-40], whereas changes in the supraspinal areas remain poorly understood [13-15, 41,-43,]. We postulate that the robust differences in pain and stress responses for group III infants may result from persistent differences in spinal and supraspinal processing, whereas differences observed in group II infants may signify the more subtle/transient changes resulting from altered supraspinal processing. The physi-

Table 3b Final outcome multiple regression analysis

	Postoperative measures											
	SQRT (COMFORT)			**LN (VAS)**			**LN (epinephrine)**			**LN (norepinephrine)**		
	ß	**Beta**	**Sign**	**ß**	**Beta**	**Sign**	**ß**	**Beta**	**Sign**	**ß**	**Beta**	**Sign**
Interept	3.75		0.00	1.01		0.26	4.45		0.00	5.98		0.00
Group II	0.05	0.04	0.54	0.12	0.09	0.20	0.45	0.10	0.13	0.24	0.14	0.05
Group III	0.27	0.28	0.00	0.23	0.21	0.01	1.05	0.29	0.00	0.28	0.21	0.02
Age[1]	$-4x10^{-4}$	-0.04	0.56	$-1x10^{-3}$	-0.08	0.22	0.03	0.68	0.00	$-3x10^{-3}$	-0.22	0.00
Age x Age[1]							$-6x10^{-6}$	-0.46	0.00			
Gestation[1]	$7x10^{-3}$	0.07	0.45	$4x10^{-3}$	0.03	0.69	0.02	0.05	0.53	$-6x10^{-3}$	-0.04	0.65
SSS total [1]	$-7x10^{-3}$	-0.06	0.40	$-3x10^{-3}$	-0.03	0.69	0.04	0.10	0.13	0.02	0.16	0.04
Duration mechanical ventilation[1]	-0.06	-0.19	0.01	-0.06	-0.18	0.01	-0.24	0.21	0.00	0.02	0.04	0.61
Baseline norepine-phrine con-centration[1]							$-1x10^{-3}$	0.19	0.00	$2x10^{-4}$	0.23	0.00
Morphine[2]												
First order[1]	0.02	0.35	0.00	0.04	0.47	0.00						
Second order[1]	$-2x10^{-3}$	-0.21	0.01	$-2x10^{-3}$	-0.31	0.00						
Adjusted R square	0.25			0.37			0.42			0.17		

Table 4 Estimated values outcome variables

	Group, Estimated means (95% CI)		
	I	II	III
Peroperative			
Fentanyl (µg/kg/h)	5.7 (5.2–6.1)	5.4 (3.4–7.3)	7.3 (5.4–9.2)
Epineprhrine (nmol/L)	79 (58–107	78 (28–229)	311 (110–875)
Norepineprhine (nmol/L)	360 (319–405)	392 (241–640)	509 (315–822)
Postoperative			
COMFORT	14 (14–15)	14 (13–16)	16 (14–18)
VAS[27]	27 (26–29)	31 (24–39)	34 (27–43)
Epinephrine (nmol/L)	85 (67–108)	134 (60–300)	245 (112–535)
Norepinephrine (nmol/L)	393 (357–432)	501 (356–704)	520 (372–726)

Note. These values are estimations of the outcome variables using the raw regression coefficients (see Table 3). To calculate these values, age was set at 58 weeks, gestation period at 38 weeks, SSS at 10, and baseline epinephrine and norepinephrine plasma concentrations were set at the average concentrations of 60 and 380 nmol/L, respectively. Morphine intake during the first 24 h was set at the average intake of 12.75 µg/kg/h which corresponds with the orthogonal polynomial value first and second order values of 0.003 and −327.79. The duration of mechanical ventilation was set at zero.

ological effect of these supraspinal differences may be suppressed by the general anaesthesia during surgery, thus no differences occurred in the fentanyl requirements or catecholamine responses at the end of surgery between groups I and II.

Conditioning[8] as a consequence of previous surgery and ICU stay might offer another explanation for the higher morphine requirements of group II. Additional post-hoc analyses showed that group II infants, like group III infants, had significant higher COMFORT and VAS scores during the first 9 h postoperatively. After this period, however, these pain assessment scores remained higher in group III infants, whereas those in groups I and II were similar. Perhaps nurses interpreted the infants' behavioural distress in the early postoperative period as evidence for pain, which is also the most plausible explanation [44].

Our findings are consistent with those reporting that infants with unilateral hydronephrosis developed abdominal hypersensitivity in the lateral and contralateral side in the dermatome of the injured region[7]. Other findings suggest that these alterations are not restricted to the dermatome. Circumcised infants showed a stronger pain response to immunization at 4–6 months of age as compared to uncircumcised infants[3]. Closer inspection shows that circumcision affects dermatome L3 while subsequent vaccinations were given in the thigh (dermatomes L2/L3). It is unclear whether this long-term hypersensitivity occurred because of neuroanatomical proximity to the dermatome injured during circumcision12 or whether it resulted from prior vaccinations at the same

Table 5 Model performance

		Original model	Prior morphine	previous adverse hospital events	Previous severe bowel disease
		F value	Improvement (F value compared with F value original model)		
Intra-operative measures	Fentanyl µg/kg/h	3.83	0.02	0.54	0.44
	Epinephrine nmol/L	8.52	0.50	2.08	0.77
	Norepinephrine nmol/L	6.34	0.08	0.03	0
Postoperative measures	COMFORT	7.71	0.88	1.13	0.50
	VAS[27]	12.74	0	0.67	0
	Epinephrine nmol/L	15.81	0.43	0.43	0.05
	Norepinephrine nmol/L	5.62	0	0	0.12
		χ2	Improvement (χ2 compared with χ2 original model)		
Postoperative	Morphine intake	49.49	0	0.55	1.13

Note. This table shows whether F-values improved after adding one of the following confounding variables: prior morphine, previous adverse hospital events, or previous severe bowel disease. Improvement in F-value was calculated by the formula: $([SSE_{\text{original model}} - SSE_{\text{model with extra confounding variable}}]/[df_{\text{model with extra confounding variable}} - df_{\text{original model}})/[MSE_{\text{model with extra confounding variable}}]$. If the F-value of the model with the extra confounding variable improved by >3.90 then this model performed significantly better than the original model. With regard to the model for postoperative morphine intake, predictability is only improved when the χ2 of the model with the extra confounding variable is >3.84 than the χ2 of the original model.

site[3]. Like the results of the present study, there is converging evidence that pain from diverse sources and experience (i.e. surgery or NICU pain) may not have generalized effects on the pain system with significant long-term implications [5, 16, 17]. On the other hand, these long-term changes may present in quite unexpected ways, which have been unexplored in follow-up studies of these vulnerable infants[41, 45].

An explanation for these findings might include changes in the peripheral nervous system during early infancy. Cutaneous injury in neonatal rats stimulated a profound sprouting response of the surrounding local sensory nerve terminals leaving an area of permanent hyperinnervation[11, 46]. These neuroanatomical changes were associated with long-lasting hypersensitivity and lowered mechanical thresholds in the previously injured area[10, 11]. Early injury or inflammation in infant rats also altered the phenotype of primary sensory neurons located in the dorsal root ganglia[9]. Human infants with established unilateral exposure to pain have not only lower sensory thresholds at the injured area8 but also at the unaffected contra-lateral side[7, 47]. These studies, however, reported the acute effects of tissue injury and visceral hypersensitivity, whereas we have found previously undocumented long-term hypersensitivity related to prior surgical injury.

Concomitant changes may also occur in the spinal cord. Unilateral hind paw inflammation caused by injection of CFA (25 µl) in neonatal rat pups was associated with marked increases in the density of dorsal primary afferents during adulthood, as compared with untreated adult rats. This increased density was not restricted to the dermatome of injury but also extended several segments caudally and, to a lesser extent, on the contralateral side[12, 14, 48]. When given lower doses of CFA or carrageen injections, neuroanatomical changes at the spinal cord were absent[14]. Behavioural studies further documented that neonatal exposure to CFA 25 µl resulted in long-lasting hypersensitivity to a diversity of pain stimuli[12, 18]. The clinical relevance of the persistent hind paw inflammation caused by CFA injections has been questioned[19, 49] and is unlikely to explain the clinical findings from this study.

We assessed pain sensitivity by means of plasma catecholamine concentrations, well-known as highly sensitive, but non-specific, markers for pain and stress in neonates. Factors such as the surgical stress severity[26,50], infection[50], or area of surgery[20] may affect the epinephrine and norepinephrine responses. Hence, we controlled for severity of surgical stress. We did not adjust for sepsis, because 'localized or generalized infection' has no effect on catecholamine plasma concentrations[20]. Plasma concentrations of epinephrine and norepinephrine is higher following surgery in the upper abdomen in comparison with other body locations[20]. In our study, however, 14% of the group III infants underwent upper abdominal surgery in comparison with about 31 and 37% of the group II and I infants, respectively, which would tend to underestimate the observed differences between groups. Moreover, the history of adverse hospital events did not differ between groups II and III. Because group III infants had greater stress responses than infants in the other groups, we assume that the higher catecholamine responses reflect a greater pain sensitivity.

Our findings suggest that group III infants were more sensitive to pain than those in group I. Reasons why group II infants needed more morphine remain unclear, but may be related to an uncoupling of opioid receptors following acute surgical pain. Clinically, however, the differences in morphine requirements between groups III/II and I were small; i.e. 1.1–1.5 µg/kg/h. The differences in COMFORT and VAS scores between h.

groups III and II/I were ≤1.5 points and ≤5 mm, respectively, and for fentanyl ≤2.3 μg /kg/h. Pain scores in the postoperative period, even among the children who were re-operated in the same dermatome, remained low. The average COMFORT and VAS scores did not suggest that children suffered pain as both remained below the cut-off points of 17 points and 40 mm, respectively[24]. Others, in contrast, showed that infants were more sensitive in the area of injury even 3 months after surgery[7]. These authors, however, did not indicate whether the observed differences were subtle or clinically relevant.

In conclusion, these preliminary data demonstrate that surgery in early infancy leads to prolonged pain hypersensitivity in the area of injury, which may last for up to 3 years after the initial surgical tissue injury. The magnitude of differences noted between these groups, however, suggest that this prolonged pain hypersensitivity may not be clinically substantial. Future studies focusing on the development of pain thresholds and subsequent pain behaviours may further improve our understanding of how surgical operations in early infancy can alter subsequent pain processing and their clinical and neurobiological implications.

Acknowledgements

The study was supported by the Netherlands Organisation for Health Research and Development (ZonMw 940-31-076).

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Chapter 3

Long term alterations in pain sensitivity 8 years following neonatal surgery

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Submitted

Abstract

Exposure to pain during the first months of life may result in hypersensitivity, Experimental animal studies confirm findings in humans, demonstrating hypersensitivity to be due to peripheral and spinal/supraspinal neuroanatomical alterations. We performed a cross-sectional study to explore whether major surgery in early infancy results in prolonged hypersensitivity. Thermal detection and pain thresholds of children who had undergone major abdominal or thoracic surgery within the first three months of life were compared, with those of age and gender matched controls. Pain stimuli were induced by a Thermal Sensory Analyser, using a Peltier contact thermode. Cold/heat detection, cold/heat pain and suprapain thresholds were determined. With regard to the latter, pain scores were assigned with the use of a Visual Analogue Scale. Structural equation modeling was used for data analysis. 57 former patients and 57 controls participated. Former patients were hyposensitive to temperature detection at the scar ($p<0.05$). They were more sensitive to cold and heat pain at the scar and contralateral side than controls ($p<0.01$ for cold and $p<0.05$ for heat). Detection and pain thresholds on the hand did not differ, nor did the suprapain thresholds. Children operated upon within the first month of life demonstrated greatest hypo- and hypersensitivity. These findings may indicate the occurrence of spinal and supraspinal changes following neonatal surgery. The long-term consequences of surgery in early infancy are most pronounced in the region of tissue damage and the contralateral side. These effects portend limited clinical but important neurobiological differences.

1. Introduction

The processing of somatosensory pain in newborn infants differs from that in adults[1]. Newborn infants have lower pain thresholds, are more vulnerable to developing sensitization and lack the inhibitory activity of the descending pathways[2, 3]. In contrast to adult age, tissue damage and pain exposure in early infancy result in prolonged hypersensitivity, i.e. extending beyond the period associated with healing of the initial tissue injury[4-12]. Confirmatory experiments demonstrated that tissue damage or inflammation at neonatal age disturbs the normal development of nociceptive neuronal circuits, resulting in structural and functional neuroanatomical changes[13-15]. Following tissue damage the nerves die and the surrounding fibers induce a sprouting response, overcompensating for the damage[14-16]. Effects are not confined to the periphery, but extend to the spinal cord and supraspinal areas as well[17-20]. Depending on the degree and duration of inflammation, anatomical changes are not restricted to the corresponding spinal level of tissue damage, but even spread caudally[17, 19, 21]. Recent findings in humans suggest that alterations at the periphery and spinal cord may even extend to supraspinal levels[12].

Prolonged hyperalgesia induced by tissue damage seems to be confined to the region of tissue injury. Infants and children demonstrated altered pain responses only when subsequent pain was induced in the dermatome damaged at neonatal ages, not when induced in any other site[6, 8, 10, 12, 22]. Hypersensitivity also extends to the contralateral side, although to a lesser degree[10, 19, 23]. Andrews and colleagues showed that 3 months after surgery both the area of incision and the unaffected contralateral side of the body demonstrated greater tenderness to mechanical stimuli in comparison with matched controls[10]. Our earlier findings suggest that adequate morphine administration may not prevent this prolonged hyperalgesia. It is still unknown whether this will recover at a later age.

To date, the maximum documented follow up after neonatal tissue damage is four years[12]. We set up a study in children who as newborn infants had undergone surgery 8 years ago in order to determine if major tissue damage during early infancy affects the development of pain processing, specifically assessing pain sensitivity. Furthermore, we aimed to identify possible differences between children who underwent surgery in the first month of life and those operated upon in the second or third month.

2. Methods

2.1 Subjects

Subjects were children who had undergone major abdominal or thoracic surgery (i.e. stoma 46%, correction CHD 14%, correction esophagus atresia 14%, adhesiolysis 7%, remaining procedures like pancreatic resection, balock procedure etc 19%) during the first 3 months of life in the Erasmus MC Sophia Children's Hospital between April 1996 and August 1999. They had participated in a double blind, randomized, clinical trial, showing that the iv morphine administration postoperatively was adequate, as assessed by validated behavioral pain measures and hormonal stress responses. This has been documented in a number of publications [24-28]. These children did not receiven any epidural or local analgesics. All children were followed according to the national neonatal follow-up criteria for a median duration of 8 years (range 6 to 11)[12, 29]. These children also participated in a trial evaluating biobehavioural pain responses at age 4 years[12, 29]. Subjects for the present study were recruited from this group.

Age and gender matched controls who had not been exposed to surgical procedures in infancy and childhood were recruited from two local elementary schools in the reference area of our hospital. We also matched for ethnicity as far as possible.

Exclusion criterion for both patients and controls was cognitive impairment. The 'Central Committee on Research Involving Human Subjects' approved the study protocol, and signed informed parental consent was obtained [12, 29].

2.2 Testing algorithm

Pain stimuli were induced by the computer-controlled thermal sensory analyzer (TSA-II 2001; Medoc Ltd. Advanced Medical Systems, Ramat Yishai, Israel) with a Peltier-based contact thermode (30x30mm). This device was used to assess alterations in somatosensory processing by three different tests, i.e. detection thresholds for heat and cold, pain thresholds for heat and cold, and suprapain thresholds for heat and cold pain. Cold and heat detection thresholds were determined, by the temperature at which the child sensed a change into either cold or heat. Cold and heat pain thresholds were determined by the temperatures at which sensations were first perceived as painful.

The used testing algorithm for the detection and the pain thresholds was the reaction-time inclusive Method of Limits (MLI). The thermode baseline temperature was set at 32°C. To prevent any tissue damage, stimulation temperatures had a potential range of -10 to 50°C. The temperature change rate was kept constant at 1.0°C/sec, with a 10°C/sec return rate. If the child did not report pain at (one of) the extreme temperatures of -10 and 50°C, pain thresholds were set at these extreme temperature(s).

The pain threshold values, finally, were used to determine the exposure temperatures for the supra-pain test. This supra-pain test consisted of a series of 5 stimuli ranging from 2 degrees below to 2 degrees above the pain threshold for heat pain and ranging from 4 degrees below to 4 degrees above the pain threshold for cold pain. Each stimulus lasted 6 seconds, after which children were asked to rate their pain intensity on a VAS scale[30]. The stimuli were delivered sequentially with a delay of 30 sec between successive stimuli.

2.3 Procedure

Some time before testing, we sent eligible children and their parents or caregivers a letter (child and parent version) explaining the purpose of the study and the methodology used. We next called them to provide further details if required and to ask whether they would consent in participating. On the day of testing, in the presence of one parent or caregiver we explained the overall procedure inviting the child to feel the thermode (at baseline level of 32°C). We again pointed out the right to withdraw permission for the continuation of the test at any time. Neither the child nor the parent/caregiver was given access to the computer screen nor were they informed of any personal results[31]. The experimental procedure was similar to that in a study by Meier and colleagues[31] and took place in a separate, quiet room. The ambient room temperature was kept between 18-20 °C. Before actual testing, we conducted a rehearsal session during which all children were trained in pain threshold measurement. The final tests were started only when we were sure that the child fully understood our explanations and the testing algorithm. All tests were carried out by one trained investigator, R. Schouw.

During the period needed for testing of all children, evaluation moments were inserted to ascertain consistency in the given instructions and testing procedure. All patients and controls were tested in random order.

Before testing, skin temperature was measured at the thenar eminence of the non-dominant hand, in order to ascertain that it exceeded 25°C. The thermode was placed on the stimulation surface and was secured by a Velcro band. Detection thresholds, pain thresholds, supra-pain thresholds were subsequently determined at 3 sites: the thenar eminence (reference site), nearby the scar and the intact contralateral side. As the dermatome of surgery varied among the former surgical patients, the control children were matched to test location as well. In order to minimize bias by reaction time, the thermode was placed on the non-dominant hand (thenar eminence C6).

To increase reliability, all detection and threshold stimuli were administered in series of four with a delay of 15 sec between successive stimuli [32, 33]. The thresholds were calculated as the means of four test results. For determining of detection thresholds children were exposed to 4 cold stimuli, after 30 seconds followed by 4 warm stimuli. Next, we assessed 4 cold and 4 heat pain thresholds, again with a 30 seconds interval [32, 33]. An auditory cue was given to indicate the start of the next stimulus. Suprapain testing then started after a 60 seconds interval. Five stimuli were given at 30 seconds intervals. After 5 minutes the same procedure was repeated for the scar and then, again after 5 minutes, for the contralateral location.

2.4 Data analysis

In this study we evaluated three different outcome parameters; i.e. detection threshold, pain threshold and suprapain thresholds. Structural equation modeling (SEM), conducted with Mplus for Windows, was used to explore structural relationships between the two groups (n=57 per group) for the detection and the pain thresholds and to evaluate the existence of a developmental window. The structural model concerns the direct and indirect relationships between independent variables and dependent variables[34]. This approach involves the examination of several models in order to identify the most plausible model, based on theory as well as published data. If the final model is robust, then the structural relations estimated by the model will produce correlations close to the ones that exist in the data. Changes in the model produce a new set of parameters and estimates, which can be similarly tested.

Alterations in repeated supra-pain thresholds for patients and controls (n=51patients, n= 47 controls) were evaluated using multivariate analysis of variances (MANOVA) for repeated measurements.

2.4.1 Strategy of SEM analysis

The processes of fitting SEM started with the construction of a theoretical baseline model, in which it was hypothesized that patients differed from controls at all levels, i.e. cold and heat temperatures and all three locations. The main objective therefore was to evaluate the presence of group differences and their specific location (Tables 1 and 2).

In the next step, it was hypothesized that differences in thresholds were only present for cold or heat between patients and controls not specified for locations (model 1). The second step consisted of evaluating differences in pain thresholds per location, i.e. only present at the hand, the scar, contralateral side, or combinations of these sites

(model 2 to 8). Each model's fit was evaluated by examining the parameter estimates and measures of overall fit. The following performance measures were used to test the models: (1) chi-square (including degrees of freedom and p-value) for model fit: a non-significant value indicates that the model at issue cannot be rejected, (2) chi-square for model fit divided by degrees of freedom: a value of <1.5 is acceptable[35], comparative fit index (CFI): a value of > 0.90 suggests a good fit, maximum is 1.00[36], (4) Tucker Lewis Index 36: a value of >0.90 suggests a close fit, maximum value is 1.00, (5) standard root mean squares of residuals (SRMR): a value of < 0.05 indicates a good fit, (6) root mean squares of approximation (RMSEA): a value of 0.05 indicates a close fit. A model was rejected when it did not meet all criteria of goodness of fit. Models were compared by means of X^2 differences. The model finally accepted, was the one, which had the best fit and allowed meaningful and substantively interpretation of the parameters.

In order to determine possible differences in somatosensory processing depending on age at surgery we stratified children in the index group to surgery in the first month of life and surgery in the second or third month of life. We used SEM to compare these two groups.

The influence of possible confounders (sex and age) was also evaluated using SEM.

3. Results

3.1 Clinical data

Of the 85 eligible children who had been included in the original study[26], 57 were recruited for these measurements (36 boys and 21 girls). Of the 71 eligible control children 57 participated.

Mean age at testing was 7.8 years (SD 1.1). All had been born at term (37.3 wks, SD 1.3) with a mean birth weight of 2946g (SD 450g), mean age at surgery 1 month and mean hospitalization for 40 days (SD 37). The control group included 58 children with a mean age at testing of 8 years (SD 1.3). All had been born at term (mean 38.5 wks, SD 2.0) with a mean birth weight of 3320g (SD 614g).

Overall twenty-four parents refused consent, three children were diagnosed with Down's syndrome and fifteen patients were lost to follow-up. No significant differences in surgical procedure, number of days admitted to the hospital (mean 41 days, SD 28), gestation age (mean 37,1 weeks, SD 1) or birth weight (mean 3000, SD 593) could be demonstrated between participants and non-participants.

3.2 Thermal Detection thresholds

Correlations for both cold and warmth detection thresholds were measured at three different locations, showing that the locations correlated well with each other (Table 3). Table 1 shows the result of 6 tested models. The X^2 differences for all tested models were subsequently evaluated (model 0 vs 1, model 0 vs 2 and so on). Models 1, 2, 4, 5 and 8 significantly worsened the structural relations compared to baseline model 0 ($p<0.05$ to $p<0.001$) and were therefore rejected. Of the accepted models 3, 5 and 7, the ratio of X^2/df and the other fit indices indicated model 7 as the best fitting and thus the most plausible one. This justified the conclusion that detection thresholds were similar

Table 1 SEM Detection thresholds

Model	Description	Hand		Scarr		Contralateral		X^2	df	X^2/df	p	CFI	TLI	RMSEA	SRMR
		C	H	C	H	C	H								
0	Patients differ from controls	-	-	-	-	-	-	34.34	15	2.29	0.00	0.92	0.84	0.17	0.31
1	No differences for cold or heat at the three locations	+a	+b	+a	+b	+a	+b	64.39	25	2.58	0.00	0.83	0.80	0.19	0.33
2	No differences per location	+a	+a	+b	+b	+c	+c	219.80	24	9.16	0.00	0.42	0.28	0.37	0.20
3	No differences at the hand	+a	+a	-	-	-	-	34.60	17	2.03	0.01	0.93	0.87	0.15	0.31
4	No differences at the scar	-	-	+a	+a	-	-	38.60	16	2.41	0.01	0.91	0.82	0.18	0.32
5	No differences at the contralateral side	-	-	-	-	+a	+a	35.19	17	2.07	0.01	0.92	0.87	0.16	0.31
6	No differences at the hand and scar	+a	+a	+b	+b	-	-	37.55	16	2.35	0.00	0.92	0.81	0.16	0.30
7	No differences at the hand and contralateral side	+a	+a	-	-	+b	+b	35.26	18	1,95	0.01	0.94	0.88	0.15	0.31
8	No differences at the scar and contralateral side	-	-	+a	+a	+b	+b	38.80	15	2.59	0.01	0.92	0.82	0.17	0.30

Note of legend: C indicates cold stimuli, H indicates heat stimili, (-) indicates that thresholds are incomparable,+a, +b and +c respectively indicate that these thresholds are comparable.

Table 2 SEM Pain thresholds

Model description								Performance Measures							
Model	Description	Hand		Scarr		Contralateral		X^2	df	X^2/df	p	CFI	TLI	RMSEA	SRMR
		C	H	C	H	C	H								
0	Patients do not differ from controls	-	-	-	-	-	-	18.38	15	1.23	0.25	0.99	0.98	0.07	0.08
1	No differences for cold or heat at the three locations	a	b	a	b	a	b	34.97	25	1.40	0.09	0.97	0.97	0.10	0.15
2	No differences per location	a	a	b	b	c	c	36.10	24	1.50	0.05	0.97	0.96	0.11	0.18
3	No differences at the hand	a	a	-	-	-	-	19.38	18	1.08	0.37	1.00	0.99	0.04	0.09
4	No differences at the scarr	-	-	a	a	-	-	30.29	18	1.68	0.03	0.96	0.94	0.13	0.17
5	No differnces at the contralateral side	-	-	-	-	a	a	31.31	18	1.74	0.03	0.96	0.94	0.13	0.18
6	No differences at the hand and scarr	a	a	b	b	-	-	32.74	22	1.49	0.07	0.97	0.96	0.11	0.15
7	No differences at the hand and contralateral side	a	a	-	-	b	b	34.49	22	1.57	0.04	0.96	0.95	0.11	0.16
8	No differences at the scarr and contralateral side	-	-	a	a	b	b	33.46	22	1.52	0.06	0.96	0.96	0.11	0.20

Note of legend: C indicates cold stimuli, H indicates heat stimili, (-) indicates that thresholds are incomparable,+a, +b and +c respectively indicate that these thresholds are comparable.

at the hand and contralateral side between patients and controls, but differed at the scar. However the differences between patients and controls for cold and heat detection at the scar were small, i.e. 1.4°C and 1.5°C, respectively.

Table 3 Correlation matrix detection thresholds

	a.	b.	c.	d.	e.	f.
a. Cold Hand	1.00					
b. Warm Hand	-.079	1.00				
c. Cold Scarr	0.46	-0.67	1.00			
d. Warm Scarr	-0.28	0.41	-0.74	1.00		
e. Cold Contra	0.41	-0.56	0.72	-0.46	1.00	
f. Warm Contra	-0.38	0.44	-0.69	0.48	-0.78	1.00

3.3 Thermal Pain thresholds

Thresholds for both groups are reported in Table 4. The overall observed correlations for both cold and heat pain threshold were measured at three different locations, showing that predominantly the scar and contralateral side were strongly correlated (correlation coefficient > 0.70). This was also true for cold and heat pain at the scar site (Table 5).

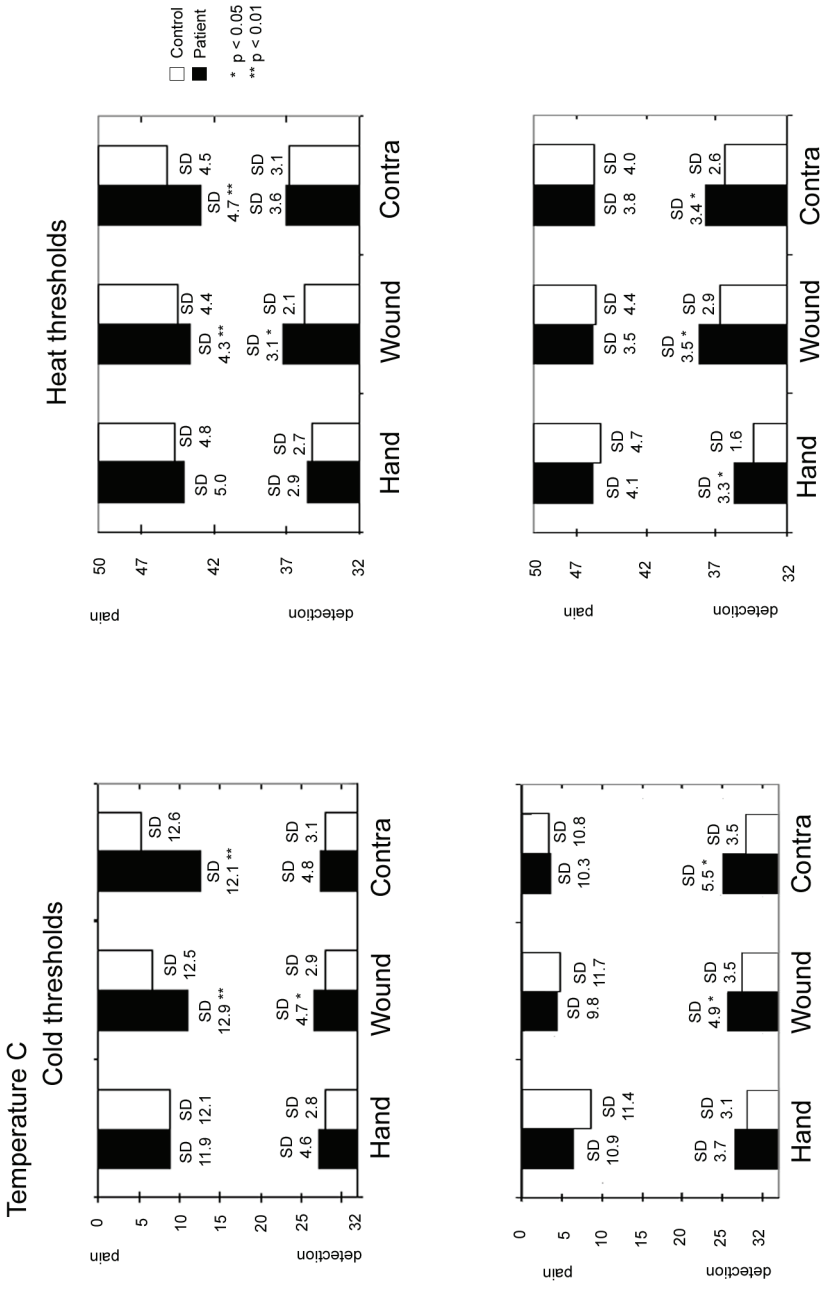
Table 2 shows the results of the 9 tested models. The differences in X^2 for all tested models were subsequently evaluated and compared with the baseline model 0 (model 0 vs model 1, model 0 vs model 2 and so on). Models 2 and 4 to 8 significantly worsened compared to model 0 ($p<0.05$ to $p<0.001$) and were therefore rejected. Examining the accepted models 1 and 3, the ratio of X^2/df and the other fit indices indicated model 3 as the best fitting and thus the most plausible one. Accordingly, we concluded that the assumption of model 3, which implied that pain thresholds are only similar at the hand, is correct. As the hypotheses of model 4 to 8 were incorrect, this indicated that former patients have altered pain thresholds at the scar and contralateral side. Figure1 shows that the differences between patients and controls at for cold and heat pain thresholds at the scar were 4.4°C and 0.9°C, respectively, and at the contralateral area 7.3°C and 2.3°C, respectively.

Possible differences in variances between the two groups were evaluated using the models described above. As all models described a good fit (p values ranged from 0.19 to 0.72) no differences in pain threshold variations between surgical patients and matched controls could be demonstrated.

3.3.1 Stratification of patients by age at surgery

To assess the probability of a developmental window, all index children were stratified into one of two groups, i.e. operated within the first month (n=44) and operated in the second or third month of life (n=13). Data were analyzed by using the same SEM hypotheses as described above. The age groups differed in pain thresholds only at the scar site, showing that the group operated on within the first month of life was hypersensitive to cold pain (Figure 2). No differences in detection and pain thresholds could be observed for the other locations.

Figure 1 Detection and Pain thresholds



Note of legend: Mean detection and pain thresholds for cold and heat for Patients versus Control group are represented by the black and white bars respectively. The standard deviation is represented above and below the individual bars.

Table 4 Mean Detection and Pain thresholds

	Number	CDT Hand	WDT Hand	CDT Scar	WDT Scar	CDT Contra	WDT Contra
Patients	57	27.3	35.5	26.5	37.3	27.4	37.0
Controls	57	28.1	35.2	27.9	35.8	28.0	36.9
		CPT Hand	**HPT Hand**	**CPT scar**	**HPT Scar**	**CPT Contra**	**HPT Contra**
Patients	57	8.9	44.1	11.1	43.6	12.6	42.9
Controls	57	8.7	44.7	6.7	44.5	5.3	45.2

Note of legend: CDT = cold detection threshold, WDT = warmth detection threshold, CPT = cold pain threshold, HPT = heat pain threshold

Table 5 Correlation matrix pain thresholds

	a.	b.	c.	d.	e.	f.
a. Cold Pain Hand	1.00					
b. Heat Pain Hand	-0.61	1.00				
c. Cold Pain Scar	0.52	-0.50	1.00			
d. Heat Pain Scar	-0.46	0.69	-0.74	1.00		
e. Cold Pain Contra	0.49	-0.39	0.75	-0.56	1.00	
f. Heat Pain Contra	-0.45	0.53	-0.59	0.72	-0.73	1.00

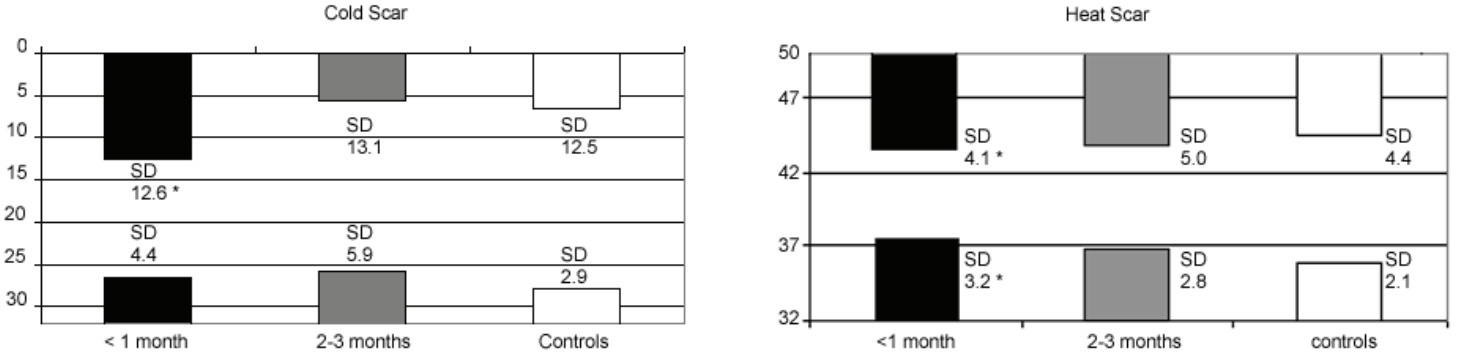
3.3.2 Other determinants of pain thresholds

SEM pain threshold analysis showed girls to be significantly more sensitive to cold and heat pain than boys. In addition, pain thresholds increased with age. The influence of gestational age and low birth weight could not be assessed in this study, as all children were term born and had normal birth weights (>2500 g).

3.4 Supra-pain threshold stimuli

Table 6a shows the means (95% CI) of the children's corresponding 5 VAS scores per location. All VAS scores increased with increasing stimulus intensity. Table 6b shows the results of multivariate significance testing for group differences. There were no significant differences between the two groups in tolerance for cold and heat pain at the various locations. There is, however, a significant temperature effect, which is due to the increasing intensity of the subsequent stimuli.

Figure 2



Note of legend: Developmental window is shown for the wound location. Mean detection and pain thresholds for cold and heat for Patients (P) versus Control group (C) are represented by the black and white bars respectively. The standard deviation is represented above and below the individual bars.

Table 6a VAS scores

Location	Patients Mean (95% CI)	Controls Mean (95% CI)
Cold Hand	6.1 (5.2-6.9)	5.3 (4.5-6.1)
Heat Hand	6.1 (5.3-6.8)	5.9 (5.2-6.6)
Cold Scar	5.0 (4.1-5.9)	5.4 (4.6 to 6.3)
Heat Scar	5.0 (4.1-5.9)	5.9 (5.1 to 6.7)
Cold Contralateral	5.1 (4.1-6.0)	4.7 (3.8 to 5.5)
Heat Contralateral	5.6 (4.6- 6.7)	5.9 (4.9 to 6.8)

Table 6b Multivariate significance testing

Effect	F	p
Location	2.83	0.07
Location x Group	2.75	0.07
Temperature	6.24	0.02
Temperature x Group	2.44	0.13
Location x Temperature	1.94	0.16
Location x Temperature x Group	0.06	0.94

4 Discussion

During these tests all children tolerated the procedure well, cooperated smoothly, and expressed willingness to participate on a second occasion. Surgery within the first three months of life in combination with adequate analgesia was found to be associated with hypoesthesia for heat and cold detection at the site of the scar and with hypersensitivity for heat and cold pain both at the scar and the contralateral side, suggesting that pain processing is permanently distorted. This hypersensitivity was predominant in children operated on within the first month of life, suggesting the existence of a developmental window.

Our study demonstrates that differences in pain processing may even persist up to 11 years after neonatal surgery. Our group earlier already showed that altered pain sensitivity may have been established during infancy. Children who, within the first three years of life, underwent subsequent surgery in the same dermatome as operated upon as a neonate, showed greater pain sensitivity than did children operated upon another dermatome[12]. Children and controls showed no differences in pain behavior when undergoing a vaccination in a different dermatome (the thigh) than the one previously operated upon[22]. This latter finding is in contrast with the finding that circumcised infants responded more vigorously to immunization at 4-6 months of age as compared to uncircumcised infants[6]. Closer inspection of this article shows, however, that circumcision affects dermatome S3 while subsequent vaccinations were given in the thigh. Experiments in neonatal rat pups confirm these findings, for unilateral hind pay inflammation invoked by injection of 25 µl Complete Freud Adjuvant induced marked increases in the density of dorsal primary afferents during adulthood, which was not restricted to the dermatome of injury but also extended into adjacent spinal segments[19, 21, 38]. Long-term effects of early injury may therefore be due to a combination of peripheral/spinal

and supraspinal changes. These long-term developmental changes in peripheral/spinal mechanisms of subsequent pain processing have been characterized reasonably well[13, 19, 39-43], whereas changes in the supraspinal areas remain poorly understood[17, 21, 44-47].

In our study, children developed hypoesthesia for the detection of temperature, although only restricted to the scar. We assume that this may be due to subtle changes resulting from altered supraspinal processing. Preliminary findings from a similar study also suggest that hypoesthesia following neonatal thoracic surgery is restricted to the site of tissue damage and does not extend to the contralateral side[48]. Experimental studies in animals only induce pain but not alterations in detection thresholds for warmth and cold[15, 19, 44].

All index children had all received adequate peri-operative analgesia as evidenced by behavioral and hormonal stress measures[26]. This suggests that tissue damage and not nociception may be responsible for the long-term alterations. Experiments in animals point in this direction as well. Another explanation might be the working mechanisms of morphine. Morphine acts on the μ opioid receptor thereby inhibiting spontaneous cellular discharge. This will decrease both calcium transmembrane transport and release of nociceptive neurotransmitters. There is growing evidence, however, that morphine does not completely blocks this process, and that enough substance p is released to activate the NK1 receptor[49, 50]. Subsequently protein kinase C is activated, which leads to phosphorylation of the P2X3 receptor. This process results in increased peak amplitude following a nociceptive stimulus[51]. As a consequence the NMDA receptor becomes activated. This receptor is directly involved in causing excitotoxic damage and may lead to the development of spinal neuro-anatomical changes[52].

Our findings are consistent with those studies reporting that pain nociception during early infancy is not restricted to the site of tissue damage, but extends to the contralateral side[10, 23]. Experiments in animals have demonstrated this hypersensitivity to coincide with greater density of afferent nerves in laminae I and II of the dorsal horn. This process extends to the contralateral side as well, although to a lesser extent[19, 21]. In an experimental setting with newborn rats, these neuroanatomical changes were most pronounced when nociception was induced at a postnatal age of 7 days (P7) and did not develop when induced at P14. These ages correspond with a term-born and a one-year-old child, respectively. Our findings confirm the existence of this developmental window as only the children operated on within the first month of life demonstrated a significant hypersensitivity to pain. It might be questioned whether premature neonates would show more severe hypersensitivity seeing that their central nervous system is even more vulnerable than that of term born neonates. Only few experimental animal studies so far have addressed this question, no human data are available[15, 19].

Besides thermal detection and pain thresholds, we studied suprapain stimuli. For practical reasons, i.e. to limit testing times, supra-pain thresholds were not administered in random order. We did not find any differences in pain reports between groups. This might be attributed to the method used to assess supra-pain sensitivity, as for both groups intensity of the stimuli was determined by the previously measured pain thresholds. Our data show that there was a temperature effect as pain report increased with increasing stimulus intention in both groups. As the pain thresholds at the scar and contralateral side of index children were about 1°C lower than those in the control children, the former would report higher VAS scores when both groups would be exposed to the same temperature. As suprapain stimuli are thought to influence supraspinal pain

processing, future studies are needed to gain better insight into neuro-anatomical alterations in these regions following neonatal pain exposure.

Our findings suggest that former patients are less sensitive to temperature detection but more sensitive to pain at the area of previous surgery than controls. Clinically, however, the differences were small. The 1.4 and 1.5°C differences found for cold and heat detection thresholds, respectively, are unlikely to be of clinical significance. For cold and heat pain thresholds at the scar, differences were 4.4°C and 0.9°C, respectively, and at the contralateral area 7.3°C and 2.3°C. Until now, no studies have been performed evaluating the impact of these differences on daily life. Their magnitudes suggest that this prolonged hypersensitivity, especially for cold pain, may be clinically substantial. However, to confirm this assumption, further studies are required.

In conclusion, our findings are consistent with surgery in early infancy leading to prolonged pain hypersensitivity in the area of injury as well as the contralateral side, which may last for up to 11 years after the initial surgical tissue damage. As children also develop thermal hypoesthesia these findings suggest that neonatal surgery permanently alters the spinal processing of thermal pain. Although differences in detection and pain thresholds were significant, the impact on clinical practice remains to be demonstrated. Future studies focusing on the developmental window may further improve our understanding of how tissue damage during early infancy alters subsequent pain processing and whether these changes predispose patients to the development of chronic pain.

Acknowledgements
The study was supported by the Netherlands Organisation for Health Research and Development (ZonMw 940-31-076).

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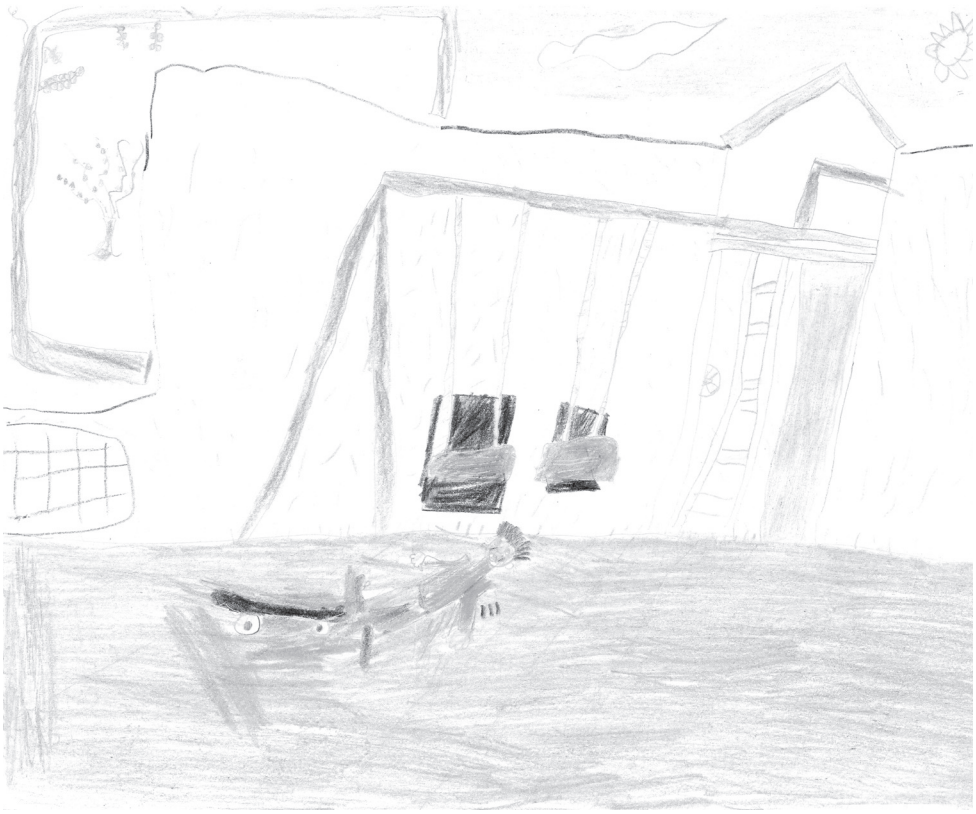
Chapter 4

Long term consequences of early injury are more pronounced in former preterm infants compared with term infants



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Submitted

Abstract

Repeated tactile stimulation decreases reflex thresholds in preterm neonates, suggestive of short-term sensitization, whereas term newborns develop habituation. Whether preterm children at later ages are still more sensitive than full-term children remains unclear. A longitudinal case-control study was performed in 8-year-old children, previously admitted to the neonatal intensive care unit (NICU) as newborns during 1996-1999. Thermal detection and pain thresholds and experiences of supra-threshold pain were measured using a thermal sensory analyzer in 53 former NICU patients (39 preterm and 14 full-term) and 53 age- and gender-matched healthy, term-born controls. Overall, patients were hyposensitive to temperature detection at both locations and hypersensitive to cold and heat pain at the heel. Predominantly the preterm-born patients showed hyposensitivity to heat detection at the hand, to cold detection at the heel ($p<0•05$) and hypersensitivity to cold pain at the heel ($p<0.05$) when compared with term-born controls. Term-born children were hypersensitive during the suprapain threshold test at both locations, whereas ex-preterm children only showed hypersensitivity for cold pain at the heel. Eight years after NICU admission, patients are still hypersensitive to pain at the area of previous tissue damage in the heel. Children born preterm are more sensitive than those born full-term. Our data suggest that possible neuroanatomical alterations have developed which can be associated with a local hypersensitivity and global hyposensitivity to thermal stimuli following early pain, which are more prominently in preterm neonates. Future studies should focus on prevention of long-term effects of early pain exposure as a means to diminish hypersensitivity.

1. Introduction

Like adults, newborns exposed to pain will develop short-term hypersensitivity at the site of tissue injury[1-6]. In neonates however, this hypersensitivity may persist for years after the short period associated with healing of the initial tissue damage[7-13].

Confirmatory findings in animals demonstrated that neonatal pain disturbs normal development of the nociceptive neural circuits, causing structural and functional neuro-anatomical changes, both at the peripheral[14, 15] and spinal cord level[16, 17]. In animal studies hyposensitivity[18, 19] and hypersensitivity[8, 14, 20, 21] have been reported following neonatal pain exposure. Also in humans these incoherent results exist [2, 9, 12, 22-25].

In one of the few studies, which directly compared preterm and full-term infants many months after neonatal pain exposure, during finger lance, immediate hypersensitivity was found in the preterm group, but they did show a faster recovery. This suggests both initial hypersensitivity and overall hyposensitivity[26]. Also animal studies describe a baseline hyposensitivity but hypersensitivity following reinflammation at adult age[27-29]. All these controversial results make long-term effects of neonatal pain exposure so little understood in humans.

One important factor in the development of neuroanatomical alterations seems to be the moment of pain exposure, as animal studies identified a developmental window of time. The effects of wounding were most pronounced when induced during postnatal days (P) 0-7, declining when performed at P14 or P21[6, 15, 16]. P6 to P9 in the rat is believed to correspond to 40 weeks gestational age in humans; P14 to a one-year-old infant[27, 30, 31].

These experimental animal studies suggest that premature birth makes neonates more susceptible to pain. This is also suggested by human data, as human infants born prematurely show different short-term reaction patterns to subsequent tactile stimuli than do term born neonates[32]. They not only show a higher sensitivity of the flexion reflex threshold [32], their amplitudes of response are also higher[2, 23, 32, 33] and they have larger receptive fields[23, 32]. While preterm neonates (less than 35 weeks gestational age) show decreasing thresholds following repeated pain exposure, those of over 36 weeks of gestation develop habituation[23, 32]. Repeated painful stimulation however is more complex, and remains hardly understood.

To examine the impact of neonatal pain exposure during the first weeks of life on long-term processing of somatic painful stimuli, we tested thermal sensory alterations in 8-year-old preterm-born and term-born NICU graduates. We hypothesized that neonatal pain exposure in the absence of analgesia results in long-term alterations in pain sensitivity and that difference in thermal detection or thermal pain thresholds may occur between preterm-born and term-born patients.

2. Methods

2.1 Subjects

This prospective case-control study included part of a cohort of children (n=53) who were admitted to the NICU of the Erasmus MC - Sophia Children's Hospital between 1996 and 1999 and were now 8 years of age. They had undergone artificial ventilation for respiratory distress syndrome during the first weeks of life without receiving morphine. All children had been followed closely according to the guidelines of the Dutch neonatal follow-up program[13, 34]. The exclusion criteria were 1) morphine administration 2) cogni-

tive impairment, 2) deafness, 3) blindness, and 4) neonatal convulsions. Age and gender matched control children who had no neonatal hospital experience were recruited from two local elementary schools within the hospital's referral area. The study protocol was approved by the 'Central Committee on Research Involving Human Subjects', and written informed parental consent was obtained.

2.2 Testing algorithm

Pain stimuli were induced by the computer-controlled thermal sensory analyzer (TSA-II 2001; Medoc Ltd. Advanced Medical Systems, Ramat Yishai, Israel) with a Peltier-based contact thermode (30x30mm). This device was used to assess alterations in somatosensory processing by three different tests, i.e. detection thresholds for heat and cold, pain thresholds for heat and cold, and suprapain thresholds for heat and cold pain. Cold and heat detection thresholds were determined, by the temperature at which the child sensed a change into either cold or heat. Cold and heat pain thresholds were determined by the temperatures at which sensations were first perceived as painful.

The used testing algorithm for the detection and the pain thresholds was the reaction-time inclusive Method of Limits (MLI). The thermode baseline temperature was set at 32°C. To prevent any tissue damage, stimulation temperatures had a potential range of -10 to 50°C. The temperature change rate was kept constant at 1.0°C/sec, with a 10°C/sec return rate. Subsequently if the child did not report pain at (one of) the extreme temperatures of -10 and 50°C, pain thresholds were set at these extreme temperature(s).

The pain threshold values, finally, were used to determine the exposure temperatures for the supra-pain test. This supra-pain test consisted of a series of 5 stimuli ranging from 2 degrees below to 2 degrees above the pain threshold for heat pain and ranging from 4 degrees below to 4 degrees above the pain threshold for cold pain. Each stimulus lasted 6 seconds, after which children were asked to rate their pain intensity on a VAS scale[35]. The stimuli were delivered sequentially with a delay of 30 sec between successive stimuli.

2.3 Procedure

Some time before testing, parents received oral and written information about the study. On the day of testing, in the presence of one parent or caregiver we explained the overall procedure inviting the child to feel the thermode (at baseline level of 32°C). The experimental procedure was similar to that in a study by Meier and colleagues[36] and took place in a separate, quiet room. Ambient room temperature was between 18-20 °C. Before actual testing, we conducted a rehearsal session during which all children were trained in pain threshold measurement. The final tests were started only when we were sure that the child fully understood our explanations and the testing algorithm. All tests were carried out by one trained investigator, R. Schouw. She was not blinded for the groups, patient-control, however, she was blinded for their medical background data like gestation age, birth weight, duration of hospital admission. During the period needed for testing of all children, evaluation moments were inserted to ascertain consistency in the given instructions and testing procedure. All patients and controls were tested in random order.

Detection thresholds, pain thresholds, supra-pain thresholds were subsequently determined at 2 sites: the thenar eminence (reference site) and the foot. First we measured skin temperature at the thenar eminence of the non-dominant hand, in order to ascertain that it exceeded 25°C. We chose the nondominant hand in order to minimize bias by reaction time.

To increase reliability, all detection and threshold stimuli were administered in series of four with a delay of 15 sec between successive stimuli [37, 38]. The thresholds were calculated as the means of four test results. For determining of detection thresholds children were exposed to 4 cold stimuli, after 30 seconds followed by 4 warm stimuli. Next, we assessed 4 cold and 4 heat pain thresholds, again with a 30 seconds interval [37, 38]. An auditory cue was given to indicate the start of the next stimulus. Suprapain testing then started after a 60 seconds interval. Five stimuli were given at 30 seconds intervals. After 5 minutes the same procedure was repeated for the heel.

2.4 Data analysis

Structural equation modeling (SEM), conducted with Mplus for Windows, was used to explore structural relationships between the patient and control groups for the detection and the pain thresholds. This approach involves the examination of several models in order to identify the most plausible model and to estimate their individual parameters based on theory as well as published data. The structural model concerns the direct and indirect relationships between independent variables and dependent variables[39]. If the final model is robust, then the structural relations estimated by the model will produce correlations close to the ones that exist in the data. Changes in the model produce a new set of parameters and estimates, which can be similarly tested.

Differences in repeated supra-pain thresholds for patients and controls were evaluated using multivariate analysis of variances (MANOVA) for repeated measurements. Differences in suprapain thresholds for preterm and term-born former patients were evaluated with the use of 95% confidence intervals. A confidence interval produces a move from a single value estimate to a range of values that are plausible for the population. A major advantage of the use of 95% confidence intervals is more valid outcomes in a limited sample size.

2.4.1 Strategy of SEM analysis

The processes of fitting SEM started with the construction of a theoretical baseline model, in which it was hypothesized that patients differed from controls at all levels, i.e. cold and heat temperatures and both locations. The main objective therefore was to evaluate the presence of group differences and the specific location to which they apply (Tables 1 and 2).

The next step hypothesized that differences in thresholds were only present for cold or heat between patients and controls not specified for locations (model 1). The second step consisted of evaluating differences in pain thresholds per location, i.e. only present at the hand, the heel, or both hand and heel (models 2 to 4).

Each model's fit was evaluated by examination of parameter estimates and measures of overall fit. The following performance measures served to test hypotheses: (1) chi-square (including degrees of freedom and p-value) for model fit: non-significant values indicate that the model cannot be rejected, (2) chi-square for model fit divided by degrees of freedom: values of <1.5 are acceptable [40], comparative fit index (CFI): values

of > 0.90 suggest good fit, maximum is 1.00 41, (4) Tucker Lewis Index [41]: values of >0.90 suggest close fit, maximum value is 1.00, (5) standard root mean squares of residuals (SRMR): values of < 0.05 indicate good fit, (6) root mean squares of approximation (RMSEA): a value of 0.05 indicates close fit. A model was rejected when it did not meet all the criteria for goodness of fit. Models were compared by means of X2 differences. The model finally accepted, was the one that had the best fit and allowed for meaningful and substantive interpretation of the parameters. The influence of possible confounders (sex, gestational age, duration of ventilation and hospital admission) were also evaluated using SEM.

3. Results

3.1 Background characteristics

Of 98 eligible former patients 53 participated, as 30 were lost to follow-up and 15 parents refused consent. Mean age at testing was 8.5 years (SD 1.6). Overall boys were overrepresented. Of these patients, 39 were born preterm and 14 were term born. Mean gestational age was 33 (SD 5) (30.2 (SD 2.5) weeks for the preterm and 40 (SD 2.0) weeks for the term neonates), with mean birth weight 1884g (SD 1131g) (1289g (SD 613g) and 3365g (SD 724g) for the preterm and term-born patients respectively). Mean hospitalization was 24 days (SD 36) (37 days for preterm-born (SD 7), and 6 (SD 4) days for term-born patients). Control group children were all term-born (mean gestation 38.1 weeks, SD 2), with a mean birth weight of 3184g (SD 462).

Before starting with the analysis, the influence of possible confounders was evaluated by using SEM. Girls appeared to be more sensitive to cold and heat pain than boys. Days of intensive care unit admission did not significantly influence pain thresholds. According to our SEM analysis, the best model fit corresponded to the hypothesis "there are no differences between short (<7 days) and long admission (>7 days)" which implies cold and heat thresholds at both the hand and heel ($x2 = 7.43$, df 10, $p = 0.68$, CFI = 1.00, TLI = 1.00, RMSEA = 0.00, SRMR = 0.17). Gestation age did influence thermal sensitivity, i.e. preterm borns are more susceptible to develop alterations, which are further described in paragraph 3.2 and 3.3.

3.2 Thermal detection thresholds

The X^2 differences for all tested models were subsequently evaluated and compared with the baseline model 0 (model 0 vs1, model 0 vs 2 and so on). All tested models significantly worsened in comparison with baseline model 0 ($p<0.001$) and were rejected. Therefore, only model 0 can be accepted. This model implies that differences in cold and heat detection thresholds are present at both hand and heel (figure 1a). This figure(1) shows that the differences between patients and controls for cold and heat detection at the heel were small, i.e. 1,9°C and 1,2°C, respectively.

During post-hoc SEM analysis we compared the preterm and term subgroups with the control group. The preterm borns showed differences with control children in detection thresholds for cold detection at heel and both cold and heat detection and the foot. The term-born group only showed significant hyposensitivity to heat at the heel. Detection thresholds for each subgroup are shown in Figure 1.

Table 1 SEM Thermal Detection thresholds

Model description						Performance Measures							
Model	Description	Hand		Heel		X^2	df	X^2/df	p	CFI	TLI	RMSEA	SRMR
		C	H	C	H								
0	Patients differ from controls	-	-	-	-	6.92	6	1.15	0.33	0.99	0.99	0.05	0.14
1	No differences for cold or heat at both locations	+a	+b	+a	+b	44.61	12	3.72	0.00	0.80	0.80	0.22	0.22
2	No differences between the locations	+a	+a	+b	+b	170.67	12	14.22	0.00	0.01	0.01	0.50	2.26
3	No differences at the hand	+a	+a	-	-	134.56	9	14.95	0.00	0.22	0.04	0.51	1.32
4	No differences at the heel	-	-	+a	+a	159.79	9	17.75	0.00	0.10	0.02	0.55	1.69

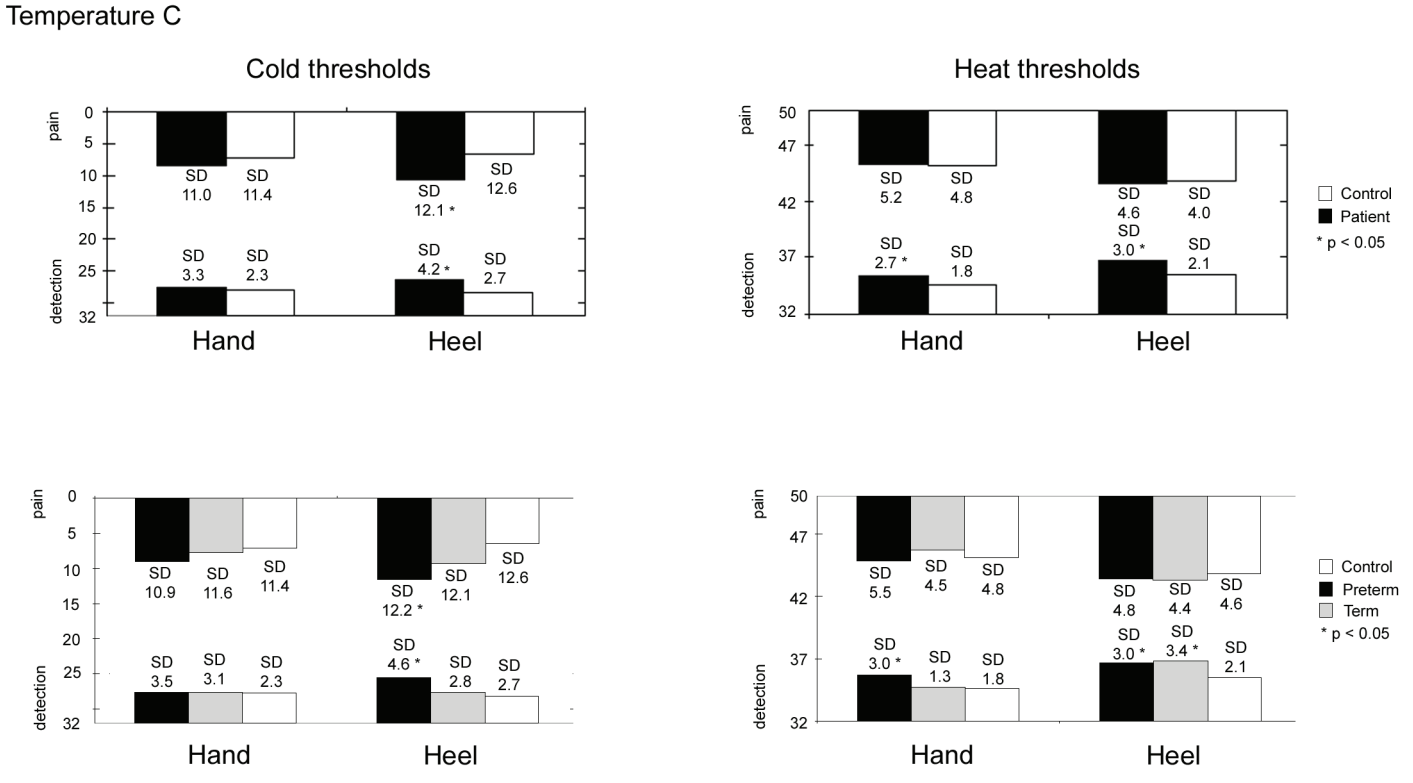
Note of legend: C indicates cold stimuli, H indicates heat stimili, (-) indicates that thresholds are incomparable,+a, and +b respectively indicate that these thresholds are comparable.

Table 2 SEM Thermal Pain thresholds

Model description						Performance Measures							
Model	Description	Hand		Heel		X^2	df	X^2/df	p	CFI	TLI	RMSEA	SRMR
		C	H	C	H								
0	Patients differ from controls	-	-	-	-	5.71	6	0.95	0.46	1.00	1.00	0.00	5.71
1	No differences for cold or heat at both locations	+a	+b	+a	+b	25.92	12	2.16	0.01	0.94	0.94	0.15	25.92
2	No differences between the locations	+a	+a	+b	+b	24.11	12	2.01	0.02	0.95	0.95	0.14	24.11
3	No differences at the hand	+a	+a	-	-	6.40	9	0.71	0.70	1.00	1.00	0.00	6.40
4	No differences at the heel	-	-	+a	+a	15.90	9	1.77	0.07	0.97	0.96	0.12	15.90

Note of legend: C indicates cold stimuli, H indicates heat stimili, (-) indicates that thresholds are incomparable,+a, and +b respectively indicate that these thresholds are comparable.

Figure 1 Thermal detection and pain thresholds



Mean detection and pain thresholds for cold and heat , the standard deviation is represented above and below each bar.

3.3 Thermal pain thresholds

Table 2 shows the results of 5 models tested for thermal pain thresholds and Table 3 shows pain thresholds for each subgroup.

The X^2 differences for all tested models were subsequently evaluated and compared with baseline model 0 (model 0 vs model 1, model 0 vs 2 and so on). Models 1, 2 and 4 significantly worsened in comparison with model 0 ($p<0.05$ to $p<0.01$) and therefore were rejected. Model 3 is the best fitting model when compared to model 0, as indicated by the ratio X^2/df and the other fit indices. Therefore, the assumption of model 3, can be accepted. This implies that there are no differences between patients and controls for cold and heat pain thresholds at the hand, but these are present at the heel, as presented in Figure 1. This figure shows that the differences between patients and controls at for cold and heat pain thresholds at the heel were 4.2°C and 0.3°C, respectively.

In order to evaluate variances between the two groups, patients versus controls, we used the same models as described above. However, as all these models described a good fit (p values ranged from 0.19 to 0.80), no alterations in pain threshold variations occurred between former patients and their controls.

During post-hoc analysis we compared both the preterm and term born subgroup with the control children by using the SEM models as described before. For the term born patients, the model "no differences in pain thresholds between patients and controls" described the best fit (figure1). In the preterm borns however, the evaluation of model fit showed that two models of the group comparison preterm versus control children showed a good fit, i.e. "no differences in heat pain thresholds" and "no differences in pain thresholds at the hand". This indicates that preterm borns only differ from control children for cold pain at the foot, i.e. they are more hypersensitive to pain (figure 1).

Table 3 Pain Thresholds

	Number	Mean CPT Hand	Mean HPT Hand	Mean CPT Heel	Mean HPT Heel
Preterms	39	9.0	44.9	11.5	43.4
Term borns	14	7.8	45.7	9.3	43.3
Controls	53	6.9	45.3	6.5	43.9

Note of legend: CPT = cold pain threshold, HPT = heat pain threshold

3.4 Suprapain threshold stimuli

The mean VAS scores (95% CI) for patients and controls at each location are presented in Table 4. All VAS scores increased with increasing stimulus intensity. Group differences were analyzed with the use of MANOVA (Table 4), showing no significant differences between patients and controls in pain tolerance for cold or heat pain at the two locations. Nevertheless, pain tolerance stimuli were based on the previously measured individual pain thresholds. Since the patients had lower pain thresholds, they also received lower intensity stimuli in pain tolerance testing.

Subgroups (preterm and term) were analyzed by 95% confidence intervals. Preterm-born patients compared with controls only differ in cold supra-threshold pain. Children in the preterm group reported higher VAS scores for perceived pain during the first two stimuli. With increasing stimulus intensity VAS scores tend to converge (Figure 2). The term-born patients show alterations at both locations for heat and cold stimulation. In this group, again the first two stimuli are different (Figure 2).

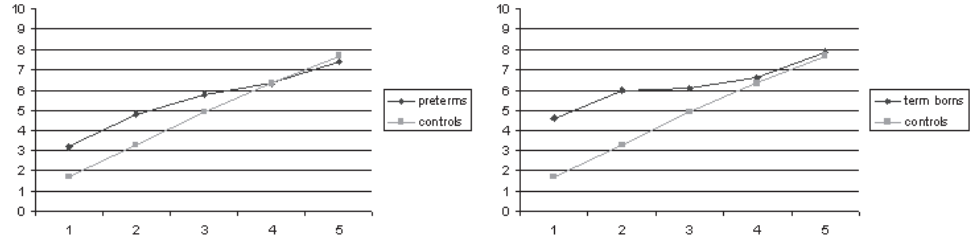
Table 4 VAS scores in cm

Location	Patients Mean (95%CI)	Controls Mean (95%CI)
Hand cold	5.8 (5.1 to 6.5)	5.1 (4.3 to 5.8)
Hand heat	5.7 (5.1 to 6.3)	5.0 (4.4 to 5.7)
Foot cold	5.7 (5.0 to 6.4)	4.9 (4.1 to 5.6)
Foot heat	5.7 (5.0 to 6.3)	4.8 (4.1 to 5.5)

Multivariate significance testing

Effect	F	p
Location	0.40	0.53
Location x Group	0.21	0.63
Temperature	0.14	0.71
Temperature x Group	0.00	0.97
Location x Temperature	0.00	0.97
Location x Temperature x Group	0.01	0.94

Figure 2 Suprapain sensitivity



Note of legend: heel preterms versus controls (left) and for term borns versus controls (right). 1=2°C below heat pain threshold or 4°C above cold pain threshold, 2=1°C below heat pain threshold or 2°C above cold pain threshold, 3=pain threshold, 4=1°C above heat pain threshold or 2°C below cold pain threshold, 5=2°C above heat pain threshold or 4°C below cold pain threshold

4. Discussion

We found that pain exposure in the absence of analgesia during the first weeks of life led to diminished sensitivity to heat and cold detection thresholds at 8 years of age. Increased sensitivity to heat and cold pain at the heel was noted as well. The finding that pain thresholds at the hand did not significantly differ between former patients and controls suggests that neonatal pain permanently distorts somatosensory processing of thermal stimuli in the area of neonatal tissue damage. Effects in terms of hyposensitivity for temperature detection and hypersensitivity for pain were more pronounced in preterm children compared with term born children, suggesting the likelihood of a developmental window. Studies in rodents also demonstrate the existence of this developmental window [15, 16].

Our findings show that the effects on pain thresholds are restricted to the area of neonatal pain exposure and are consistent with previous studies showing that surgical pain results in a localized altered pain sensitivity [9, 12, 13, 42]. Confirmatory animal experiments have shown that altered pain processing is associated with increased density of spinal afferents at the dermatome of tissue damage and adjacent spinal segments[15, 16, 28] Both human and experimental animal studies also demonstrated increased pain sensitivity and spinal morphological alterations on the contralateral side, yet less pronounced[9, 16]. In this study we refrained from testing on the contralateral side, as all infants received heel stick in both feet alternately. Therefore both feet were equally exposed to pain. In order to limit the testing time we decided that one foot was sufficient to determine whether alterations in pain sensitivity were present. As both heels received the same amount of pain, we expect the same differences in the non-tested foot.

In this study we found that hypersensitivity for cold pain was even greater in the former premature born children than in the former full term born children. Recently, similar findings were demonstrated in 8-9 year-old children who underwent major surgery neonatally. These children had greater hypersensitivity for cold as well as heat pain than those who underwent major surgery at the age of 2 to 3 months suggesting that long-term effects of neonatal pain are more pronounced in the preterm-born patients and diminish with increasing gestational age[43]. Experimental animal studies, confirmed these findings and showed that spinal neuro-anatomical changes were most pronounced when induced at P0-7 [15, 16, 27].

Magnitude of pain exposure may have influenced our findings. As the prematurely born children overall needed longer duration of ICU stay, they likely had undergone more painful procedures in comparison with the hospitalized term born neonates[44-48]. This prolonged and increased pain exposure makes it more likely that preterm borns suffered more pain and as a consequence reported increased pain sensitivity. However, when evaluating the effect of NICU admission days, this could not be confirmed.

Our data on temperature detection are not consistent with data from our previous study, in which children operated on as neonates appeared to be less sensitive to temperature detection at the surgical wound[43]. However, all these children were born full-term. Their hyposensitivity may therefore have resulted from nerve damage following surgery. This stimulates a sprouting response form distant axons, starting a few days after wounding[27]. These axons may be specifically nociceptive and therefore do not transmit subtle detection thresholds. Alternatively, the more generalized hyposensitivity in our present study may be explained by subtle changes in supraspinal processing among preterm-born children, a field which is still poorly understood [8, 18, 49-52].

A limitation of our study may be that supra-threshold pain testing was not randomised. However, in order to control for anticipation, children were not informed that the subsequent stimuli would increase in intensity. Generally, pain reports increased with increasing pain stimuli, and did not differ between patients and controls. Subgroup analysis showed, however, that term-born patients reported more pain for the first two stimuli than control children. This also suggests that supra-pain thresholds in the former patients, both prematurely and term born children, follow a different pattern, with some kind of ceiling effect. Perhaps certain compensatory mechanisms will prevent unwanted hypersensitivity during supra-threshold pain stimuli. As supra-threshold pain stimuli activate supraspinal pain processing, it is most likely that these compensatory mechanisms are located in the supraspinal areas. However, to our knowledge, studies concerning this issue have not yet been reported.

Our findings suggest that former patients are less sensitive for temperature detection but more sensitive to pain at the area of previous tissue damage, i.e. the heel. Clinically, however, the differences in detection and pain thresholds were small, i.e. 1.9 and 1.2°C for cold and heat detection thresholds. Therefore, it is unlikely that this will be clinical substantial. For cold and heat pain thresholds at the heel, differences were 4.2°C and 0.3°C, respectively. Until now, no studies have been performed evaluating the impact of these differences during daily life. The magnitude of these differences, suggests that this prolonged hypersensitivity, especially for cold pain, may be clinical substantial. However, to confirm this assumption, further studies are required.

In conclusion, our data demonstrate that neonatal pain exposure leads to prolonged hypersensitivity at the site of tissue damage, lasting up to 10 years. Effects were more pronounced in preterm-born children compared with term-born children, suggesting that the latter's CNS may be more mature. The development of hyposensitivity for temperature detection, points at altered supraspinal processing having resulted in a permanently distorted pain-warning signal. Future studies focussing on the development of supraspinal alterations may further improve our understanding about pain processing and its long-term effects.

Acknowledgements
The study was supported by the Netherlands Organisation for Health Research and Development (ZonMw 940-31-076).

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Chapter 5

Long term alterations in thermal detection and pain thresholds following neonatal morphine exposure, a study in former ECMO patients

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Abstract

Pre-emptive analgesia in neonates, using morphine infusions, can prevent pain but does not prevent the development of long-term hypersensitivity to pain in the area of tissue damage. High-dose morphine therapy in neonatal period causes altered development of the central nervous system in animal studies. To investigate whether these alterations cause long-term hypo- or hypersensitivity to pain in humans, we performed a case-control study comparing pain sensitivity in 8 year-old between children who received morphine during extra-corporeal membrane oxygenation (ECMO) as neonates and controls without previous hospital experiences. We recruited 60 children (mean age 8 years, SD 1.1) who had received morphine (20 mcg/kg/h for a median of 9 days) during veno-arterial ECMO for neonatal respiratory or circulatory insufficiency between 1996 and 1999. Their thermal sensitivity was compared with 60 age- and gender-matched controls. A thermode was sequentially attached to the non-dominant hand, the right and left sides of the neck (i.e. site of ECMO cannulation and contralateral side). Thermal detection and pain thresholds were assessed by steadily increasing/decreasing the thermode's temperature at a rate of 1°C per second (exposure range -10°C to 50°C) and suprapain thresholds were also determined. We found that the patients were hyposensitive to the detection of cold and heat temperatures at all three anatomical locations in comparison with the control children. No differences in cold and heat pain thresholds could be demonstrated at any location when compared with controls. Moreover, also supra-pain thresholds were not different from controls at any of these locations. Concluding, long-term alterations in thermal pain sensitivity did not occur following neonatal high-dose morphine exposure during ECMO. These children developed a global hyposensitivity for thermal detection, possibly resulting from alterations in supraspinal processing, which need further investigation.

1. Introduction

Brain development is characterized by an increased plasticity during early postnatal life [1-3]. Alterations in normally occurring activity patterns during this "critical period of development" make the nervous system more susceptible to perturbation than at any other time of life. In contrast to adults, tissue injury in neonates results in both a global hyposensitivity for temperature detection and hypersensitivity to pain, persisting for years after the tissue damage has healed [4-7]. This hypersensitivity may result from pain-induced excitotoxic damage, presenting as a permanent distortion of nociceptive neuronal circuits, due to structural and functional, peripheral and spinal neuroanatomical changes [1, 8-10]. These neuroanatomical changes consist of sprouting of fibers into the wounded area after nerve damage, eventually overcompensating for the damage [9-11].

Neonatal pain is treated by pre-emptive administration of opioids[12] to prevent pain-related stress and short-term morbidity [13]. Paradoxically, postoperative morphine does not prevent these children from developing long-term hypersensitivity for pain in the area of tissue damage [4, 5, 14]. Studies in adults provide growing evidence that morphine does not completely block the local nociceptive signals which eventually lead to pain hypersensitivity via changes in synaptic signaling and intracellular signal transduction mechanisms [15].

Apart from treatment of postoperative pain, opioids are also commonly administered for sedation in neonates undergoing ECMO [4, 16]. During ECMO cannulation in our hospital, morphine 100 µg/kg and midazolam 0.2 mg/kg are given[17] followed by continuous infusions of 10 µg/kg/h morphine with 0,1 µg/kg/h increments based on COMFORT and VAS scores[13, 18]. It is unknown whether high dose morphine infusions administered during ECMO (median 20 µg/kg/h for a median duration of 9 days) affect the development and maturation of the nervous system as noted from animal studies,[1, 3, 10, 19-21]. Due to the paucity of data on the long-term effects of opioid therapy on human brain development, we performed a case-control study in former ECMO patients and matched controls.

2. Patients & Methods

2.1 Patients

We performed a cross-sectional study of children admitted to the ICU between 1994 and 1999 either at Erasmus MC - Sophia Children's Hospital in Rotterdam or at Radboud University Nijmegen Medical Center in Nijmegen, the Netherlands. These children underwent Veno-Arterial Extra Corporeal Membrane Oxygenation (ECMO) for various problems – i.e. meconium aspiration syndrome, persistent pulmonary hypertension, or sepsis, according to standardized protocols based on international guidelines. They all received analgesics and sedatives for a prolonged period in the absence of severe pain as assessed by COMFORT and VAS scores. Since then, all children have been followed by the Dutch neonatal follow-up program [4, 14].

The present study took place in the years 2002 to 2005, when these children had reached a mean age of 8 years. Age and gender matched controls who had no neonatal hospital experience were recruited from two local elementary schools within the referral area of the Erasmus MC-Sophia Children's Hospital. The study protocol was approved by the 'Central Committee on Research Involving Human Subjects' and signed informed parental consent was obtained.

2.2 Testing algorithm

Alterations in somatosensory processing were assessed by three different tests, i.e. thermal detection thresholds and pain thresholds for heat and cold, and suprapain thresholds for heat and cold pain using a thermal sensory analyzer (TSA-II 2001; Medoc Ltd. Advanced Medical Systems, Ramat Yishai, Israel). This is a precise, computer-controlled device capable of generating and recording a response to a highly repeatable thermal stimulus. A Peltier-based contact thermode (30x30mm) was used to apply cold or heat. The entire thermode-stimulating surface was kept in contact with the skin by a Velcro band.

The baseline thermode temperature was set at 32°C. To prevent any tissue damage, thermode temperatures had a potential range of -10 to 50°C. Cold and heat detection thresholds were determined by the temperature at which the child sensed a change into either cold or heat, respectively. Cold and heat pain thresholds were determined by the temperatures at which sensations were first perceived as painful. The pain threshold values were used to determine the exposure temperatures for the suprapain test, which is explained below. The temperature change rate was kept constant at 1.0°C/sec, with a 10°C/sec return rate. Interstimulus intervals were fixed at 15 seconds. To increase reliability, a series of four stimuli were administered and thresholds were calculated as the means of four test results[22, 23]. The thresholds were calculated as the means of four test results. If the child did not report pain at (one of) the extreme temperatures of -10 and 50°C, pain thresholds were set at these extreme temperature(s).

The suprapain test consisted of a series of 5 stimuli ranging from 2 degrees below to 2 degrees above the pain threshold for heat pain and ranging from 4 degrees below to 4 degrees above the pain threshold for cold pain. Each stimulus lasted 6 seconds, after which children were asked to rate their pain intensity on a VAS scale[24]. Excluded from this latter test were the children whose pain thresholds had been set at the extreme temperatures.

Tests were done at three body locations: the thenar eminence of the non-dominant hand (reference site), the previous ECMO canula insertion site (i.e. the right anterior neck) and the intact contralateral side (left anterior neck).

2.3 Procedure

Before testing, we sent eligible children and their caregivers a letter (child and adult version) explaining the purpose of the study and the methodology to be used. We next called them to provide further details if required and to request their consent for participation. On the day of testing, in the presence of one caregiver we explained the overall procedure inviting the child to feel the thermode (at baseline level of 32°C). We again pointed out their right to withdraw permission for the continuation of the test at any time. The children or caregivers were not given access to the computer screen, nor were they informed of any personal results[25]. The experimental procedure was similar to that in a study by Meier and colleagues[25]. The ambient room temperature was kept between 18-20 °C. Before actual testing, we conducted a rehearsal session.

Skin temperature was measured at the thenar eminence of the non-dominant hand; this location was chosen in order to minimize bias by reaction time. For testing, the children were instructed to press the button as soon as they experienced cold or heat

sensations. Next, the first pain threshold (either cold or heat) was determined in a series of four stimuli, with an auditory cue to indicate the start of the next stimulus. After a five-minute interval, the other pain threshold was assessed, also in a series of four.

2.4 Data analyses

In this study we evaluated three different outcome parameters; i.e. detection threshold, pain threshold, and pain intensity at suprapain thresholds. Structural equation modeling (SEM), conducted with Mplus for Windows, was used to explore structural relationships between the two groups for the detection and pain thresholds. This approach involves the examination of several models in order to identify the most plausible model, based on theory as well as published data, and to estimate their individual parameters. The structural model concerns the direct and indirect relationships between independent variables and dependent variables[26]. If the final model is robust, then the structural relations estimated by the model will produce correlations close to the ones that exist in the data. Changes in the model produce a new set of parameters and estimates, which can be similarly tested. Alterations in repeated supra-pain thresholds for patients and controls were evaluated using multivariate analysis of variances (MANOVA) for repeated measurements.

2.4.1 Strategy of SEM analysis

The process of fitting SEM started with the construction of a theoretical baseline model (model 0) (tables 1 and 2). This model represents the hypothesis that thermal detection/ pain thresholds are not altered in children neonatally exposed to prolonged morphine treatment compared to those who lack this history. Next, the temperature specificity (i.e. were alterations only present for heat, or cold, or both temperatures, model 1), the exact location or combinations of locations, and extent of alterations between patients and controls were determined (models 2 to 8).

To identify the most plausible model, we evaluated each model's fit by examining the parameter estimates and measures of overall fit. Several performance measures were used to test these models: (1) chi-square for model fit (including degrees of freedom and p-value; a non-significant value indicates that this model cannot be rejected), (2) chi-square for model fit divided by degrees of freedom: a value of <1.5 is acceptable [27], (3) comparative fit index (CFI): a value of > 0.90 suggests a good fit, maximum is 1.00[28], (4) Tucker Lewis Index (TLI): a value of >0.90 suggests a close fit, maximum value has to be 1.00, (5) standard root mean squares of residuals (SRMR): a value of < 0.05 indicates a good fit, (6) root mean squares of approximation (RMSEA): a value of 0.05 indicates a close fit. A model was rejected if it did not meet all these criteria for goodness of fit. Models are compared by means of X^2 differences between the tested and the baseline model. The model finally accepted, was the model that had the best fit and allowed meaningful and substantive interpretation of the parameters[29].

3. Results

3.1 Background characteristics

Of the 72 eligible children, 60 participated (35 boys and 25 girls). Four children were lost to follow-up and eight parents refused consent. Of the enrolled patients, 35 had undergone neonatal ECMO in Rotterdam and 25 in Nijmegen. Mean age at testing

Table 1 Model description and performance measures detection thresholds

Model description								Performance Measures							
Model	Description	Hand		Scarr		Contralateral		X^2	df	X^2/df	p	CFI	TLI	RMSEA	SRMR
		C	H	C	H	C	H								
0	Patients differ from controls	-	-	-	-	-	-	30.14	15	2.01	0.01	0.98	0.91	0.13	0.15
1	No differences for cold or heat at the three locations	+a	+b	+a	+b	+a	+b	115.00	25	4.60	0.00	0.73	0.68	0.25	0.33
2	No differences per location	+a	+a	+b	+b	+c	+c	219.30	14	15.66	0.00	0.42	0.28	0.37	2.20
3	No differences at the hand	+a	+a	-	-	-	-	178.00	18	9.89	0.00	0.55	0.21	0.39	1.15
4	No differences at the scar	-	-	+a	+a	-	-	189.00	18	10.50	0.00	0.49	0.15	0.40	1.46
5	No differnces at the contralateral side of the neck	-	-	-	-	+a	+a	185.30	18	10.29	0.00	0.50	0.17	0.40	1.41
6	No differences at the hand and scar	+a	+a	+b	+b	-	-	211.40	21	10.07	0.00	0.44	0.19	0.39	1.99
7	No differences at the hand and contralalteral side of the neck	+a	+a	-	-	+b	+b	209.70	21	9.99	0.00	0.44	0.20	0.39	1.96
8	No differences at the scar and contralateral side of the neck	-	-	+a	+a	+b	+b	202.8	21	9.66	0.00	0.46	0.23	0.38	1.80

Note of legend: (-) indicates that thresholds are not comparable, + a, +b and +c respectively indicate that these thresholds are comparable.

Table 2 Model description and performance measures pain thresholds

Model description								Performance Measures							
Model	Description	Hand		Scarr		Contralateral		X^2	df	X^2/df	p	CFI	TLI	RMSEA	SRMR
		C	H	C	H	C	H								
0	Patients differ from controls	-	-	-	-	-	-	15.85	15	1.06	0.39	1.00	1.00	0.03	0.17
1	No differences for cold or heat at the three locations	+a	+b	+a	+b	+a	+b	22.07	25	0.89	0.62	1.00	1.00	0.00	0.17
2	No differences per location	+a	+a	+b	+b	+c	+c	23.85	24	0.99	0.47	1.00	1.00	0.00	0.19
3	No differences at the hand	+a	+a	-	-	-	-	16.90	18	0.94	0.53	1.00	1.00	0.00	0.17
4	No differences at the scar	-	-	+a	+a	-	-	19.38	18	1.08	0.37	1.00	1.00	0.04	0.19
5	No differnces at the contralateral side of the neck	-	-	-	-	+a	+a	20.78	18	1.15	0.29	0.99	0.99	0.05	0.19
6	No differences at the hand and scar	+a	+a	+b	+b	-	-	21.58	21	1.03	0.42	1.00	1.00	0.02	0.18
7	No differences at the hand and contralalteral side of the neck	+a	+a	-	-	+b	+b	23.43	21	1.12	0.32	1.00	0.99	0.04	0.18
8	No differences at the scar and contralateral side of the neck	-	-	+a	+a	+b	+b	23.85	24	0.99	0.47	1.00	1.00	0.00	0.19

Note of legend: (-) indicates that thresholds are not comparable, +a , +b and +c respectively indicate that these thresholds are comparable.

was approximately eight years for ECMO patients and controls (SD 1.1 and 1.3, respectively). All patients were born at term (39 wks, SD 2.1) and with normal birth weight (mean 3333g, SD 618g). Total duration of admission for neonatal ECMO was 22 days (SD 21). The control group included 60 children, born at term (mean 38 wks, SD 1.4) and with normal birth weight (mean 3454g, SD 559).

3.2 Thermal detection thresholds

Both cold and heat detection thresholds were measured at three different locations, with good correlation of thresholds at all three locations.

Table 1 shows the result of 8 tested models. Our baseline hypothesis, that patients do not differ from controls, was evaluated and represented by model 0. This model was rejected because of a bad fit, noted from multiple parameters. Therefore, we concluded that significant differences in thermal detection thresholds occur between patients and controls. In order to verify these finding, all proposed models were subsequently evaluated (model 0 vs 1, model 0 vs 2 and so on). Firstly, the temperature specificity of the alterations in detection thresholds was determined (model 1). Evaluation of parameters revealed that model 1 had a bad fit, worse than that of model 0, and was also rejected. Accordingly, we concluded that differences in detection thresholds between patients and controls were present for both cold and hot temperatures.

Subsequently, the location of thermal detection differences between patients and controls was determined (models 2 to 8). Evaluation of the parameters for models 2 to 8 revealed that all these models had a bad fit, each of which performed worse than the fit for model 0 when comparing X^2 differences. Therefore all these models were rejected. These analyses imply that thermal detection thresholds are different between patients and controls, for both heat and cold stimuli at all three locations (figure 1). In general, patients had higher thresholds than controls, suggesting a global thermal hypoesthesia, with significant differences for the right and left neck areas for cold detection and for the thenar eminence and the left neck area for heat detection ($p<0.05$).

3.3Thermal pain thresholds

Mean (SD) pain thresholds for both groups are reported in Table 3 showing good correlations for both cold and heat pain thresholds measured at all three locations.

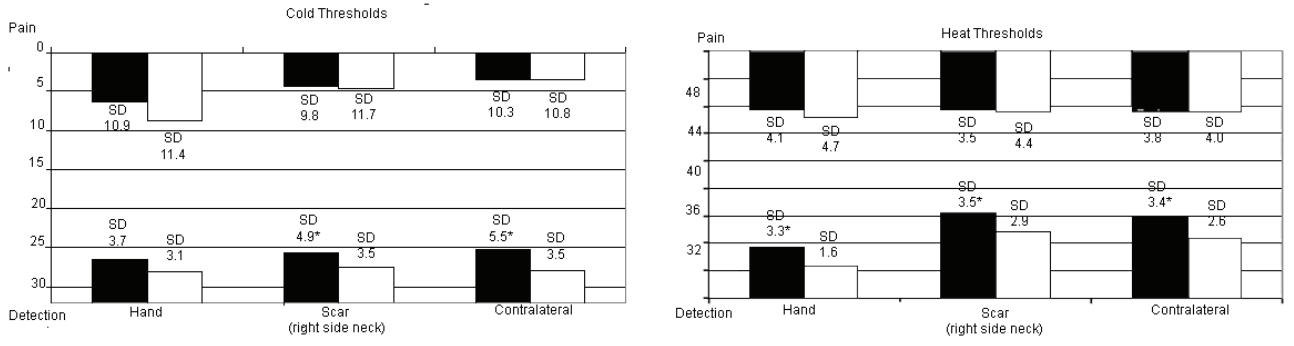
Table 2 shows the result of the 9 tested models. Our baseline hypothesis, patients differ from controls, is evaluated and represented by model 0. As this model has a good fit, it cannot be rejected. Therefore we can conclude that there are no differences in pain thresholds between patients and controls. In order to verify this conclusion the differences in X2 for all tested models were subsequently evaluated and compared with the baseline model 0 (model 0 vs model 1, model 0 vs model 2 and so on). None of the models 1 to 8 was significantly better dan model 0.

Possible differences in variances between the two groups were evaluated using the same models as described above. However, all these models described a good fit (p values ranged from 0.21 to 0.80). This indicates that no long-term alterations in pain

3.4 Pain intensity with supra-threshold thermal stimuli

Table 4a shows the means (95% CI) of the children's corresponding five VAS scores per location. All VAS scores increased with increasing stimulus intensity. Table 4b

Figure 1 Heat and cold detection and pain thresholds



Note of legend: black bars represent the patients, the white bars represent the control group. * Indicates a significance of $p<0.05$.

Table 3 Mean Pain Thresholds (SD)

	Number	Mean CPT Hand	Mean HPT Hand	Mean CPT Scar	Mean HPT Scar	Mean CPT Contra	Mean HPT Contra
ECMO	30	6.4	45.8	4.4	45.8	3.6	45.7
Controls	30	8.7	45.2	4.8	45.6	3.4	45.7

Note of legend: CPT = Cold pain threshold, HPT = Heat pain threshold

shows the results of multivariate significance testing for group differences, which demonstrates that there are significant differences in pain tolerance for cold and heat pain between the various locations. However, this is the same for both patients and controls.

Table 4a Mean VAS scores

Location	Patients Mean (95%CI)	Controls Mean (95%CI)
Hand cold **Hand heat**	5.4 (4.9 to 5.8) 5.0 (4.5 to 5.6)	4.9 (4.4 to 5.3) 5.3 (4.8 to 5.9)
Scar cold **Scar heat**	4.8 (4.4 to 5.3) 5.4 (4.8 to 5.9)	5.0 (4.5 to 5.4) 4.9 (4.4 to 5.5)
Contra cold **Contra heat**	5.1 (4.6 to 5.6) 5.7 (5.2 to 6.1)	5.3 (4.8 to 5.8) 5.3 (4.8 to 5.7)

Table 4b MANOVA

Effect	F	p
Location	4.94	0.01
Location x Group	2.15	0.12
Temperature	5.76	0.02
Temperature x Group	1.69	0.20
Location x Temperature	1.41	0.25
Location x Temperature x Group	0.00	0.99

4. Discussion

To date in many neonatal intensive care units around the world, premature and term neonates placed on mechanical ventilation or ECMO routinely receive morphine[29]. Morphine has significant short-term side effects on hemodynamic stability, causing hypotension in ventilated preterm neonates[30, 31]. For the first time, we report that term neonates treated a median dose of 20 µg/kg/h of morphine for a median of 9 days during ECMO, have developed a long-term generalized hyposensitivity for temperature detection at age 8 years, but no alterations in pain sensitivity.

We were surprised to find no differences in pain thresholds between children treated with ECMO and matched controls. In our previous studies, despite adequate treatment with morphine[32], neonatal pain exposure, was found to result in localized hypersensitivity to pain [4, 5, 13]. It must be noted, however, that the children in the present study received 2 to 4 times higher morphine dosages during ECMO than those in our previous studies, evaluating neonatal morphine exposure for non-cardiac surgery [4, 32]. In addition, tissue damage at the cannulation site is very limited, as there is a small wound and minimal tissue handling when compared to surgical wounds (in case of thoracotomy or laparotomy). These children are thus unlikely to have suffered excessive pain during the neonatal period. This suggests that only substantial pain would generate permanent hypersensitivity in the area of neonatal tissue damage.

In adults, paradoxically, opioid administration can result in hypersensitivity, since opioid-induced analgesia shares the same underlying mechanisms as pain-induced

hypersensitivity. Both pain and opioids can activate the N-methyl-D-aspartate (NMDA) receptor [33]. Activation will initiate an intracellular cascade, including translocation and activation of protein kinase C (PKC)[34]. PKC increases the amplitude of response following a nociceptive stimulus, which then contributes to the development of hypersensitivity[14]. We questioned whether in neonates this may lead to long-term sequelae.

Clinical studies in humans as well as animal experiments have shown that neonatal pain disturbs normal development of the nociceptive neural circuits probably caused by excessive NMDA receptor activation. As a consequence, this causes structural and functional neuroanatomical changes, both at a peripheral and spinal level [1, 8, 31, 35-37]. These changes may lead to hypersensitivity persisting up to 10 years following neonatal tissue damage [4-7, 38]. Since we were unable to detect differences in pain thresholds, newborns may be less sensitive to develop opioid-induced hypersensitivity than adults. Fitzgerald suggested that neonates might have more 'sleeping' NMDA receptors, which might explain the fact that we could not detect differences in pain thresholds. However, future studies concerning this issue are required[39].

ECMO treatment in combination with morphine administration was found to result in a generalized hyposensitivity for thermal detection. Nevertheless, clinical differences were small, ranging from 1 to 2.5 °C, suggesting that this hyposensitivity may not have major implications for daily life. Yet this finding suggests that neuroanatomical changes have indeed occurred. A burning question is whether these alterations are due to morphine alone or result from other factors associated with ECMO treatment (e.g. the administration of additional sedatives to prevent restlessness, severity of illness, and hypoxia).

Experimental findings in animals have shown that during late ontogenesis, morphine will induce apoptosis in the cerebral cortex [40, 41]. As a consequence, there is a decreased neuronal density in the primary somatosensory cortex and hypothalamus, which can lead to a generalized altered sensitivity. Hu et al.[42], found that this morphine-induced apoptosis in vitro is found with morphine concentrations of 8-10 M and 10-12 M, respectively, which are much higher than those in ECMO treated children[4]. Contradictory findings suggest that μ-opioid receptor activation may even prevent neuronal apoptosis in vitro [41, 43, 44] or improve the outcomes of birth asphyxia[45]. As changes in supraspinal processing remain poorly understood [30, 46-48], further studies are required.

Apart from morphine, neonates on ECMO also receive GABA agonists like midazolam. This group of drugs has recently been shown to trigger widespread apoptotic neuro-degeneration throughout the developing brain, at least when administered to immature rodents during the period of brain growth spurt [49]. The dosages used in these studies however, far exceed the dosages used during ECMO [15]. Therefore, it seems unlikely that sedative drugs should have induced neuroanatomical alterations in supraspinal processing which lead to generalized thermal detection hyposensitivity in our patients.

In this study, the observed hyposensitivity for temperature detection appeared to be generalized. By contrast, other recent observations show that children who had undergone major surgery at neonatal age in combination with morphine administration develop hyposensitivity that is restricted to the dermatome of previous wounding [5, 50]. Differences in dosage and duration of morphine administration could explain this discrepancy. While infants on ECMO in our study had received a median morphine infusion of 20 μg/kg/h for 5 to 17 days, surgical neonates had only received 5-10 μg/kg/h for 1-2 days[12].

Besides detection and pain thresholds, we studied suprapain stimuli. In order to restrict total testing time, suprapain threshold testing was not randomized [5, 38]. To control for anticipation, children were not informed that the intensity of subsequent stimuli will be increased. VAS pain scores increased with increasing painful stimuli, but consistent with our previous study pain scores did not differ between patients and controls[5].

In conclusion, we were not able to demonstrate long-term alterations in pain sensitivity following neonatal high and prolonged morphine exposure to high-dose morphine in the neonatal period, associated with relatively minor tissue damage. Yet, as morphine-exposed children had developed hyposensitivity to temperature detection, supraspinal processing might have undergone alteration. Other factors, such as the administration of additional sedatives to prevent restlessness during ECMO, duration of admission and severity of illness, may be of importance as well. Future studies focusing on supraspinal neuroanatomical alterations will improve our understanding of pain processing in the human neonate and its long-term clinical consequences.

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Chapter 6

Alterations in pain perception 8 years following neonatal ICU admission

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Abstract

Introduction
Neonatal pain exposure appears to have long-term consequences such as alterations to pain perception, including later hyper- and hypo-sensitivity. Confirming these findings, experimental animal studies have shown that neonatal, but not later, injury can lead to reorganization of the nervous system, lasting into adulthood. However, there are few studies in humans describing functional pain perception of children who differed in neonatal exposure to pain. In this study we examined whether children with varying neonatal pain and analgesia exposure showed different pain perception and coping at age 8 years. A cross-sectional study was undertaken in which children age 6 to 11 years responded to pictures depicting pain situations and to a pain coping questionnaire. This study included 4 groups of children: a Surgery group (full-term infants neonatally exposed to pain in the presence of morphine, n=57), a Pain group (infants exposed to pain during neonatal intensive care in the absence of morphine, n=53), Morphine group (full-term infants who underwent neonatal extra corporeal membrane oxygenation [ECMO], n=60) and a Control group (full-term infants without previous hospital experience, n=170). We found that children in the Pain group rated all pain related cartoons higher (both affect and intensity) and used fewer problem focused avoidance techniques and approach, but responded with more emotion-focused techniques, which are considered inadequate. Children in the Surgery group rated only the medical-setting pictures as more painful, and children in the ECMO group responded no differently than the controls. Concluding neonatal pain exposure in infants in the absence of morphine leads to alterations in pain perception and coping. The specific neuronal pathways which could have contributed to this phenomenon are still unknown. Nevertheless, although the differences are significant, they remain small. Therefore, it is unlikely that these children will experience problems later in childhood. Further studies are needed to gain more insight into factors influencing pain coping in former critically ill newborns, and whether these differences are limited to infants born preterm.

1. Introduction

Pain and stress are common phenomena occurring in neonates admitted to the neonatal intensive care unit (NICU), particularly those born prematurely who spend a long time in the hospital[1-3], and infants who undergo surgery. Apart from short-term effects such as homeostasis disturbance[4] and hyperalgesia[5-7], prolonged neonatal pain exposure may also lead to conditioning and to long term changes in sensitivity to pain[8-11]. The magnitude of these effects appears to depend on the period of brain development when the pain exposure occurred. For example, preterm children exposed to pain inherent to their Neonatal Intensive Care Unit (NICU) admission developed greater hypersensitivity to pain than their term counterparts also exposed to pain inherent to their NICU stay[12]. Therefore, there appears to be a critical developmental window during which newborns are extremely vulnerable to the long-term effects of repetitive pain. Similarly, experimental animal studies have shown that pain applied in the neonatal period induces long lasting or permanent changes not seen when pain is applied at later ages[13]. Moreover, the direction (hyposensitivity or hyposensitivity) and extent of changes depends on the timing, and whether the site is re-inflamed[14]. In animals, alterations spread to peripheral[15, 16], spinal[17, 18] and supraspinal levels [19, 20] of the rat nervous system and can result in permanent reorganization[21].

Besides neuroanatomical changes in the somatosensory cortex, it is likely that the affective, motivational and cognitive evaluative dimensions of pain might be affected by neonatal pain exposure. Grunau[22] demonstrated that former extremely low birth weight (ELBW) children rated medical pain intensity significantly higher than psychosocial pain at the age of 8 years, unlike full birth weight controls. Also, duration of neonatal intensive care unit (NICU) stay for the ELBW children was correlated with increased pain affect ratings in recreational and daily living settings[22]. Moreover, the way children perceive and experience pain is also influenced by their use of pain coping strategies[23, 24]. Coping includes dynamic cognitive and behavioral efforts to manage specific events that are appraised as exceeding the resources of the person[25]. During childhood, children experience numerous painful events, inherent to daily living[23]. However, they develop more efficient coping styles as they grow up[23] learning to interpret each pain experience within a constantly changing frame of reference as they are exposed to a wider diversity of pain intensities, frequencies, locations and levels of unpleasantness. Also, coping styles develop more efficiently or maladaptively following pain experiences[26]. It is suggested that the cortical areas controlling the sensory, attentional, premotor and affective functions of pain are integrally related to motivation and emotions and may be associated with immediate efforts to cope with, escape or avoid the pain and pain-evoking situation[27]. To address child pain experience, it is important to evaluate coping, as well as perception of pain.

The goal of this study was to examine whether pain and coping differ in children with different histories of neonatal pain, analgesia and hospitalization, compared to healthy controls. Children age 7-9 years responded to pictures depicting pain situations, and to a pain coping questionnaire.

2. Methods

2.1 Design

A cross-sectional study was undertaken using a case-control design with concurrent controls in both the Erasmus MC – Sophia (surgery group, pain group and 35 ECMO children) and Radboud University Nijmegen Medical Center (25 ECMO children).

2.2 Subjects

We included four cohorts of children with different pain history. The first cohort (Surgery group), comprised children born full-term (37-40 weeks gestation), between 1996 and 1999, who had undergone major abdominal or thoracic surgery during the first 3 months of life. Surgery had been stoma placement in 46%, congenital diaphragmatic hernia repair in 14%, esophageal atresia correction in 14%, adhesiolysis in 7%, and other procedures, including pancreatic resection and Blalock procedure, in 19%. Analgesia had been given by standard protocol, as published earlier by our group[28]. The second cohort (Pain group), predominantly comprised of children born preterm between 1996 and 1999, admitted to the Neonatal Intensive Care Unit (NICU) during the first weeks of life. These children underwent artificial ventilation for pulmonary problems such as hyaline membrane disease and bronchopulmonary dysplasia. None of these children had received morphine during the period of artificial ventilation.

The third cohort (ECMO group) included children who in the period 1993-1998 underwent Extra Corporal Membrane Oxygenation (ECMO) for meconium aspiration syndrome, persisting pulmonary hypertension or sepsis, during their first weeks of life. They all had received analgesics and sedatives for a prolonged period in the absence of overt pain. The fourth cohort consisted of controls. All former patients were compared with age and gender matched controls without neonatal medical history. We also matched for ethnicity as far as possible. The control children were recruited from two local elementary schools in the referral area of Erasmus MC-Sophia. There were no differences in socio-economic status between subjects and controls.

Exclusion criteria for all cohorts included cognitive impairment and major sensory or motor impairments (e.g. cerebral palsy, deafness, blindness). All patients were followed according to neonatal follow-up guidelines.

2.3 Measures

Pediatric Pain Inventory: We assessed children's pain perception with the use of the Paediatric Pain Inventory (PPI)[29]. This projective test presents a series of 24 cartoon-like pictures in which a child undergoes potentially painful events across four different settings. These settings are: Medical (MED). i.e. getting an injection, lying in a hospital bed beside an intravenous bottle, receiving stitches, getting medicine from a nurse, sitting in a wheelchair in a hospital, and having a cast put on in a physician's office; Recreational (REC), i.e. being hit by a baseball while batting, falling off a skateboard, having wrecked a bicycle, dropping a bowling ball on foot, run over by another football player, falling out of a tree; Daily Living (ADL), i.e. catching a finger in a door, getting an electric shock, getting stung by bees, cutting hand while peeling fruit, pulling off a bandaid, burning hand on the stove; and Psychosocial (PSY), i.e. being scolded by a policeman, laughed at by schoolmates for misspelling a word, striking out in a baseball game, reprimanded by a teacher, fighting with another child, being excluded from a game. Settings

were randomized among the 24 pictures[22]. Pain intensity was measured with the Color Visual Analogue Scale (CAS) and pain affect was measured with the Facial Affective Scale (FAS) which were validated by McGrath [30].

Pain Coping Questionnaire: We assessed coping with the use of the Pain Coping Questionnaire (PCQ), validated by Reid in 1998[31]. The PCQ comprises three higher-order scales: Approach, including the subscales of information seeking, problem solving, seeking social support, and positive self-statements; Problem-focused Avoidance, including subscales of positive self-statements, behavioural distraction, and cognitive distraction; and Emotion-focused Avoidance, including externalizing and internalizing/catastrophizing subscales. The children were instructed that "every one has had a time when they have been hurt or in pain for a few hours or longer. For example, you might have had a headache, a stomach ache, a bad muscle pull, pain in your joints, back pain, an earache etc. Below are some things that people might say, do or think when they are hurt or in pain. We are interested in the things you do when you are in pain for a few hours or days" [31]. Then, using Likert scales (never, hardly ever, sometimes, often, very often), the children rated how frequently (never, hardly ever, sometimes, often, very often) they used these 39 coping strategies.

2.4 Procedure

The questionnaires were presented as part of a standardized follow-up assessment for long-term alterations in pain sensitivity at the age of 8 years. The PCQ was sent to the children 2 weeks before the day of testing, they completed it at home and returned it on the day of testing. The PPI, in combination with the CAS and FAS, was administered on the day of testing. The instructions provided with the CAS and FAS were adapted for use with the pictures. The investigator (RS) placed a PPI picture on the table, and presented the CAS to the child, asking: "Look at the picture. How much do you think that hurts? How much pain do you think he/she is feeling?" Next the investigator removed the CAS and presented the FAS along with the same picture, saying: "look at all the faces. Now look at the picture. Which face looks like how he/she feels deep down inside, not the face you show to the world?" [22] The procedure was then repeated for the other pictures. Investigator RS performed all testing. As this study was part of a larger follow-up study, she was not blinded to the group. This study protocol was approved by the 'Central Committee on Research Involving Human Subjects'.

2.5 Data analysis

In the primary analyses, first patients, irrespective of group, were compared to control children. When significant differences were found, subgroup analyses were performed subsequently.

A pain intensity score and a pain affect score for each setting were generated by summing scores on the VAS and FAS respectively. For the PPI, pain intensity and affect were analysed separately, using univariate analysis of covariance (ANCOVA) with setting (Medical, Recreational, Daily Living and Psychological) as a repeated measures factor. Statistical assessment of coping strategies was done using analysis of variance (ANOVA). For all analyses, first patients, irrespective of their subgroup, were compared to control children. When significant differences were found, subgroup analyses were performed.
Prior to the primary data analyses, correlations among potential covariates were evaluated. As gestational age and birth weight were highly correlated it was not possible to

use both items in our analysis. Therefore, we used the worst of these two. As also total admission days and days spent on the ICU were also highly correlated, we used the same procedure. Age, gestational age at birth and duration of ICC admission were entered as covariates in each analysis.

3. Results

3.1 Background characteristics

Of 85 eligible children for the Surgery group included in a previous study [28], 57 participated in this study. Parents of 10 children did not give consent, 3 children had Down's syndrome and 15 were lost to follow-up. Of 98 eligible children for the Pain group, 53 participated, 30 were lost to follow-up and parents of 15 did not consent. Of 72 eligible children for the ECMO group, 60 were included, with 4 lost to follow-up and parents of 8 did not consent. Comparison of each group's participants and non-participants for age, gestation age, birth weight, duration of hospital admission and morphine administration revealed no significant differences. Background characteristics for the children participating in this study for each group are presented in Table 1.

In total 170 subjects and 170 controls were tested. Median age at testing for all groups was approximately eight years (total range 6-11) and more boys were included than girls in all groups. Gestational age varied from term in the surgery and ECMO groups to pre-term in the pain group. Birth weight shows an analogous distribution, as most children in the pain group had a birth weight < 2500g. All control children had been born full term with birth weight >2500g. Total duration of ICU admission ranged from 15 days for the ECMO children to 22 days for the surgery group. The groups did not differ in age at testing.

3.2 Pediatric Pain Inventory

3.2.1 Pain Intensity: assessed by CAS

Analysis of CAS test results showed a significant main effect for Setting ($p<0.05$), i.e. pain intensity related to REC and PSY settings was rated higher than ADL and MED settings, respectively ($p<0.01$). The main overall effect for Group was significant ($p<0.01$), as shown in Fig. 1. There was no significant main effect for sex. Between-group post-hoc comparison showed that the Surgery group rated medical (MED) pain higher than Control children ($p<0.05$). The CAS ratings were not significantly correlated with time spent in the ICU and gestational age. The Pain group, however, rated pain in all settings higher than Control children ($p<0.05$). Also in the Pain group, CAS ratings were not significantly correlated with time spent in the ICU and gestational age. No differences were found between the ECMO group and the control children. In all three groups the covariate age, however, was statistically significant ($p<0.01$), as CAS ratings increased with increasing age.

3.2.2. Pain Affect: assessed by FAS

FAS scores by setting for each group are displayed in Figure 2. There were significant main effects for Setting ($p<0.01$), and Group ($p<0.05$), but no interaction. Pain affect scores for the REC and PSY settings were rated significantly higher than those for the ADL and MED Settings. Between-group post-hoc comparisons showed that the Surgery

Table 1 Background characteristics

	Surgery patients N=57	Pain patients N=53	ECMO patients N=60	Controls N=170
Age (in years)				
Median	8	9	7	8
(25-75 th percentile)	7-9	7-9	7-8	7-9
Boys/girls	36/21	37/16	35/25	108/62
Gestational age (in weeks)				
Median	38	31	40	38
(25-75 th percentile)	37-40	29-36	38-41	36-39
Birth weight (in grams)				
Median	2930	1425	3405	3400
(25-75 th percentile)	2240-3625	955-2890	2937-3800	2930-3830
Negative hospital experiences:				
**** Length ICU stay (in days)***				
Median	22	11	15	0
(25-75 th percentile)	12-48	4-21	9-28	
**** Length of total stay (in days)***				
Median	26	13	16	0
(25-75 th percentile)	15-52	5-26	8-30	
**** morphine***				
Median dosage (µg/kg/h)	10	none	20	none
(25-75 th percentile)	10-15		10-30	
Median duration (in days)	4		9	
(25-75 th percentile)	2-6		2-12	

group rated medical affect (MED) higher than Control children ($p<0.01$). Duration of ICU stay, sex and gestational age did not significantly influence pain affect scores. The Pain group on the other hand rated all settings higher than Control children ($p<0.01$). Also duration of ICU stay, sex and gestational age did not significantly influence pain affect scores. No differences were found for the ECMO children. Again the covariate age was statistically significant ($p<0.01$) in all three groups, with increasing scores for the older children.

The CAS and FAS scores were highly correlated in all groups, ranging from 0.86 to 0.95 for the four groups.

3.3 Pain Coping Questionnaire

Overall, significant differences in coping strategies between Groups were seen in all three higher order scales: Approach ($p<0.05$), Problem-focused Avoidance ($p<0.05$) and Emotion-focused Avoidance ($p<0.01$), as shown in Fig. 3. Sex did not influence coping strategies. Post-hoc group comparison showed that only the Pain group accounted for the differences in all the higher order scales ($p<0.01$). These children used fewer Ap-

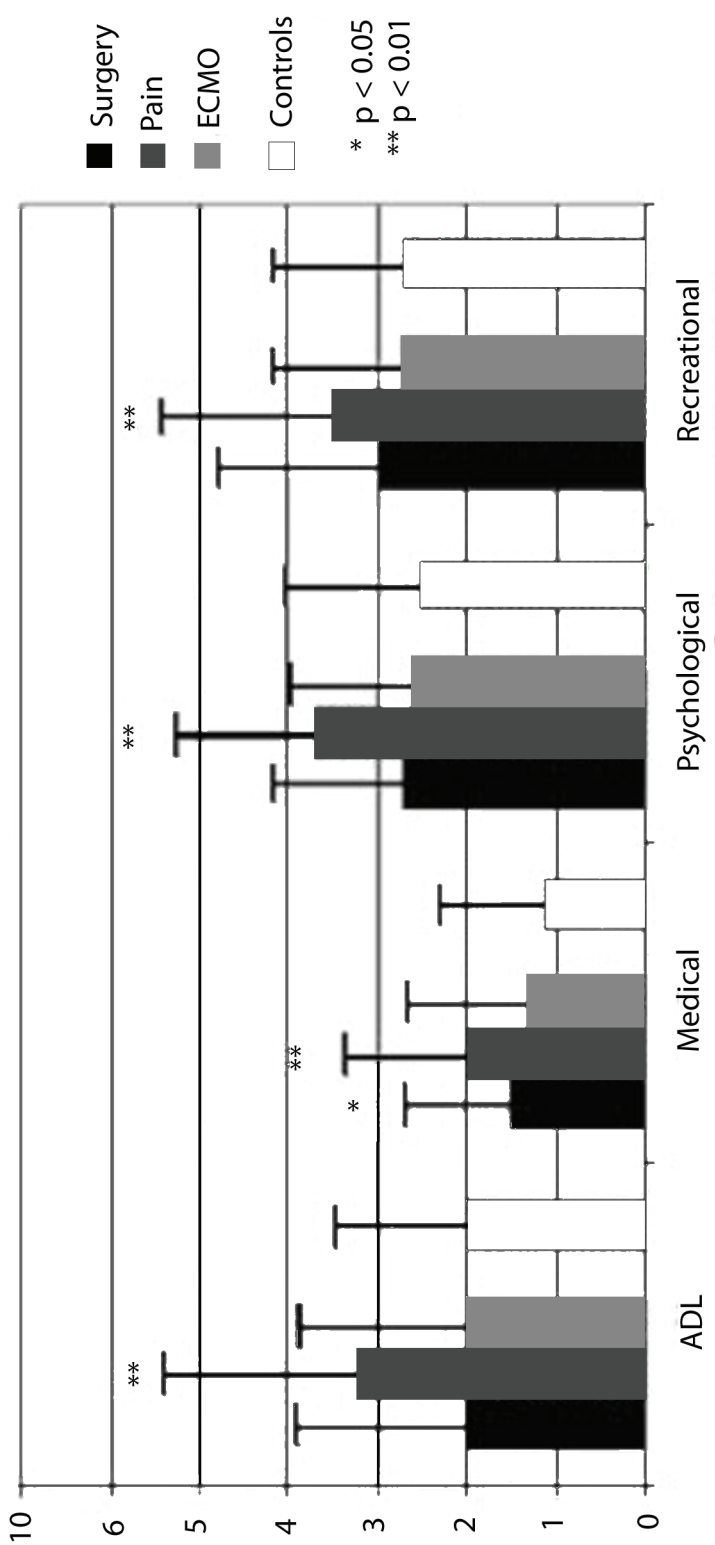
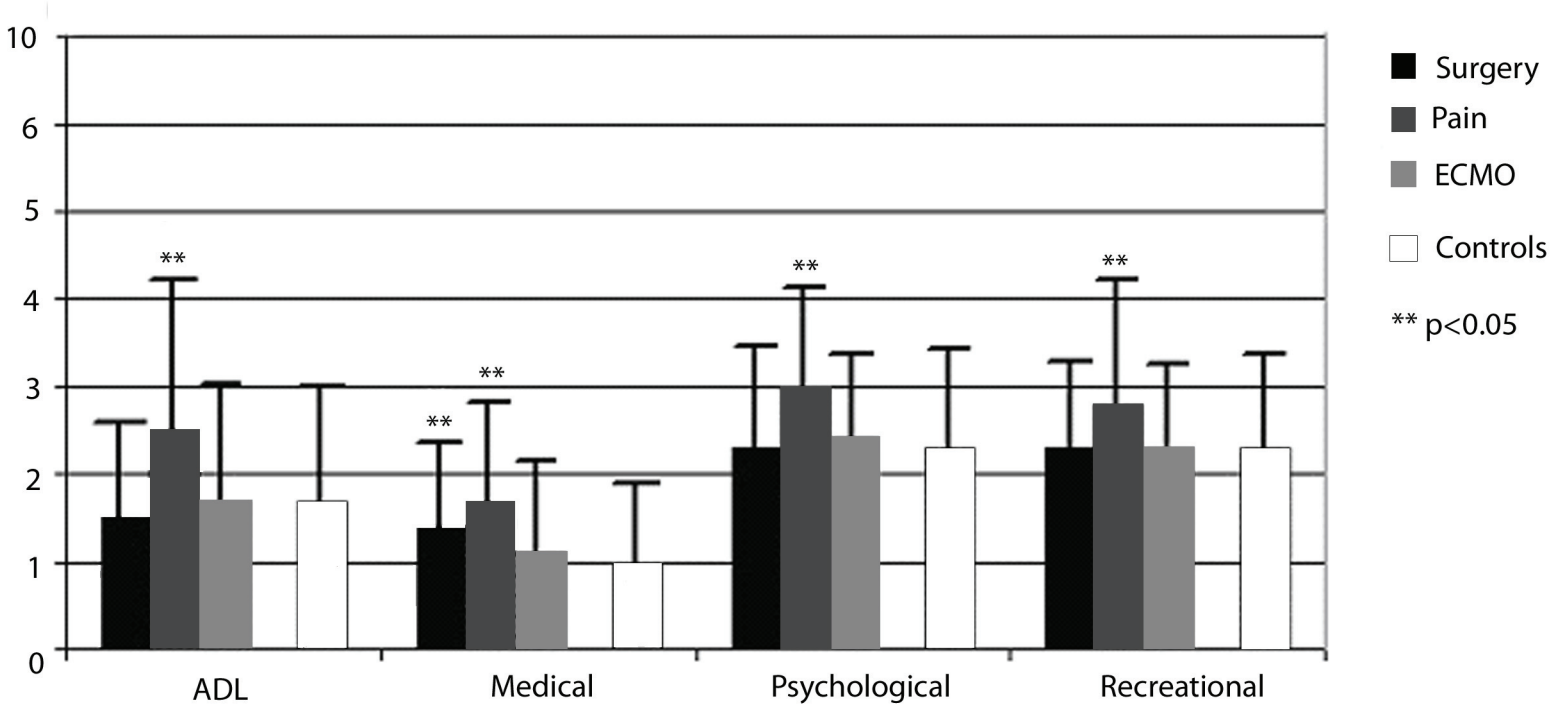


Figure 1 Color analogue scale ratings (range 0-10) of pain intensity

Note of legend: bars represent means, the lines represent the SD

Figure 2 Facial Analogue Scale ratings (range 0-10) of pain affect



Note of legend: FAS ratings (range 0-10), bars represent means, the lines represent the SD

proach- and Problem-focused Avoidance strategies, and demonstrated more Emotion-focused Avoidance. Duration of ICU stay, gestational age and age were not significantly associated with pain coping in the Pain group. No differences in coping strategies were found for the Surgery and ECMO groups, compared to Controls. Again, this was not influenced by age, gestational age or duration of ICU admission.

Pain Intensity and Affect in response to the PPI pictures were correlated with Coping Strategy scores (intensity and affect) which revealed low correlations, ranging from 0.01 to 0.48 for the three higher order scales.

4. Discussion

The goal of this study was to compare pain perception and coping in several groups of children who had neonatal hospital exposure with varying pain and analgesia. We found that children who had been in neonatal intensive care primarily due to preterm birth (Pain group) rated all forms of depicted pain across all settings (Medical, Activities of Daily Living, Psychosocial and Recreation) significantly higher (both affect and intensity), compared to control children. Moreover, they commonly used fewer problem-focused avoidance techniques and fewer approach techniques, but responded with more emotion-focused avoidance techniques. The latter, however, are considered inadequate techniques. Children in the surgery group rated only the medical cartoons as more painful when compared with controls and children in the ECMO group responded like the controls. These findings were noted even after adjusting for differences in gestational age at birth, age, and duration of ICU admission. However, in contrast to the children in the pain group, children in the surgery group all had received adequate per-operative pain relief[28].

Our findings suggest that hospitalization in the neonatal period does not in itself lead to altered pain perception. The question is however, whether inefficient coping and increased pain perception are due to neuro-anatomical alterations supraspinally, induced by neonatal pain exposure, or to hypoxia or conditioning8. Long-term supraspinal developmental changes as a consequence of neonatal pain exposure remain poorly understood[6, 17, 20, 32-35]. In the case of neuropathic pain, however, Killackey and colleagues demonstrated that peripheral disturbances such as nerve sectioning influence supraspinal levels[36] . Others found that sectioning of a peripheral nerve in rodents significantly reduced the corresponding area within the primary somatosensory cortex[36, 37]. These findings suggest that repetitive neonatal pain without analgesia leads to altered processing of pain and pain-related behaviours. Consistent with numerous animal studies, developmental changes in the supraspinal pain system including the somatosensory cortex and parts of the limbic system involved in the emotional component of pain may result from untreated pain in the neonatal period. Increased pain intensity and affect ratings in the pain group, as noted in this study, are in concordance with this hypothesis. As the limbic system is characterised by high numbers of μ opioid receptors[38], we may assume that morphine inhibits increased activity in the limbic system due to pain exposure. This mechanism could have prevented the occurrence of long-term alterations in pain perception in the surgery group.

We also found that administration of prolonged, high-dose analgesics (20 μg/kg/h for a median of 9 days) in the absence of overt pain in case of ECMO treatment, as assessed by validated pain scores such as COMFORT and VAS scale in a previous study, did not lead to alterations in pain intensity, affect and pain coping strategies. This sup-

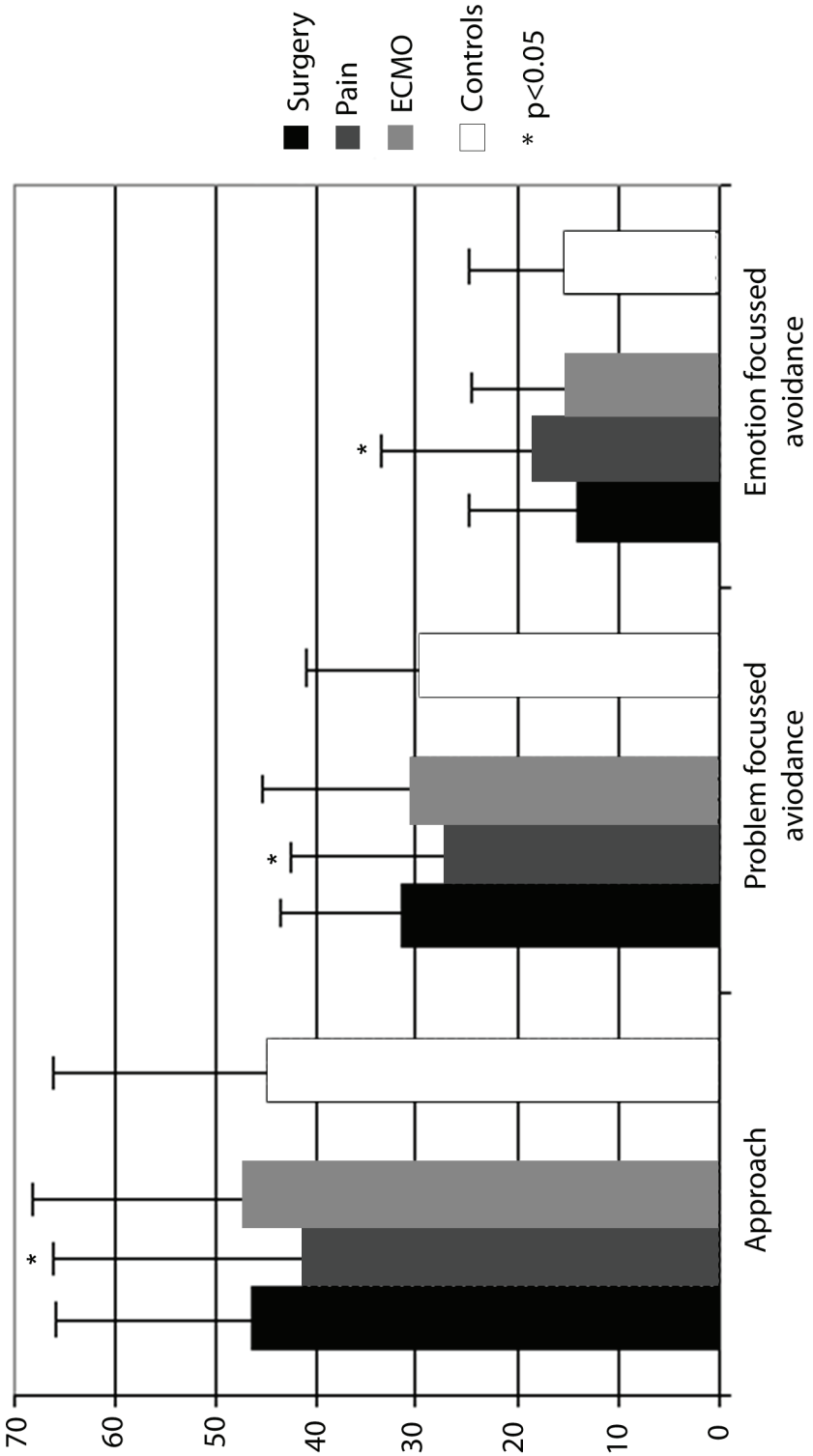


Figure 3 Pain Coping

Note of legend; bars represent means, the lines represent the SD

ports our hypothesis that pain exposure – which could have been prevented by morphine – is responsible for the observed differences.

Could other factors explain these differences? It seems unlikely that severity of illness could explain the differences between groups, since babies in the ECMO group had greater and babies in the surgery group had lesser severity of illness as compared to babies in the respiratory group. These differences remained even after controlling for most of the other clinical factors (Table 1), suggesting that the observed differences in pain intensity, affect and coping were associated with the differences in neonatal pain exposure between the three groups.

Conditioning might also explain the increased pain intensity and affect scores. Newborns exposed to repeated heel lances in the first 24 to 36 hours of life learned to anticipate pain and exhibited more intense pain responses during subsequent venapuncture than normal control infants[8, 39]. The surgery group children in our study had been repeatedly exposed to pain during infancy and could possibly have anticipated pain as most of them required multiple subsequent hospital visits. Nevertheless, as our study did not use 'real' stimuli, a role for conditioning seems unlikely.

In this study, we were unable to relate prematurity with high pain intensity and affect scores and alterations in pain coping. On the other hand, Grunau et all reported that medical pain significantly differed between patients and controls. This finding might have been influenced by a type 2 error due to insufficient power, as their groups included 47 patients versus 37 controls. Furthermore, these patients obviously had more hospital experiences than the healthy controls. As pain is known to be interpreted against a background of previous pain experiences [23], this could have influenced their pain intensity and affect ratings. Moreover, in Grunau's study duration of ICU stay for the ELBW children was related to increased pain affect rating in the recreational and daily living settings. We did not find such an effect, perhaps because ICU stay in our study was much less: 25-46 days versus 97 days in Grunau's study.

Apart from neonatal hospital admission and subsequent hypersensitivity, other factors logically influence coping and the expression of pain intensity and affect. Coping is directly influenced by a child's personality, appraisal and the social context. Indirectly, the event that causes pain also bears on the coping strategy to be used[40]. Cognitive level also plays an important role in paediatric pain coping. The Piagetian theory proposes four main stages of cognitive development, each of which evokes a different perception of and response to pain[41, 42]. Therefore coping and interpretation of pain is a multifactorial event in which parents, learning and the social environment are instrumental [40-42].

In conclusion, neonatal pain exposure in the absence of morphine seems to lead to alterations in pain perception and coping, as these children report increased pain intensity and affect scores, use fewer avoidance and problem-focused distraction techniques, yet more emotion-focused avoidance. The specific neuronal pathways which could have contributed to this development remain unknown. Although the differences between patients and controls are significant, their clinical importance remains uncertain. Whether these children will experience problems during childhood due to increased pain perception and inefficient coping styles or whether they develop greater vulnerability to chronic pain problems during adulthood remains to be investigated. Thus, further research is necessary to gain more insight in the factors that influence pain processing and pain coping patterns in former NICU patients.

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Chapter 7

Chronic pain and somatic complaints 8 years following neonatal intensive care unit admission

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Abstract

Many adults report chronic pain following surgery. A Dutch study found a 20% general incidence of chronic pain in children, but it is unknown whether, like in adults, the incidence of chronic pain is higher for children who underwent neonatal surgery or opioid administration. Apart from chronic pain, early pain exposure could also result in somatization, i.e. the tendency to experience and rapport physical complaints which cannot be related to medical history. The present study aimed to determine the development of chronic pain and the incidence of somatization as assessed by the CSI questionnaire 8 years later. Four cohorts were included, i.e. 57 children neonatally exposed to pain treated with morphine, 53 children neonatally exposed to pain during artificial ventilation without morphine, 60 eight year olds treated with morphine in the absence of overt pain and a control group consisting of healthy children without previous hospital experiences c. All children were approximately eight years old at the moment of testing. To determine chronic pain and somatization, the children's parents completed two questionnaires on their children, i.e. the Structured Pain Questionnaire and the Children's Somatization Inventory respectively. We found that nineteen percent of the parents reported that their child suffered from pain for more than 3 months. This was equally distributed among both patients and controls. Chronic pain at present, however, was more frequent (more than once a week) among former patients than among the controls (39 vs 21%). The number of somatic symptoms was relatively low, and did not differ significantly between patients and controls (three vs two). According to the Children's Somatization Inventory questionnaire, only four children were classified as suffering from somatization disorder, all four were former patients (one surgery, two pain, one ECMO group). We conclude that neonatal pain exposure does not seem to predispose for chronic pain and somatization (as assessed by the CSI). Nevertheless, former patients suffering from chronic pain experience this pain more frequently, report higher pain VAS scores and a higher frequency of somatic complaints (according to the CSI), when compared to chronic pain sufferers without previous hospital experiences. Future studies should focus on degrees of impairment and utilization of medical services for children who were repeatedly exposed to pain during their neonatal hospital admission.

1. Introduction

Adults may suffer from chronic postoperative pain for years after operative procedures such as thoracotomy and laparotomy[1, 2,3]. Even analgesics cannot prevent that 41 to 66 % of all surgical patients experiences persistent post-operative pain up to one year after surgery[1, 4]. While the causes of chronic pain following surgery still remain unclear, several risk factors have nevertheless been identified[5]. For one, pre- and postoperative pain increase the risk of developing chronic post-surgical pain[6-9], due to operation-related nerve damage[10, 11], but also genetic and psychosocial factors play a role in chronic pain development [5].

Perquin and colleagues found a 20% prevalence of chronic pain in healthy Dutch children aged 0-18 years[12]. Like for adults, still little is known about the etiology. One explanation might be neonatal pain exposure. Several studies have demonstrated that pain exposure during early infancy has longer-lasting effects than pain exposure in adulthood, and result in persistent hypersensitivity during childhood[13, 14]. Confirmatory findings in animals have shown permanent peripheral, spinal and supraspinal neuroanatomical alterations following neonatal wounding [15, 16]. There even seems to be a developmental window during which children are especially vulnerable for adverse events like pain. This is demonstrated by preterm children being more hypersensitive to pain than full-term children following neonatal tissue damage [14]. The question, however, is whether, apart from alterations in pain sensitivity, newborns exposed to pain will also have a higher chance to develop chronic pain (defined as existing for >three months) later in life.

Also opioids can result in an (opioid-induced) hypersensitivity to pain in adults [4]. In children, prolonged analgesic administration in the absence of overt pain and extensive tissue damage is reported in newborns who are treated with extracorporeal membrane oxygenation (ECMO). Dosing may be 2-4 times higher and for a longer period than when given following non cardiac surgery[14]. It is unknown whether this opioid induced hypersensitivity develops in children and would contribute to chronic pain development. To fill the knowledge gap, we conducted a study aiming at determining whether neonatal pain and/or morphine exposure results in chronic pain development.

Moreover, the presence of hypersensitivity to pain is also reported to influence children's somatic complaints[17]. A somatization disorder is characterized as a chronic condition in which there are numerous physical complaints, like headache, abdominal pain, limb pain, back pain etc, without detectable or known organic basis. This disorder however, is a DSMIV definition which can only be diagnosed by a psychiatrist and is relatively rare in children and adolescents[18]. In our study we aimed to evaluated whether neonatal pain and/or morphine exposure contributed to the development of somatic complaints and whether there was an indication for somatization disorder (>13 symptoms) by using the children's somatization inventory.

2. Methods

2.1 Design

A cross-sectional study was undertaken in both the Erasmus MC – Sophia Children's Hospital (surgery, pain and 35 ECMO children) and Radboud University Nijmegen Medical Center (25 ECMO children). Parents of these children and parents of control children completed questionnaires on chronic pain and somatic symptoms.

2.2 Subjects

We included four different cohorts. The first cohort consisted of children who between 1996 and 1999 had undergone major abdominal or non cardiac thoracic surgery during the first 3 months of life (surgery group). Surgery had been stoma placement in 46%, congenital diaphragmatic hernia repair in 14%, esophageal atresia correction in 14%, adhesiolysis in 7%, and other procedures, including pancreatic resection and Blalock procedure, in 19%. Pain was adequately treated with analgesics, which had been given by standard protocol[19].

The second cohort (pain group) consisted of children admitted to the Neonatal Intensive Care Unit (NICU) during the first weeks of life who were all were exposed to pain inherent to their high technological medical care. However, none of them had received any morphine during this period. These children underwent artificial ventilation for pulmonary problems such as hyaline membrane disease and bronchopulmonary dysplasia between 1996 and 1999.

The third cohort (ECMO group) consisted of children who in the period 1993 -1998 underwent ECMO for meconium aspiration syndrome, persisting pulmonary hypertension or sepsis, during their first weeks of life. All these children had received high dosages of analgesics (20µg/kg/h) and sedatives for a prolonged period (9 days) in the absence of overt pain.

Overall exclusion criteria were; (1) cognitive impairment, (2) major sensory or motor impairments. All patients were followed according to neonatal follow-up guidelines.

All former patients were compared with age and gender matched controls (fourth cohort) without neonatal medical history. We also matched for ethnicity as far as possible. The control children were recruited from two local elementary schools in the referral area of Erasmus MC-Sophia. There were no differences in socio-economic status between subjects and controls.

2.3 Questionnaires

We used the structured pain questionnaire, which was designed for parents by Perquin and colleagues, to evaluate the occurrence of chronic pain in children and adolescents (age 0-18)[12]. If the answer to the first question 'Did your child experience pain in the previous twelve months?' was no, no further questions were asked. If yes, parents were asked to provide additional information on duration, frequency, location and intensity. From a list of possible locations (head, abdomen, limb, ear, throat, back, unknown and elsewhere) parents were asked to tick all locations where their child had experienced pain in the previous three months. Parents were asked to provide details for the pain that troubled their child most. Pre-coded categories serve to assess frequency of occurrence (<1x/month, 1x/month, 2-3x/month, 1x/week, 2-6x/week, each day) and the duration of pain (<4 weeks, between 4 weeks and 3 months, > 3 months). Pain intensity was assessed with a Visual Analogue Scale.

We used the Children's Somatization Inventory (CSI) questionnaire to evaluate the occurrence of somatic complaints and the possibility to develop a somatization disorder. Garber and colleagues developed this 36-item CSI by including somatic symptoms from the Hopkins Symptom Checklist, one additional somatic symptom (constipation), and non-overlapping symptoms from the DSM-III criteria for somatization disorder[20]. Parents were asked to rate magnitude of each symptom in the past 2 weeks on a four-point

scale ranging from 0 (not at all) to 3 (a whole lot). The total CSI score reflects the intensity of somatic complaints reported. According to this CSI questionnaire, a total number of 13 symptoms or more is associated with the development of a somatization disorder[20].

2.4 Procedure

The questionnaires were presented within the framework of a standardized follow-up assessment concerning long-term alterations in pain sensitivity at the age of 8 in children neonatally exposed to pain or morphine. Both questionnaires were sent to the parents of patients and controls 2 weeks before scheduled hospital appointment. Parents were asked to complete the questionnaires and return it on the appointed day. The control children participated in a larger case-control study as well, involving hospital visit for testing[14]. This study protocol was approved by the 'Central Committee on Research Involving Human Subjects'.

2.5 Data analysis

Chronic pain was defined as recurrent or continuous pain for more than 3 months; non-chronic pain was defined as pain lasting less than 3 months[12]. Data are partly represented in frequencies. The Structured pain questionnaire was analyzed by logistic regression analysis with age and sex as covariates, with chronic pain as outcome. The CSI differences were tested by analysis of variance (ANOVA), with threshold for statistical significance as less than 0.05. Patients were compared with controls first, after which subgroup analyses were performed.

3. Results

3.1 Background characteristics

Of 85 eligible children for the surgery group included in a previous study [19], 57 participated in this study. Parents of 10 children did not give consent, 3 children had Down's syndrome and 15 were lost to follow-up. Of 98 eligible children for the pain group, 53 participated, 30 were lost to follow-up and parents of 15 did not consent. Of 72 eligible children for the ECMO group, 60 participated, with 4 lost to follow-up and parents of 8 not consenting.

Table 1 presents background characteristics for participants and controls. In total 170 subjects and 170 controls were tested. Median age at testing for all groups was approximately eight years (total range 6-11). Overall there was a predominance of boys. Gestation age varied from term in the surgery and ECMO groups to pre-term in the pain group. Birth weight shows an analogous distribution, as only in the pain group median birth weight was below 2500g. All control children had been born full term with birth weight >2500g. Total duration of ICU admission ranged from 15 days for the ECMO children to 22 days for the surgery group.

3.2 Structured pain questionnaire

For 36% of all children parents reported pain In the previous 12 months, with an even distribution between patients and controls (37% surgery group, 38% pain group, 32% ECMO group and 36% for the controls) (Table 2). Sex, gestation age, birth weight and duration of hospital admission did not influence the occurrence of pain in the past 12 months. However, age had a significant effect ($p<0.05$) on the prevalence, which was

Table 1 Background characteristics represented in Means and 25-75 percentile range

	Surgery group N=57	Pain group N=53	ECMO group N=60	Control group N=170
Age (in years)				
Median	8	9	7	8
(25-75 th percentile)	7-9	7-9	7-8	7-9
Boys/girls	36/21	37/16	35/25	108/62
Gestational age (in weeks)				
Median	38	31	40	38
(25-75 th percentile)	37-40	29-36	38-41	36-39
Birth weight (in grams)				
Median	2930	1425	3405	3400
(25-75 th percentile)	2240-3625	955-2890	2937-3800	2930-3830
Negative hospital experiences:				
**** Length ICU stay (in days)***				
Median	22	11	15	0
(25-75 th percentile)	12-48	4-21	9-28	
**** Length of total stay (in days)***				
Median	26	13	16	0
(25-75 th percentile)	15-52	5-26	8-30	
**** morphine***				
Median dosage (µg/kg/h)	10	none	20	none
(25-75 th percentile)	10-15		10-30	
Median duration (in days)	4		9	
(25-75 th percentile)	2-6		2-12	

higher among the older children (>8 years).
Of all parents who reported that their child had suffered pain in the previous 12 months, non-chronic pain (lasting less than 3 months) was reported for 81% of the patients (15% surgery group, 15% pain group, 14% ECMO group, 56% controls) and chronic pain (longer than 3 months) for 19% of the patients (6% surgery group, 5% pain group, 5% ECMO group, 8% controls). Chronic pain occurrence did not differ between patients and controls.

Frequency of chronic pain in both patients and controls was at least once a week for 42%, less than once a month for 0%, and less than weekly but more than once a month for 58%. Frequency at least once a week was significantly more prevalent ($p<0.01$) in patients suffering from chronic pain than for controls with chronic pain (37% patients vs. 21% controls).

The occurrence and frequency of chronic pain was not influenced by age, sex, gestation age, birth weight and duration of hospital admission. Subgroup analysis showed that weekly pain was reported more frequently in the surgery group (57% ver-

sus 25% pain group, 26% ECMO group and 21% controls). Also, the intensity of chronic pain was significantly higher ($p<0.05$) in the surgery group than in the control group, with mean VAS score 4.3 surgery vs. 2.5 controls. Table 2 shows the mean scores specified for patient subgroups.

Table 2 Subgroup characteristics evaluated by the Structured Pain Questionnaire

	Surgery group N=57	Pain group N=53	ECMO group N=60	Control group N=170
Pain in previous year	37 %	38%	32%	36%
Number of chronic pain symptoms				
Mean	1.6	1.9	1.8	1.8
25-75 percentile range	1.0-3.0	1.0-4.0	1.0-4.0	1.0-4.0
Duration of pain > 3 months	29%	25%	26%	13%
Weekly pain	57%	25%	26%	21%
Pain intensity (VAS in cm)				
Mean	4.3	3.7	3.7	2.5
25-75 percentile range	2.0 – 6.3	1.3 – 5.6	1.3 – 5.0	0.6 – 4.0

3.3 Children´s Somatization Inventory

Overall, patients showed higher total CSI score than the controls ($p<0.01$). All three patient subgroups showed significantly higher total scores ($p<0.05$) when compared with control children (3 surgery, 3 pain, 3 ecmo vs. 2 for the control children).

The number of symptoms did not significantly differ between patients and controls. Parents reported a mean of 2 (range 0-12) symptoms for the control children and a mean of 3 symptoms (range 0-23) for children in each of the patient subgroups. 40% of the children's parents reported no symptoms at all (patients and controls), 5 or more symptoms was reported by 13%, and 10 or more symptoms by 5% of the parents. Only 4 children (2 in the pain group, 1 in the surgery group and 1 in the ECMO group) reported a total of 13 or more somatic complaints. None of the children in the control group reported more than 13 symptoms. This difference (4 patients versus 0 controls) however, is not significant. These four children (three girls and one boy) were aged six, seven and ten (two children), born with a gestation age of respectively 25.5, 41.3, 41 and 43 weeks, with a birth weight of 835, 3500, 2905 and 3390 grams and total duration of intensive care unit stay of 40, 25 and 4 (two children) days respectively.

4. Discussion

The results of this study show a low prevalence of chronic pain among Dutch children, as only 19% of the parents reported that their child suffered from pain for more than 3 months. This was equally distributed among patients and controls. Former patients suffering from chronic pain, however, reported more frequently weekly pain than controls with chronic pain. Findings for somatization were similar, i.e. the prevalence was relatively low, did not differ between patients and controls, but patients more often suffered somatic complaints (which could not be explained by their medical history) than the controls. Only four children – all former patients –were classified as having somatization (assessed by the CSI), since they suffered from more than 13 somatic complaints.

In this study the prevalence for both chronic pain and somatization were lower than those reported by others[12, 20]. We feel that this is due to our method of data collection, i.e. parents completing questionnaires on their children. Parents may not always be aware of their children's minor pains[20]. Moreover, parents are known to underestimate their child's pain problems[20-22]. Children completing these questionnaires themselves could therefore possibly result in a higher prevalence. Also, the retrospective nature of both questionnaires, with risk of insufficient recall, could have resulted in a prevalence reflecting an underestimation of real problems. This might have been prevented by a prospective evaluation of pain occurrences in the shape of a pain diary[23].

The patients' background characteristics, such as group characteristics (pain adequately treated with morphine, pain in absence of morphine and morphine in absence of overt pain), gestation age, birth weight, duration of ICU admission and total hospital admission were not found to be associated with the occurrence of chronic pain complaints. This suggests that adverse neonatal events, inherent to intensive care unit admission, do not increase chances of developing chronic pain. These findings agree with those from a study by Mallen et al, which could not establish a direct relationship between birth-related factors like prematurity, fetal distress, nociceptive exposure, low birth weight and intensive care unit admission[24]. Nevertheless, the authors observed a non-significant trend to more chronic pain for low birth weight and intensive care unit admission.

Table 3 Somatization Inventory, means and 25-75 percentile range

	Surgery group	Pain group	ECMO group	Controls
Mean score **25-75 percentile range**	3 1-5	3 0-4	3 0-4	2 0 - 3
Mean number of symptoms **25-75 percentile range**	3 1-5	3 1-4	3 1-4	2 1-3
>13 somatizing symptoms	1	2	1	0

In this study, we demonstrated a similar prevalence of chronic pain (19%, children aged 6 to 11) as did Perquin and colleagues who used the same questionnaire, in 2351 Dutch schoolchildren aged 4 to 11. Among the 4- to 7–year-olds in Perquin´s study, chronic pain was less frequent than among the 8- to 11-year-olds years (19,3 % versus 23,7%)5. Accordingly, in our sample of children aged 6 to 11 years, we also found that chronic pain prevalence increased with age, in line with other studies[25-32].

Apart from the low prevalence of chronic pain in the four cohorts studied (n = 340), this study also shows that somatization according to the CSI is not common among children. As in Garber et al. study, most children reported less than 2 symptoms, far below the 13 symptoms required for somatization [20]. Only a small proportion of children (n=4) met this requirement, which is in accordance with the previously mentioned study[20].

Although numbers of symptoms did not differ between patients and controls, patients showed higher mean somatization scores. This implies that former patients had suffered more frequently from the reported symptoms than did the controls.

In sum, we can conclude that neonatal pain exposure and/or opioid exposure, does not seem to predispose for chronic pain and somatic complaints. Nevertheless, chronic pain in former patients is more frequent (i.e. more weekly pain) and has a higher VAS pain intensity than among chronic pain sufferers without previous hospital experiences. Future studies should replicate these findings in a prospective design following children up to adolescence, in order to determine whether pain as a result of hospital admission and opioid administration can predict the occurrence of chronic pain and somatization disorder.

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Chapter 8

General discussion

Approximately 200,000 children are born in the Netherlands every year (CBS 2004), 4000-5000 of whom are admitted to a neonatal intensive care unit during their first weeks of life. In particular premature birth and major congenital anomalies are important indications for hospital admission of the newborn. These consequently may suffer from two major developmentally inappropriate events; i.e. repeated exposure to pain and stress, partly disease-related and partly inherent to the high technological medical care[1-3], as well as prolonged separation from direct and continuous maternal care.

Pain exposure in early life regrettably may influence nociceptive processing for life [4]. Contributive factors are the immaturity of the central nervous system and the rapid brain development during the last trimester of fetal life [5]. Clinical studies show that prolonged pain early in life, independent of morphine administration, may have long-lasting effects on pain sensitivity [6-10] and on the individual pattern of stress hormone responses in vulnerable infants[11]. Like in animal experiments, these effects may result in persistent changes in nociceptive processing in humans as well, with implications for future pain experiences[6, 10]. As yet, however, there is little evidence for this in humans.

Anand en Scalzo[12] have published a model based, however, on experiments in rodents, that could help explain these mechanisms. They consider the central nervous system of newborns as immature. Its development and maturation is dependent on NMDA receptor activity. Neonatal pain exposure would lead to excessive NMDA receptor activation and increased release of excitatory neurotransmitters, resulting in hyperexcitability and windup. According to this model, excessive NMDA activation during the neonatal period causes excitotoxic damage and subsequent cell death. Finally on the long term this results in hyposensitivity to pain during adulthood; possibly due to compensatory mechanisms. Besides pain, also stimulus deprivation may lead to altered sensitivity and behavioural alterations. During the early brain growth spurt, the neurons bearing NMDA receptors are exceedingly sensitive. Diminished NMDA activity as a result of stimulus deprivation triggers widespread apoptotic neurodegeneration in the brain, at least in rats[13]. Long-term pain hypersensitivity has been suggested as a consequence. So far it was unknown whether it was applicable to humans as well – leading to altered pain sensitivity. In this thesis we therefore tried to delineate the long-term effects of neonatal pain exposure in a number of studies in humans in which all neonatal painful experiences were known and documented. One group of patients has been included in a randomised controlled clinical trial, evaluating continuous morphine. A second group of children were subjected to artificial ventilation and the last group of children were treated with ECMO.

Most pronounced findings

The most pronounced findings in this study were a hyposensitivity to temperature detection and a hypersensitivity to pain in the pain exposed children, irrespective of morphine administration. In the ECMO group, a generalised hyposensitivity to temperature detection was found, in absence of any alterations in pain sensitivity. In all three patient groups, no differences in suprapain thresholds could be demonstrated. Regarding pain perception and coping strategies, only the children exposed to pain in the absence of morphine (pain group), developed an increased pain perception and altered coping behaviour. Finally, the children in our study (surgery group, pain group, ECMO group),

were not more susceptible to develop chronic pain or somatization than children without previous hospital experiences (control group). The data have been summarized in table 1.

Hyposensitivity for temperature detection

An unexpected but very interesting finding in our study was development of hyposensitivity for temperature detection. Both children on mechanical ventilation neonatally without receiving analgesics (pain group) and those who underwent a surgical procedure during their first three months of life (surgery group) had higher detection thresholds for warmth and lower detection thresholds for cold stimuli. Hyposensitivity was restricted, however, to the area of neonatal wounding (i.e. heel and scar, respectively). Former preterm born children (pain group) showed greater hyposensitivity than their term born counterparts in the pain group. The term borns operated upon in the first month of life (surgery group), in turn, developed greater hyposensitivity than term born children exposed to pain at the age of 2-3 months. Smelzle-Lubiecky et al.[14] recently also reported this type of localized hyposensitivity in abstract form only concerning children aged 9-12 years who underwent thoracic cardiac surgery for patent ductus arteriosus or coarctation of the aorta neonatally[14]. An even more unexpected finding in our study was that former ECMO children had developed a generalized hyposensitivity.

The mechanisms underlying this hyposensitivity remain unclear, as, to our knowledge, no experimental animal studies have evaluated detection thresholds in animals in the long-run. This makes it difficult to interpret our findings. One explanation is that tissue damage, caused by surgery or heel stick, leads to peripheral nerve death. This would force the somatosensory cortex to reorganize. Kaas and Killackey, for one, demonstrated that peripheral nerve sectioning results in diminished representation in the responsible secondary somatosensory cortex area [15, 16]. These lesions might also extend to the primary somatosensory (SI) cortex, as experimental studies clearly indicate SI involvement in the intensity coding of tactile stimuli[17 18]. Whether this is also the case for nerve damage due to neonatal pain exposure remains unclear as no histopathological data are available from our patients for obvious reasons.

This hypothesis, however, does not explain our findings in the former ECMO children, for they suffered relatively minor tissue damage in the absence of overt pain. These children had been given relatively high dosages of morphine (20µg/kg/h) for a prolonged period of time, mainly to prevent displacement of the cannules and prevention of life threatening complications. This is in contrast with the other children studied (surgery group 10µg/kg/h, pain group none). The generalized nature of the hyposensitivity suggests other underlying mechanisms. Prolonged morphine administration, however, could have contributed, as it is associated with death of human microglia and neurons in vitro [19]. Nevertheless, as only few data are available on long-term effects of opioid therapy on human brain development, future studies are needed.

Hypersensitivity for heat and cold pain stimuli

In line with previous studies, we found that children from the surgery group who underwent subsequent surgery in the same dermatome as neonatally operated upon had greater hormonal stress responses, needed more opioids and showed more distress than the ones who underwent subsequent surgery in another dermatome. Even 10 years later, these children were still hypersensitive to pain, for they had lower cold and

Table 1 Most pronounced findings

	Detection threshold	Pain threshold	Suprapain threshold	Pain perception	Coping	Chronic pain	Somatization
Surgery	Increased, at dermatome of neonatal tissue injury	Increased at the scar and contralateral side	No significant differences	Increased medical pain perception	No significant differences	No significant differences	No significant differences
Pain	Increased, at dermatome of neonatal tissue injury	Increased at the heel	No significant differences	Increased pain perception; medical, recreational, psychological and during daily living	Altered coping strategies	No significant differences	No significant differences
ECMO	Increased, generalised	No differences	No significant differences	No significant differences	No significant differences	No significant differences	No significant differences

heat pain thresholds. This hypersensitivity, however, was restricted to the dermatome of tissue damage and its contralateral side. Hypersensitivity to pain was also observed in the pain group, suggesting that long-term alterations have developed. Again the hypersensitivity was restricted to the side of tissue damage, i.e. the heel. Boys had higher pain thresholds than girls, confirming other findings in humans[10]. Then, the effect of neonatal wounding was greatest in former preterm born children (pain group) in contrast to their term born counterparts of the pain group. The term born children operated upon during the first month of life (surgery group), in turn, developed greater hypersensitivity than children exposed to pain at age 2-3 months (surgery group). These developmental findings are in accordance with findings in animals, for neuroanatomical alterations and hypersensitivity in rats were greatest when wounds were inflicted at postnatal day (P) 0-7, but smaller at P14 and absent at P21 to resemble the weaker and transient effect in the adult[20-23].

An even more unexpected finding in our study was that former ECMO children did not develop any alterations in pain thresholds at all. In an experimental animal study, high dosages of morphine are reported to inhibit neuritogenesis, while low dosages of morphine enhance this neurite formation[24]. It could be that the dosages in our study were insufficient to inhibit neurite growth. Unfortunately the exact mechanisms remain unclear.

Our findings suggest that neuro-anatomical alterations in these children may underlie their altered somatosensory functioning. Animal data show skin wounding to result in peripheral nerve death, leaving an area of deafferentiation. Nevertheless, five days following skin wounding, a peripheral sprouting response of both Aδ and C fibres is observed, overcompensating for the damage, and resulting in hyperinnervation [21, 22]. Besides local collaterals, also sensory fibres drawn from deeper tissue and non-cutaneous nerve bundles sprout into the deafferentiated area [25]. At the spinal level, inflammatory pain was found to increase the number of primary afferents in the substantia gelatinosa of the dorsal horn. These alterations extend over several spinal levels caudally ipsi- and contralateral but do not spread throughout the whole spinal cord [23]. This latter finding in animals is in accordance with our finding of hypersensitivity being restricted to the area of wounding and the intact contralateral side.

Our results, however, are in contrast with Anand and Scalzo's model, in which they hypothesize that repetitive neonatal pain exposure, should lead to diminished pain sensitivity on the long term [5]. Some experimental studies confirm this hypothesis[26, 27], others partially agree as they find a baseline hyposensitivity but hypersensitivity during reinflammation[22, 28, 29]. Other studies do not confirm this model and demonstrate hypersensitivity[20, 30-32]. Interspecies and/or strain differences, type of neonatal pain exposure/stimulus, the environment in which the animals grow up and\or types of pain tests may all contribute to these conflicting findings, see overview in table 2. Indeed, animals that developed hypersensitivity were all Sprague Dawley rats, in contrast to those in whom hyposensitivity was found (mice and Long Evans rats). This suggests that interspecies as well as strain differences are decisive for the development of long-term effects. Besides species and strain, also type of pain exposure seems to influence the occurrence of long-term hypersensitivity. Long-term effects of inflammatory pain could only be induced by 25 µl CFA. Carrageen and 5 µl CFA on the other hand did not have any effect[33]. This suggests that causative agent and dosage also affects the occurrence of long-term hypersensitivity.

Table 2 Overview of animal studies

Author	Species / Strain		Neonatal pain exposure						
	SP[I]	LE[II]	Mouse	Skin wound	Visceral pain	Needle prick	Formalin	Laparotomy	25% Car[III]
Al-Chear 2000	+				+				
Anand 1999	+					+			
De Lima 1999	+			+					
Bhutta 2001		+					+		
Sternberg 2005			+					+	
Ren 2004	+								+
Lidow 2001	+								+

Author	Adult pain exposure					Response		
	Bowel distension	Hot Plate	Von Frey	Tail Flick	Acid abdominal constriction	Hypersensitive	Hyposensitive	Both[IV]
Al-Chear 2000	+					+		
Anand 1999		+				+		
De Lima 1999			+			+		
Bhutta 2001		+		+			+	
Sternberg 2005		+	+	+	+		+	
Ren 2004		+	+					+
Lidow 2001		+						+

Note of legend: I Sprague Dawley rats, II Long Evans rats, III25% Carrageenan, IV Basal hyposensitivity, hypersensitivity during reinflammation

Moreover, one important factor in the development of neuro-anatomical alterations seems to be the moment of pain exposure, as animal studies identified a developmental window of time. The effects of wounding were most pronounced when induced during postnatal days (P) 0-7, declining when performed at P14 or P21[12, 21, 23]. P6 to P9 in the rat is considered to correspond to 40 weeks gestational age in humans; P14 to a one-year-old infant[22, 25, 34].

Apart from pain, the children in our study also suffered from maternal separation. According to Anand's model, the environment in which animals grow up is also of importance for the development of long-term alterations. Stimulus deprivation due to maternal separation is a major problem in most animal studies, which makes results of human and animal studies difficult to compare. Experimental animals grow up in a laboratory environment, without the normally stimuli. In this early period, central nervous system maturation is influenced by activity. Stimulus deprivation would lead to a diminished neural input with neuronal nerve death as a consequence. It seems plausible that this influences long-term alterations in pain sensitivity. Yet, although the former patients in our studies suffered from maternal separation, we could not show any correlation between the length of stay and pain sensitivity. Perhaps parental rooming-in and kangaroo care might have reduced the negative effects of separation [32].

A final explanation for the paradoxal findings between our study and Anand's model might lie in the method of testing. In our study we evaluated pain thresholds. In the animal studies rats were exposed to heat until a response took place. This response may likely exceed the actual pain threshold as animals' pain responses are mostly determined by exposing them to heat until a flexion withdrawal reflex, like tail flick or paw withdrawal, occurs [26-29, 32]. For this reason, we also studied suprapain thresholds in our children.

Suprapain Thresholds

Beside pain thresholds patients were also exposed to suprapain stimuli. For this purpose, children were exposed to a series of 5 stimuli of increasing intensities. After every stimulus subjects were asked to give a pain report on a VAS scale. In contrast to our previous findings, we did not find any significant differences in pain reports between the three patient groups in comparison with the controls. However, term born subjects of the pain group paradoxically reported significantly more pain on the first two stimuli (i.e the lowest 2 intensities) in comparison with their preterm born counterparts (4,5 versus 3,5 on a VAS scale). This effect decreased with increasing intensity of the 5 subsequent stimuli.

We would like to accentuate that we used the individual pain thresholds to calculate the temperatures of suprapain exposure. By doing so, former patients were exposed to lower temperatures simply by their lower pain thresholds. Our findings suggest that they might have reported higher pain scores when exposed to the same temperatures as the controls, as methodological faults might be introduced by our set-up

A possible explanation for the lack of differences in suprapain thresholds is the method of assessment. All children were exposed to stimuli lasting for a maximum of 6 seconds. Perhaps the duration of exposure was eventually not long enough to induce suprapain stimulation. As this was the first study evaluating suprapain thresholds in children, the appropriate stimulus time should also be investigated in detail in future research projects.

The fact that the term born children showed increased pain sensitivity during suprapain stimuli might be due to their developmental state. Different parts of the brain are most vulnerable to perturbation and cell death at specific moments in time shortly after birth [13]. As shown experimentally, it is possible that the regions involved in suprapain sensitivity might be most vulnerable at term birth, which then contributes to the slightly increased suprapain sensitivity we have found.

In order to determine whether long-term neuroanatomical alterations are restricted to the somatosensory system or whether other brain areas are affected as well, we assessed by proxy if neonatal circumstances may affect pain perception and coping. Using questionnaires we found that only children of the pain group reported increased pain perception and less adequate coping behaviour in comparison with control children. The magnitude of this altered pain perception was not correlated with gestation age at birth and birth weight. The same was true for coping behaviour. Reports of pain perception and coping behaviour for children from the surgery group as well as the ECMO group did not differ in comparison with controls. As pain perception and coping in the surgery group was unaffected, the morphine they were given might have prevented these long-term alterations. Another possible explanation is provided by the large proportion of preterm borns in our pain group, as they are reported to have more problems with executive functions8. Grunau demonstrated that extremely low birth weight children, rated medical pain intensity significantly higher than psychosocial pain, unlike full birth weight controls [9]. She however included children with a birth weight of < 1000 grams.

Unexpectedly, high pain perception and distorted pain coping were not correlated in our study. Pain perception is likely to influence pain coping behaviour. However, coping is a constantly changing cognitive and behavioural effort to manage pain [35] and a child's frame of reference changes continuously during life [36]. This might contribute to the lack of correlation.

Methodological considerations

In all our studies we used Quantitative Sensory Testing (QST). This is a precise, computer-controlled device capable of generating and recording a response to highly repeatable thermal stimuli. The QST device has been used for the early diagnosis and follow-up of small fiber neuropathies and diagnosis of diabetic neuropathy. It is also an appropriate method to quantify mechanical and thermal allodynia and hypersensitivity. In children (aged 3 and older) [37-39], this device is reported to be reliable as repeated assessment of detection and pain thresholds lead to comparable results[39].
In order to limit testing time (current duration 45-60 minutes), we only evaluated thermal sensitivity, as this involves both a delta en c fibers. It is unclear whether the findings from experimental animal studies can be contributed to either c or a delta fibers or both [20, 21, 23]. As we found both a hyposensitivity for warmth and cold detection and hypersensitivity for heat and cold pain, it seems that both nerve fibbers (a delta and c fibers) are involved in long term alterations in pain sensitivity. Unfortunately, we did not induce temporal summation, therefore it is unclear whether the differences found in our studies coincide with an increased risk to develop wind up.

Whether the alterations in pain sensitivity involve superficial or deep nerves cannot be determined from our data. Two methods which are better suited for this purpose are the induction of pressure pain [40-42] or electrical stimulation. A disadvantage of

pressure pain however, is that it activates both peripheral small fibers (a delta and c fibres). Therefore, electrical stimulation is preferred. Until now, no studies have evaluated electrical pain stimuli in children, partly due to ethical considerations and partly due to negative associations of parents concerning their child being exposed to electrical pain.

Currently, there is no general agreement on standard procedures and every sensory function may be assessed in a variety of ways. Even the same test instrument may give different outcomes due to variation of stimulation parameters. In our study for example we instructed children to press a button, when pain was first perceived ("thermode now will slowly begin to warm, at a particular point it starts to hurt. When this point is reached, please press the button immediately."), which is in accordance with Zohsel [43]. Meier on the other hand, instructed the children to press the button when a particular sensation occurred [37]. This latter procedure however, results in lower pain thresholds. In addition, differences in temperature increase may also lead to different pain thresholds, for 1.5 C/s increases will lead to higher pain thresholds than temperature increases of 1.0 C/s which we used in our studies [37, 44]. Also, the size of the thermode influences pain thresholds, for the smaller the thermode, the lower the pain threshold [43]. Hilz even reported that the intratrial threshold reproducibility improved when the 2,5 cm x 5 cm probe was used instead of the 1,5cm x 2,5 cm probe [45]. Due to all these potentially confounding factors there is an urgent need to develop guidelines for a comprehensive QST protocol in order to make results comparable in future studies.

Chronic pain

We were happy to find that the children participating in our study, while being hypersensitive to pain, were not necessarily more susceptible to develop chronic pain (19% for both patients and controls) or somatic complaints (3 patients versus 2 controls) than control children without previous hospital experiences. In contrast, adult studies report 41-66% of all surgery patients to suffer from chronic pain, which may last at least for up to one year after surgery [46, 47]. The aetiology of chronic pain following surgery however, remains unclear [48-51].

One explanation for the development of chronic pain in adults might be the protein called Glial cell line-derived neurotrophic factor (GDNF)[52]. In an experimental animal study they found that following nerve damage in rats the expression of GDNF increases[52]. Although positively involved in nerve repair and growth stimulation, the protein is also reported to act as a neurotransmitter in the hypersensitivity involved in chronic pain[52]. According to Jongen, blocking of GDNF might prevent the occurrence of chronic pain while normal pain sensitivity remains present[52]. Whether the expression of GDNF in newborns differs from adults however is unknown.

Clinical implications

In this study we found a hyposensitivity to temperature detection following neonatal pain and morphine exposure. Although significant, the differences are small (maximal 1.5 degrees C). Therefore, we consider the clinical implications of thermal detection hyposensitivity to be negligible.

Regarding pain thresholds, children following neonatal pain exposure developed a hypersensitivity to pain, irrespective of morphine treatment. Although differences were significant, clinical implications are unclear. This study was the first to dem-

onstrate long-term alterations in pain sensitivity in school-aged children. No data are available regarding the clinical consequences of a 4 to 7 degrees lower pain threshold. As these differences are large we assume that they will lead to increased pain sensitivity in daily life which should be evaluated by long time follow up of these unique cohorts of former newborns exposed to a variety of painful events.

Besides these sensory differences, children exposed to pain in the absence of morphine report heightened pain perception and altered pain coping. During rehospitalization, these children might need extra analgesics, as they do not only experience more pain due to their lower pain thresholds, but also perceive this pain as worse than others do and use more stress related coping strategies, like internalizing and externalizing. However, the children participating in our study are not necessarily more susceptible to develop chronic pain or somatic complaints.

General conclusions and recommendations for future studies

Evaluation of the findings in this thesis shows that neonatal pain exposure, irrespective of morphine administration, results in long-term hyposensitivity to temperature detection and hypersensitivity to pain, limited to the region of neonatal tissue damage. On the other hand, morphine administration in the absence of overt pain leads to a generalized hyposensitivity to temperature detection without alterations in pain sensitivity. There appears to be a developmental window during which children are extremely vulnerable to develop alterations in pain sensitivity, taken the fact that preterm borns are more hypersensitive than their term born counterparts. The latter are more hypersensitive than children operated upon during the second or third month of life. Next to these somatosensory alterations, also the emotional-affective component of pain seems to be affected, as children exposed to pain in the absence of morphine show altered pain perception and coping behaviour. Fortunately, former patients did not have a higher risk of developing chronic pain.

Despite our past and present research efforts, a number of questions remain unanswered, e.g. 'Does hypersensitivity persist during adolescence and even adulthood?' and 'Can we prevent the development of hypersensitivity?'. Moreover, gaps between findings from experimental animal data and incorporation of knowledge into daily clinical practice need to be bridged.

Long-term follow up of the children studied is needed to determine whether the observed alterations will diminish with time. Clinically, it is possible to determine a child's pain threshold and prescribe analgesics accordingly. Nevertheless, it would be a better option to prevent this hypersensitivity. Here the NMDA receptor comes into the picture. This receptor is believed to be of major importance in the development of persistent neuro-anatomical alterations. Morphine, as the most frequently prescribed drug following major events in the NICU unit (endotrachial intubation, artificial ventilation [53 54] or operative procedures [55, 56]), however, is not able to prevent NMDA activation as a result of pain exposure. It would be worthwhile, therefore, to consider another analgesic agent that indeed prevents NMDA receptor activation, i.e. ketamine, and to use a combination of drugs. Prospective studies are warranted, integrating evaluation of pain threshold from the first day of admission to a NICU and followed by repeated measurements during initial admission. During re-admission pain thresholds would be determined providing a longitudinal data set which can be used for future studies. Apart from interventional standardization of QST measurements, new research projects will be

conducted integrating other non invasive methods to "visualize" pain in the brain such as fMRI to complete our picture of the long term effects of repeated neonatal pain experiences.

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Chapter 9

Summary / Samenvatting

The past twenty years have seen great progress in our knowledge of paediatric pain. Especially our understanding of neonatal pain processing and neurobehavioral development has much deepened. Today, the human central nervous system (CNS) is generally acknowledged to be very immature at birth and to show great plasticity during the first year of life. Andrews and Fitzgerald most clearly demonstrated the immaturity of the CNS in humans, in that pain on the short-term decreased the pain threshold. However, until now only few studies have evaluated the prolonged effects of neonatal pain exposure in humans, and results are conflicting. .

In order to evaluate whether pain or tissue damage in early life will lead to hypersensitivity persisting into childhood, we performed a cross-sectional study (Chapter 2). A total of 164 infants were included to determine if major surgery within the first 3 months of life increases pain sensitivity during subsequent surgery. Moreover, we wanted to evaluate whether apart from subsequent surgery in the same dermatome, also subsequent surgery in a different dermatome would alter pain sensitivity. All children received standard intraoperative and postoperative pain management. Rescue analgesic administration was guided by a treatment algorithm. Outcome measures to determine differences in pain sensitivity were assessed by the intraoperative fentanyl intake and by (nor)epinephrine plasma concentrations. Observational pain ratings from the COMFORT behaviour scale and Visual Analogue Scale (VAS), morphine intake and (nor)epinephrine plasma concentrations, served to assess differences in postoperative pain sensitivity. We found that only the infants previously operated upon in the same dermatome needed more intraoperative fentanyl, had higher COMFORT and VAS scores, had greater (nor)epinephrine plasma concentrations, and also needed more morphine than did infants with no prior surgery. The children previous operated upon in another dermatome only demonstrated higher postoperative analgesic requirements and norepinephrine plasma concentrations in comparison with infants with no prior surgery. These preliminary findings could indicate that neuroanatomical changes in the spinal and possibly also supraspinal nervous system have developed as a result of neonatal surgery. We conclude that the long-term consequences of surgery in early infancy are greatest in areas of prior tissue damage, which may portend limited clinical but important neurobiological differences.

As suggested in Chapter 2, exposure to pain during the first months of life may result in hypersensitivity. These findings are confirmed by experimental animal studies demonstrating that hypersensitivity may be caused by peripheral and spinal neuroanatomical alterations. In Chapter 3, we performed a cross-sectional study in order to investigate whether major surgery in early infancy results in prolonged hypersensitivity to pain. Thermal detection, pain and suprapain thresholds of children who had undergone major abdominal or thoracic surgery within the first three months of life were compared with those of age and gender matched healthy controls without previous hospital experiences. All thermal stimuli were induced by a Thermal Sensory Analyser with Peltier contact thermode. Both cold and heat stimuli were given. During a suprapain test, subjects assigned pain scores on a VAS. Data were analysed with structural equation modelling. A total of 57 patients and 57 controls were included. We found that former patients were hyposensitivity for both cold and heat temperature detection, only at the scar. Moreover, they were more sensitive to cold and heat pain at the scar and the contralateral,

unoperated side, when compared with the control children. No alterations in detection and pain thresholds could be determined on the hand, nor were we able to find any differences in suprapain thresholds at all three measured locations. There appears to be a developmental window in time during which children are more vulnerable to develop alterations in pain processing, as children operated upon within the first month of life demonstrated greater hypo-and hypersensitivity than children operated in the second or third months of life. Our findings indicate that spinal and supraspinal alterations have indeed developed following neonatal surgery, and are most pronounced in the region of tissue damage and the contralateral side.

Previous human studies have reported that repeated painful stimuli decrease pain thresholds in preterm neonates, suggesting short-term sensitization. In term neonates, on the other hand, habituation develops. In Chapter 4, we set out to determine whether pain exposure in the absence of morphine resulted in long term alterations in pain sensitivity. Moreover, we evaluated whether preterm children on the long term are still more sensitive to pain than full-term born children. To this aim we conducted a cross-sectional study in 8-year-old children who had been admitted to the neonatal intensive care unit (NICU) as newborns (1996 to 1999). These children were compared with age and gender matched healthy term born controls without previous hospital experiences. In these children both cold and heat detection, pain and suprapain thresholds were determined by a Thermal Sensory Analyser. A total of 53 patients (39 preterm and 14 full-term) and 53 controls were included. We found that preterm born patients were hyposensitive to heat detection at the hand and to cold detection at the heel. Moreover, the former patients were hypersensitive to cold pain at the heel in comparison with the control children. Overall, the patients were hyposensitive to temperature detection at both locations, i.e. the hand and heel, and were hypersensitive to both cold and heat pain at the heel. During the suprapain test, only the term born children were hypersensitive at both locations, whereas preterm children only showed hypersensitivity for cold pain at the heel. In conclusion, we found that even eight years after neonatal pain exposure, former patients are still hypersensitive to pain in the area of previous wounding. Our data suggest that neuroanatomical alterations have developed and are even more pronounced in preterm borns. Future studies should focus on prevention of long-term effects of early pain exposure in order to diminish hypersensitivity.

Nowadays, all neonates receive adequate pain treatment, based on the concept of pre-emptive analgesia. Although this will prevent children from experiencing pain on the short term, it does not prevent them from developing hypersensitivity to pain in the area of tissue damage in the long run. Animal studies have shown that high dosages of morphine will result in CNS alterations. However, we do not know whether this also applies to humans and whether these alterations will cause hypo- or hypersensitivity to pain in the long run. We therefore performed a cross-sectional study, described in Chapter 5, comparing pain sensitivity at age 8 years. Former patients who had received morphine (20 mcg/kg/h for a median of 9 days), inherent to their Extra Corporeal Membrane Oxygenation (ECMO) treatment, were compared with age and gender matched healthy controls without previous hospital experiences. A total of 60 patients and 60 controls were included. Cold and heat detection, pain and suprapain thresholds were determined by a Thermal Sensory Analyser. We found that patients were hyposensitive

to the detection of both cold and heat stimuli at all three locations, i.e. the hand, the right en left side of the neck. No differences in pain and suprapain threshold could be demonstrated. These data do suggest that alterations in supraspinal processing have developed, however only involving thermal detection thresholds. Future studies focussing on supraspinal neuroanatomical alterations might improve our understanding of the effect of opioid analgesics on pain processing

Besides neuroanatomical changes in the somatosensory cortex which could account for the hypersensitivity, there is some evidence that the affective motivational and cognitive evaluative dimensions of pain might also be affected by neonatal pain exposure. Therefore we evaluated whether neonatal pain and/or morphine exposure results in different pain perception and coping in Chapter 6. In a cross-sectional study we included all four studied cohorts, i.e. surgery group (n=57), pain exposed group in absence of morphine (n=53), ECMO group (n=60) and a control group without previous hospital experiences (n=170). Children were asked to complete the Paediatric Pain Inventory and the Pain Coping Questionnaire. We found that the children in the pain exposed group rated all pain related cartoons (both intensity and affect) higher than control children. Moreover, they commonly use less problem focused techniques and used less avoidance techniques to cope with pain, but responded with more emotion focused techniques which are considered to be inadequate techniques. The children in the surgery group only rated the medical cartoons as more painful. The ECMO children on the other hand, responded the same as the control children. In this study we adjusted for gestational age at birth and duration of intensive care unit admission. These data suggest that neonatal pain exposure in the absence of morphine seems to lead to alterations in pain perception and coping. Unfortunately, the specific neuronal pathways, which could have contributed to this development, are still unknown. Although the differences found between patients and controls are significant, they remain small. Therefore we think it is rather unlikely that these children will experience problems during childhood as a consequence of their increased pain perception and inadequate pain coping techniques. Further research is necessary to gain more insight into the factors that influence pain coping in former NICU patients.

Next to pain perception and coping, we assumed that also the occurrence of chronic pain and somatization could be influenced by early experiences in Chapter 7. Adults are known to frequently report chronic postoperative pain in the years after surgery. For children, however, there are hardly any data concerning development of chronic pain following neonatal surgery or pain exposure. Moreover, opioid administration in adults is reported to result in hypersensitivity to pain as well. We do not know whether this holds true for children. Next to chronic pain, also somatic complaints could develop as a consequence of neonatal pain exposure. Therefore, we also aimed to determine the occurrence of somatic symptoms. We evaluated parent's ratings of two questionnaires, i.e. the Structured Pain Questionnaire and the Children's Somatization Inventory, in the same four cohorts as described in chapter 7. We found that 19% of the parents reported that their children suffered from pain for more than 3 months. No differences could be determined between patients and controls. However, when patients' parents reported their child to suffer from chronic pain, they reported more weekly pain than the controls (39 vs 21%).. Similar findings were found for somatic complaints. Its incidence was rela-

tively low and did not differ between patients and controls (3 vs 2 symptoms). Only four children were classified as having a somatization disorder, all four were former patients. Concluding, we can assume that neonatal pain exposure does not seem to predispose for chronic pain and somatic complaints during childhood. Although, when patients do suffer from chronic pain, they will experience this pain more frequently and report a higher VAS score when compared to chronic pain sufferers without previous hospital experiences. Future studies should focus on the degree of impairment and utilization of medical services of children, who are hypersensitive to pain as a consequence of their neonatal hospital admission.

Chapter 8 is the final chapter in which all results are discussed and recommendations for future studies are made.

In de laatste twintig jaar is er grote vooruitgang geboekt wat betreft de kennis over pijn bij kinderen. Vooral ons begrip van neonatale pijn transmissie en neurale ontwikkeling is uitgebreid. Tegenwoordig is het algemeen bekend dat het humane centrale zenuw stelsel op het moment van de geboorte erg immatuur is en daarnaast een grote plasticiteit vertoont gedurende het eerste levensjaar. Andrews en Fitzgerald ondersteunen deze bevinding en beschrijven dat pijnblootstelling op de korte termijn leidt tot een afname in de pijndrempel. Tot heden zijn er maar enkele studies, die lange termijn effecten van neonatale pijnblootstelling hebben bestudeerd.

Om te beoordelen of neonatale pijn en/of weefsel schade leidt tot een persisterende hypergevoeligheid, hebben we een cross-sectionele studie uitgevoerd (Hoofdstuk 2). 164 kinderen werden geïncludeerd om te bepalen of een grote operatie gedurende de eerste 3 levensmaanden leidt tot een toegenomen pijngevoeligheid tijdens herhaalde operaties. Verder, hebben we geëvalueerd of behalve operatie in hetzelfde dermatoom, ook herhaalde operatie in een ander dermatoom leidde tot veranderingen in de pijngevoeligheid. Alle kinderen kregen standaard intra-operatieve en postoperatieve pijnbestrijding. Uitkomstmaten om veranderingen in pijngevoeligheid gedurende de operatie te bepalen bestonden uit de intra-operatieve fentanyl inname en de (nor)adrenaline plasma concentraties. Observationele pijn scores met behulp van de COMFORT gedragschaal en de Visuele Analoge Schaal (VAS), morfine inname en (nor)adrenaline plasma concentraties dienden om verschillen in postoperatieve pijngevoeligheid te bepalen. We vonden dat alleen de kinderen die voor de tweede keer in hetzelfde dermatoom geopereerd werden meer intra-operatieve fentanyl nodig hadden, hogere COMFORT en VAS scores hadden, hogere (nor)adrenaline plasma concentraties en ook meer morfine nodig hadden in vergelijking met kinderen die voor de eerste keer geopereerd werden. Kinderen die voor de tweede keer geopereerd werden, in een ander dermatoom, hadden alleen een hogere post-operatieve analgetica behoefte en noradrenaline plasma concentraties in vergelijking met kinderen zonder eerdere operaties. Deze bevindingen zouden kunnen duiden op neuro-anatomische veranderingen in het ruggenmerg en mogelijk ook supraspinale veranderingen in het centraal zenuw stelsel, welke als gevolg van neonatale chirurgie zijn ontstaan. We concluderen dat de lange termijn consequenties van neonatale operatie het grootst zijn in gebieden van eerdere weefselbeschadiging. Dit kan duiden op mogelijk beperkte klinische maar belangrijke neuro-biologische verschillen.

Zoals gesuggereerd in hoofdstuk 2 kan blootstelling aan pijn tijdens de eerste levensmaanden leiden tot hypergevoeligheid. Deze bevindingen worden bevestigd door experimentele dierstudies, die aantonen dat hypergevoeligheid veroorzaakt zou kunnen worden door perifere en spinale neuro-anatomische veranderingen. In Hoofdstuk 3, hebben we een cross-sectionele studie uitgevoerd om te bepalen of grote operaties tijdens de vroege jeugd leidden tot een blijvende hypergevoeligheid voor pijn op 8 jarige leeftijd. Thermale detectie, pijn en suprapijn drempels van kinderen die een grote buik of thorax OK hebben ondergaan tijdens de eerste 3 levensmaanden werden vergeleken met die van op leeftijd en geslacht gematchte controles zonder eerdere ziekenhuiservaringen. Alle thermale stimuli werden geinduceerd met een Thermal Sensory Analyser (TSA) met behulp van een Peltier contact thermode. Aan de kinderen werd gevraagd op een knop te drukken zodra zij warmte/ koude stimuli en warmte/koude pijn voelden. Ti-

jdens de suprapain test werd aan de kinderen gevraagd pijnscores te geven op een VAS schaal. Data werden geanalyseerd met behulp van Structural Equation Modelling (SEM). In totaal werden 57 patiënten en 57 controles geincludeerd. We vonden dat patiënten hypogevoelig waren voor koud en warmte temperatuur waarneming, alleen ter plaatse van de vroegere operatie wond. Verder waren de patiënten meer gevoelig voor koude en warmte pijn ter plaatse van het litteken en de contralaterale, niet geopereerde zijde in vergelijking met de controle kinderen. Op de hand konden geen verschillen in detectie en pijn drempels worden gevonden. Verder werden er ook geen veranderingen in suprapijn gevoeligheid aangetoond op alle drie de locaties. Er bleek een 'developmental window' in de tijd te zijn waarin kinderen meer kwetsbaar zijn ten aanzien van het ontwikkelen van lange termijnveranderingen in pijngevoeligheid, aangezien de kinderen die in de eerste levensmaand geopereerd werden, meer hypo- en hypergevoelig waren dan kinderen die in de tweede of derde maand geopereerd werden. Onze bevindingen suggereren dat spinale en supraspinale veranderingen inderdaad zijn ontstaan na neonatale chirurgie en dat deze voornamelijk aanwezig zijn op de plaats van neonatale weefsel beschadiging en de contralaterale zijde.

Eerdere studies bij pasgeborenen hebben laten zien dat herhaalde prikkels leiden tot een afname van de flexie reflex drempel bij te vroeg geborenen, wat suggereert dat korte termijn sensitizatie is ontstaan. In a term geboren neonaten echter ontstaat habituatie van de flexie reflex na herhaalde stimulatie met Von Frey haren. In Hoofdstuk 4, hebben we bepaald of blootstelling aan pijn in de afwezigheid van morfine leidt tot veranderingen in de pijngevoeligheid. Daarnaast hebben we geëvalueerd of in deze groep de te vroeg geboren kinderen op de lange termijn meer gevoeliger zijn voor pijn dan a term geborenen. Hiervoor hebben we een cross-sectionele studie uitgevoerd in 8 jarige kinderen die als pasgeborenen op de Neonatale Intensive Care Unit (NICU) opgenomen zijn geweest (in de periode 1996-1999). Deze kinderen werden vergeleken met op leeftijd en geslacht gematchte controles zonder eerdere ziekenhuis ervaringen. Bij deze kinderen werd de koude/warmte waarneming, koude/warmte pijndrempel en koude/warmte suprapijn drempel bepaald doormiddel van een Thermal Sensory Analyser. Totaal werden 53 patiënten (39 preterm en 14 a term) en 53 controles geincludeerd. De patiënten bleken hypogevoelig te zijn voor temperatuur waarneming op beide locaties, de hand en de hiel, daarnaast waren ze ook hypergevoelig voor koude en warmte pijn op de hiel. Verder vonden we dat de te vroeg geboren patiënten hypogevoelig waren voor warmte waarneming op de hand en voor koude waarneming op de hiel. Verder waren de patiënten hypergevoelig voor koude pijn op de heel in vergelijking met de controle kinderen. Tijdens de suprapijn test, waren alleen de a term geboren kinderen hypergevoelig op beide locaties, terwijl de te vroeg geborenen alleen hypergevoelig waren voor koude pijn op de hiel. Concluderend, kunnen we aannemen dat 8 jaar na neonatale pijnblootstelling, patiënten nog steeds hypergevoelig zijn voor pijn in het gebied van eerdere weefselbeschadiging. Onze data suggereren dat neuro-anatomische veranderingen zijn ontstaan en voornamelijk aanwezig zijn in te vroeg geborenen. Verder onderzoek zou moeten focussen op de preventie van deze lange termijn effecten van neonatale pijnblootstelling om deze hypergevoeligheid te voorkomen of te verminderen.

Tegenwoordig krijgen alle neonaten adequate pijnstilling, gebaseerd op het concept van pre-emptieve analgesie. Ondanks dat dit er voor zorgt dat kinderen op de korte termijn geen pijn voelen, leidt het op de lange termijn niet tot het voorkomen van hypergevoeligheid in het gebied van neonatale weefsel beschadiging. Dierstudies laten zijn dat een hoge dosis van morfine leidt tot veranderingen in het centrale zenuwstelsel. Helaas weten we niet of dit ook het geval is in mensen en of deze veranderingen leiden tot een hypo- dan wel hypergevoeligheid voor pijn op de lange termijn. Daarom hebben wen een cross-sectionele studie verricht, welke beschreven wordt in Hoofdstuk 5, waarin de pijngevoeligheid op 8 jarige leeftijd wordt beoordeeld. Patiënten die morfine (20 mcg/kg/h voor een mediane duur van 9 dagen) kregen, als gevolg van hun Extra Corporale Membraan Oxygenatie (ECMO) tijdens de eerste levensweken, werden vergeleken met leeftijd en geslacht gematchte controles zonder eerdere ziekenhuis ervaringen. Totaal werden 60 patiënten en 60 controles geincludeerd. Koude/warmte detectie, koude/warmte pijn en koude/warmte suprapijn drempels werden bepaald met behulp van een Thermal Sensory Analyser. We vonden dat de patiënten hypogevoelig waren voor de waarneming van koude en warmte stimuli op alle drie de locaties: de hand, de rechter en linker kant van de hals. Geen verschillen in pijn en suprapijn drempels konden worden aangetoond. Deze data suggereren dat veranderingen in supraspinale pijn processing zijn ontstaan, waarbij alleen de detectie drempels betrokken zijn. Toekomstige studies die focussen op supraspinale neuro-anatomische veranderingen zouden ons begrip over het effect van opioiden op lange termijn pijn processing kunnen verbeteren.

Behalve neuro-anatomische veranderingen in de somatosensore cortex welke mogelijk verantwoordelijk zijn voor de gevonden hypergevoeligheid, wordt er gesuggereerd dat de affectieve motivationele and cognitieve evaluatieve dimensies van pijn ook beïnvloed worden door neonatale pijn blootstelling. Daarom hebben we in Hoofdstuk 6 geëvalueerd of neonatale pijnblootstelling en/of morfine gebruik leidt tot een verschil in pijn perceptie en coping gedrag. In een cross-sectionele studie werden alle vier de studie cohorts zoals eerder beschreven geincludeerd, de operatie groep (n= 57), de pijn groep zonder morfine (n=53), de ECMO groep (n=60) en een controle groep (n=170) zonder eerdere ziekenhuis ervaringen. Aan de kinderen werd gevraagd om twee vragenlijsten in te vullen, de "Pediatric Pain Inventory" (PPI) en de "Pain Coping Questionnaire" (PCQ). We vonden dat kinderen in de pijn groep alle pijngerelateerde plaatjes van de PPI hoger scoorden (op intensiteit en affect) dan de controle kinderen. Verder gebruikten zij minder probleem gefocuste technieken en minder vermijdingstechnieken bij het omgaan met pijn, echter reageerden zij wel met meer emotie gefocuste technieken, die als inadequaat worden beschouwd. De kinderen in de operatie groep scoorden alleen de medische plaatjes van de PPI als meer pijnlijk (intensiteit en affect) dan de controles. De ECMO kinderen echter, reageerden hetzelfde als de controle kinderen. In deze studie hebben we gecorrigeerd voor zwangerschapsduur en duur van intensive care unit opname. Deze data suggereren dat neonatale blootstelling aan pijn in de afwezigheid van morfine leidt tot veranderingen in pijn perceptie en coping gedrag. Helaas zijn de specifieke neuronale banen die mogelijk van invloed kunnen zijn, tot op heden onbekend. Ondanks dat we verschillen tussen patiënten en controles hebben gevonden, blijven ze klein. Daarom vermoeden wij dat het onwaarschijnlijk is dat deze kinderen problemen

zullen ervaring tijdens hun jeugd als gevolg van hun veranderde pijn perceptie en inadequate pijn coping technieken. Verder onderzoek is nodig om meer inzicht te krijgen in de factoren die van invloed zijn op coping gedrag in voormalige NICU patiënten.

Behalve pijn perceptie en coping, kan mogelijk ook het optreden van chronische pijn en somatizatie beïnvloed worden door vroege pijn ervaringen. Volwassenen rapporteren frequent chronische pijn in de jaren na een operatie. Bij kinderen echter, zijn er weinig gegevens bekend over de ontwikkeling van chronische pijn na neonatale operatie of pijnblootstelling. In volwassenen kunnen opioiden eveneens leiden tot een hypergevoeligheid voor pijn. We weten niet of dit ook het geval is in kinderen. Behalve chronische pijn, kunnen ook somatizatie klachten optreden als gevolg van neonatale pijn blootstelling. Daarom was het doel van deze studie (Hoofdstuk 7) naast het optreden van chronische pijn, ook het voorkomen van somatizatie klachten te evalueren. Hiervoor hebben we twee vragenlijsten gebruikt, de "Structured Pain Questionnaire" (SPQ) en de "Children's Somatization Inventory" (CSI). Beide vragenlijsten werden ingevuld door de ouders van dezelfde cohorten kinderen als beschreven in hoofdstuk 6. We vonden dat 19% van de ouders rapporteerde dat hun kinderen meer dan 3 maanden pijn hadden. Hiervoor konden geen verschillen worden gevonden tussen patiënten en controles. Echter, de ouders van patiënten rapporteerden veel vaker wekelijks pijn dan de ouders van controle kinderen (39 versus 21%). Voor somatische klachten werd hetzelfde gevonden. De incidentie was relatief laag en verschilde niet tussen patiënten en controles (3 symptomen voor patiënten versus 2 voor de controles). Vier kinderen hadden een gestoorde somatizatie (>13 klachten), alle vier waren patiënten. Concluderend kunnen we zeggen dat neonatale pijnblootstelling niet leid tot een hogere kans op chronische pijn en somatizatie klachten tijdens de jeugd. Wanneer patiënten echter meer dan 3 maanden pijnklachten hebben, zijn dit vaker wekelijkse pijnklachten dan bij de controle kinderen. Verder geven de ouders van patiënten ook een hogere VAS score voor de pijn die hun kind ervaart dan ouders van controle kinderen. Verdere studies zijn nodig om een beter inzicht te krijgen in het ontwikkelen van chronische pijn en de consequenties hiervan als gevolg van neonatale ziekenhuis opname.

Hoofdstuk 8 is het laatste hoofdstuk waarin alle resultaten worden besproken en suggesties voor toekomstige studies worden gegeven.

Dankwoord

Eindelijk is het dan zover, mijn boekje is af. Toch is dat een raar idee. Vier jaar aan gewerkt, en dan ineens is het zover, het is klaar! Natuurlijk was het nooit zo ver gekomen zonder de hulp van velen en die wil ik graag bedanken!

In eerste plaats Prof dr D. Tibboel. Beste Dick, ik wil je bedanken omdat je mij de mogelijkheid hebt gegeven om onderzoek te doen. Ik kwam als onderzoeksstudent en zag promoveren in eerste instantie niet zitten. Mede door je gedrevenheid en enthousiasme ben ik toch gebleven. Dankzij je vertrouwen was het mogelijk om dit onderzoek tegelijkertijd met mijn co-schappen te combineren, bedankt voor alles.

Mijn co-promoter, dr. JWB Peters. Beste Jeroen, bedankt dat je altijd voor me klaar stond. Het maakte niet uit hoe laat het was, 's avonds of in het weekend, ik kon je altijd bellen. Je enthousiasme heeft me zeker gestimuleerd om dit onderzoek tot een goed einde te brengen.

De leden van de kleine commissie: Prof dr. J Klein, Prof dr J Passchier en Prof dr A van der Heijden. Hartelijk dank voor het snelle beoordelen van mijn manuscript en de waardevolle bijdragen. Tevens wil ik ook Prof dr. J van Goudoever, Prof dr GJ Fleuren, dr. K Allegaert en dr. O Wilder-Smith bedanken voor hun bereidheid zitting te nemen in de grote commissie.

Alle ouders en kinderen die aan dit onderzoek hebben meegedaan en natuurlijk de basisscholen: De Klim-op uit Capelle aan den IJssel en de Sint Bernardusschool uit Oud-Gastel, zonder jullie was dit onderzoek niet mogelijk geweest, bedankt.

Lieve Isabella, jammer dat je er niet bij kan zijn. Ik had je graag als paranimf naast me gehad. Ik weet zeker dat je een te gekke tijd zult hebben in Harvard. Tot snel!

Lieve Kim, vriendinnen sinds de dag dat jij de zwarte en ik de witte koningin was. Ik ben blij dat je mijn paranimf bent.

Lieve Eva, ik had vroeger nooit gedacht dat je op deze dag naast me zou staan, fijn dat je mijn paranimf wilt zijn.

Lieve Janine en Joanne, bedankt voor alle gezelligheid en discussies, ik zal jullie missen in Leiden!

Lieve Saira en Bert, al die avondjes 'catannen', goede (soms ook wat minder goede) films, lekker eten, en natuurlijk de organisatie van de jeugdcommissie, waren belangrijk om even het onderzoek te vergeten en lekker te ontspannen. Saira bedankt voor al het werk dat je in het ontwerp en de aanleg van onze tuin hebt gestopt, daar had ik zelf nooit tijd voor gehad.

Lieve Susanne en Lisette, bedankt voor alle inspannende ontspanning. Roeien met jullie (en Saira en Bert natuurlijk) zorgt voor de nodige gezelligheid in de boot.

Lieve Beau en Vince, jullie altijd enthousiaste begroeting maakten elke vervelende dag weer goed.

Kamergenoten en Collega's. Sandra, samen waren we altijd twee vroege vogels om de files voor te zijn. Bedankt voor alle gezelligheid! Heleen en Joke bedankt voor alle gezellige lunches en kopjes thee tussendoor! Tom, Mark, Monique, Patricia, Sebas, Ilse, Maaike S, Emile, Merel, Liesbeth, Niels, bedankt voor jullie gezelligheid en support

Algesiologisch instituut, Pijnkenniscentrum en ZonMW bedankt voor de financiële steun voor een deel van dit onderzoek.

Beste Ko, dankzij je snelle en nauwkeurige redigerende acties is mijn boekje nu een stuk leesbaarder geworden.

Dr. Duivenvoorden, beste Hugo, bedankt voor je hulp bij de opbouw van dit boekje, de data analyses en alle leuke discussies en gesprekken tussendoor.

Dr. Van Heijst, beste Arno, bedankt voor je hulp bij het includeren van een deel van jullie ECMO kinderen en de snelle beoordeling van hoofdstuk 6.

Lieve pap en mam, zonder jullie onvoorwaardelijke steun was ik nooit zo ver gekomen. Het was fijn dat jullie altijd voor me klaar stonden!
Lieve mam, niemand had ooit verwacht dat je er vandaag niet bij zou zijn, maar ik weet zeker dat je trots op me bent, daar waar je nu bent.

Lieve familie, bedankt voor jullie steun en interesse.

Lieve Jurgen, bedankt dat je er altijd voor me was. Jij wist altijd mijn problemen, stress en irritatie weer in het juiste perspectief te zetten, waardoor ze een stuk minder belangrijk leken.

Curriculum Vitae

Renata Schouw werd geboren op 19 September 1980 te Roosendaal en Nispen. In 1998 behaalde ze haar VWO diploma aan het Markland College te Oudenbosch. Hierna is ze begonnen met de studie Geneeskunde aan de Erasmus Universiteit Rotterdam. Tijdens haar studie heeft ze gewerkt als datamanager bij de Kinderoncologie van het Erasmus MC - Sophia Kinderziekenhuis. Voor haar doctoraal scriptie heeft zij een onderzoek uitgevoerd naar de validatie van een pijnmeet instrument (EDIN schaal) voor pasgeborenen met chronische pijn als gevolg van necrotiserende enterocolitis (NEC) op de afdeling Kinderheelkunde van het Erasmus MC - Sophia Kinderziekenhuis. In augustus 2004 heeft zij haar co-schappen afgerond met het artsexamen.
Van september 2002 tot augustus 2006 heeft zij onderzoek verricht naar de lange termijn effecten van neonatale pijnblootstelling welke in dit proefschrift zijn beschreven. Dit onderzoek is uitgevoerd op de afdeling Kinderheelkunde van het Erasmus MC - Sophia Kinderziekenhuis. Sinds Augustus 2006 is ze in opleiding tot patholoog in het LUMC.