SHADOWS OF THE PAST

Does neonatal morphine use foreshadow neuropsychological functioning, stress response and pain sensitivity at primary school age?

Johanna de Graaf

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Does neonatal morphine use foreshadow neuropsychological functioning, stress response and pain sensitivity at primary school age?

schaduwen van het verleden

Voorspelt neonataal morfine gebruik het neuropsychologisch functioneren, de stressrespons en de pijngevoeligheid op basisschoolleeftijd?

Proefschrift

ter verkrijgen van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de rector magnificus
Prof.dr. H.G. Schmidt
en volgens besluit van het College van Promoties.

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door

Johanna de Graaf

geboren te Leiderdorp

Ter nagedachtenis

n=18*

***Van de 150 kinderen die deelnamen aan de originele studie zijn 18 kinderen overleden. In dit proefschrift wordt deze groep slechts weergegeven in een emotieloze, statistische term: n=18.**

Promotiecommissie

Promotor	Prof.dr. D. Tibboel
Overige leden	Prof.dr. I.K.M. Reiss Prof.dr. F.J.P.M. Huygen Dr. E.M.W.J. Utens
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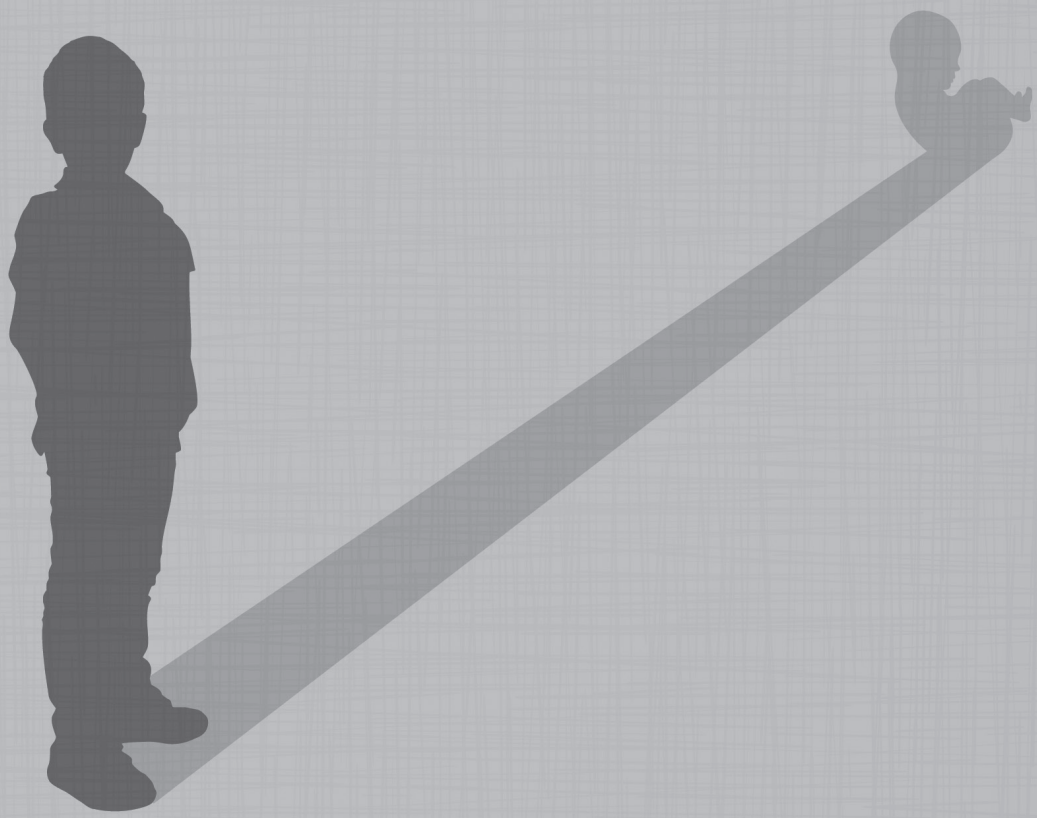
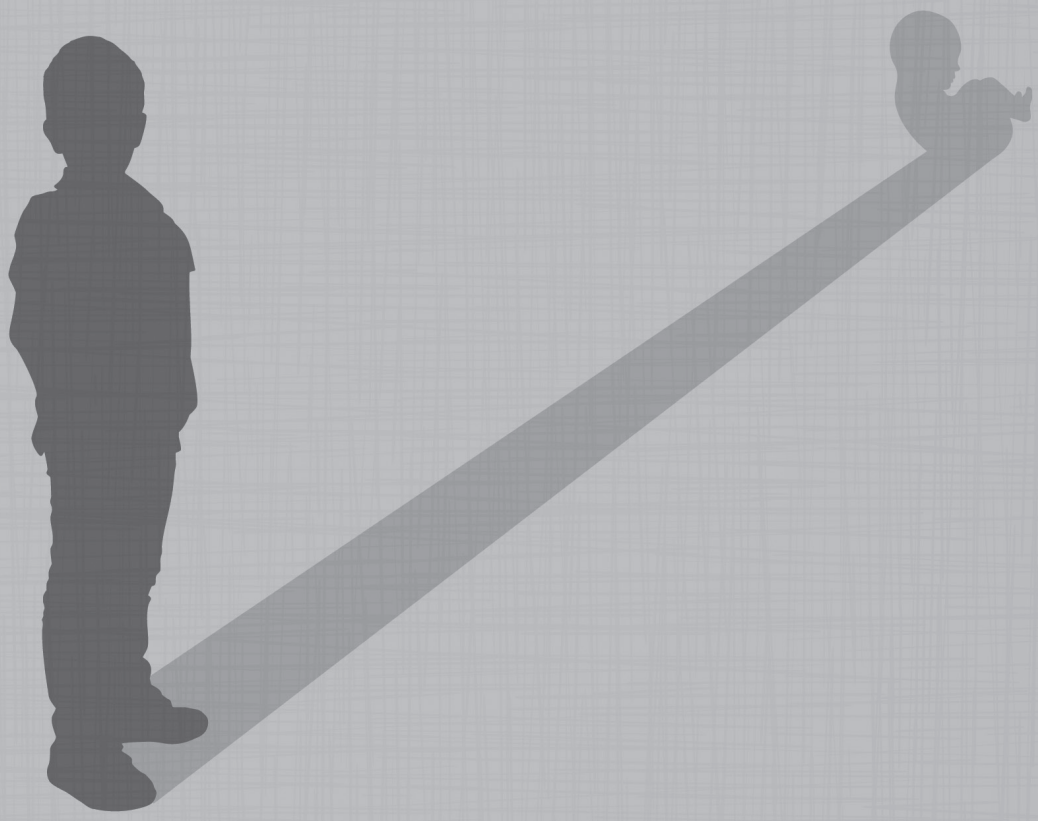


Chapter 1

General introduction



Critically ill newborns often need to undergo invasive procedures that may bring discomfort and pain. This is very worrying because at newborn age the brain undergoes, in a relatively short time, an extreme growth and transformation[2,21,27], which makes the brain more susceptible to perturbation than at any other time of life. From a study performed in our Neonatal Intensive Care Unit (NICU) – at the Erasmus MC-Sophia Children's hospital Rotterdam, the Netherlands – we learned that patients on average experienced an astonishingly high number of 14 painful procedures per day. Up to 65% of these patients did not receive appropriate analgesic therapy[22].

At the time of that study, some ten years ago, pain treatment generally consisted of opioids as these had been proven effective for postoperative pain relief in preterm born infants[1]. However, there was no evidence that they would be equally effective for other types of pain than postoperative pain. To learn more about the effects of morphine administration to critically ill newborns we set up a next study together with the Isala Clinics Zwolle. This randomized controlled trial (RCT), in which 150 neonates on ventilatory support received either continuous morphine or placebo, demonstrated no beneficial effect of continuous morphine on neurological outcome or pain and was therefore not recommended as *'standard of care'* in this vulnerable population[22-24]. This was later confirmed by a larger RCT, the so-called NEOPAIN trial[3], and a meta-analysis[5].

Long-term effects of morphine

Today, morphine is still commonly used to treat neonates' postoperative pain or other severe pain, in spite of its adverse short-term side-effects for example respiratory depression[6]. Besides, we cannot rule out the possibility of negative long-term effects[7,16,18,26], although positive effects has been suggested as well[10,11]. Based on the scarce literature about the long-term effects of morphine we should focus on three areas: (1) brain development, (2) the stress system, and (3) pain perception.

Brain development

Animal studies have shown alarming effects of neonatal morphine administration on brain development. Rodents treated with morphine in the neonatal period showed increased neuro-apoptosis affecting both neurons and microglia[4,16] and showed alterations in the hippocampus[9]. These changes are suggested to be an explanation for long-term learning problems found in rodents in adulthood[18]. No human studies, however, could confirm these learning problems. MacGregor et al. (1998) evaluated children who participated in a RCT of morphine versus pancuronium[17]. They did not find adverse effects of neonatal morphine use on cognitive functioning, motor abilities and behavior 5-6 years later. In addition, Roze et al. (2008) also found no association between prolonged sedation and/or analgesics use at neonatal age and cognitive or neuromotor outcomes at 5 years of age[20].

Stress system

Regarding long-term effects of neonatal morphine use, the stress system should be studied as well because our original RCT[20] and previous RCTs[16-17] showed that neonatal morphine use reduced noradrenaline and adrenaline levels. Stress at older ages is usually assessed by levels of cortisol. This stress-hormone is the predominant glucocorticoid in humans and one of the end-products of the hypothalamic-pituitary-adrenal (HPA) axis. Studies have shown higher levels of cortisol in preterm born children than in term born children from the age of 8 months up to 14 years[8,14]. On the other hand, Grunau and colleagues showed that neonatal morphine use had no effect on cortisol response at 32 weeks post-conceptional age and 8 months of age[12,13].

Pain perception

Another line of research has focused on the effects of neonatal pain on the development of pain pathways and thermal sensitivity[15,19,25,28]. Grunau and colleagues found preliminary evidence that morphine may have a positive ameliorating effect on later pain reactivity in very low birth weight neonates at 32 weeks postconceptional age[11] and at 8 months of age[10]. However, this effect could not be confirmed in a later study[13].

General objective

Taken together, studies in this areas show contradictory results which leaves the long-term effects of morphine – both in animals and in humans – subject to ongoing debate. Most human studies will not help resolve this issue, however, because they were designed to investigate the long-term effects of pain in relation to gestational age and not to examine specific morphine protocols[10-13]. Morphine served as a co-variable only, which was controlled for. However, it cannot be ruled out that associations between morphine and the outcome variables were masked due to high correlations between morphine and for example severity of illness or gestational age[20]. Therefore, a follow-up study of a RCT in which morphine treatment was compared with placebo is needed to evaluate exclusively the long-term effects of morphine without interference of other neonatal components. Our RCT describing the effects of continuous morphine infusion in ventilated neonates[22-24] offers this unique opportunity. In this thesis, we provide a detailed analysis of the long-term effects of neonatal morphine use on the child's development at the ages of 5 and 8 or 9 years. The follow-up schedule below details the different domains tested at the various ages. We address the question whether the morphine use has either a protective or harmful effect on (1) brain development in terms of psychological and neuropsychological development; (2) the stress system; and (3) pain perception later in life. Or, in other words; is morphine a shadow of the past?

Outline of the thesis

This thesis consists of three parts. **Part I** is dedicated to the long-term effects of neonatal morphine infusion on psychological and neuropsychological development. At five years we evaluated intelligence, visual motor integration, behaviour, and health-related quality of life (**chapter two**). At 8 or 9 years we added the evaluation of the executive functions (**chapter three**).

Part II evaluates the long-term effect of morphine use on the hypothalamic-pituitary-adrenal (HPA) axis, by studying the diurnal rhythm of cortisol of children who participated in the original RCT and a control cohort of healthy term born children without a history of hospitalization (**chapter four**).

Part III deals with pain detection thresholds and pain thresholds in the context of morphine use. First we established whether quantitative sensory testing, using the Thermal Sensory analyser (TSA), would be feasible in 5-year-old children and whether intelligence would play a role herein (**chapter five**). Next we tested the 8- or 9–year-old children with the TSA to examine the effect of morphine use on pain detection thresholds and thresholds, as well as to evaluate the occurrence of chronic pain (**chapter six**).

The concluding **chapter seven** presents a general discussion of the main findings and conclusions, the implications thereof, and recommendations for follow-up.

	0 years	5 years	8/9 years
Physical evaluation	Growth		
	Neurological examination		Neurological examination
	Blood pressure		
(neuro)psychogological evaluation		Intelligence	
		Visual motor integration	
		Behavioural and emotional problems	
		Quality of life	
			Academic achievements
			Executive functions
Stress	Adrenaline/noradrenaline		
		Cortisol levels	
Pain	Pain scores		
		Chronic pain	
		Detection thresholds	
			Pain thresholds

Figure 1. Follow-up schedule

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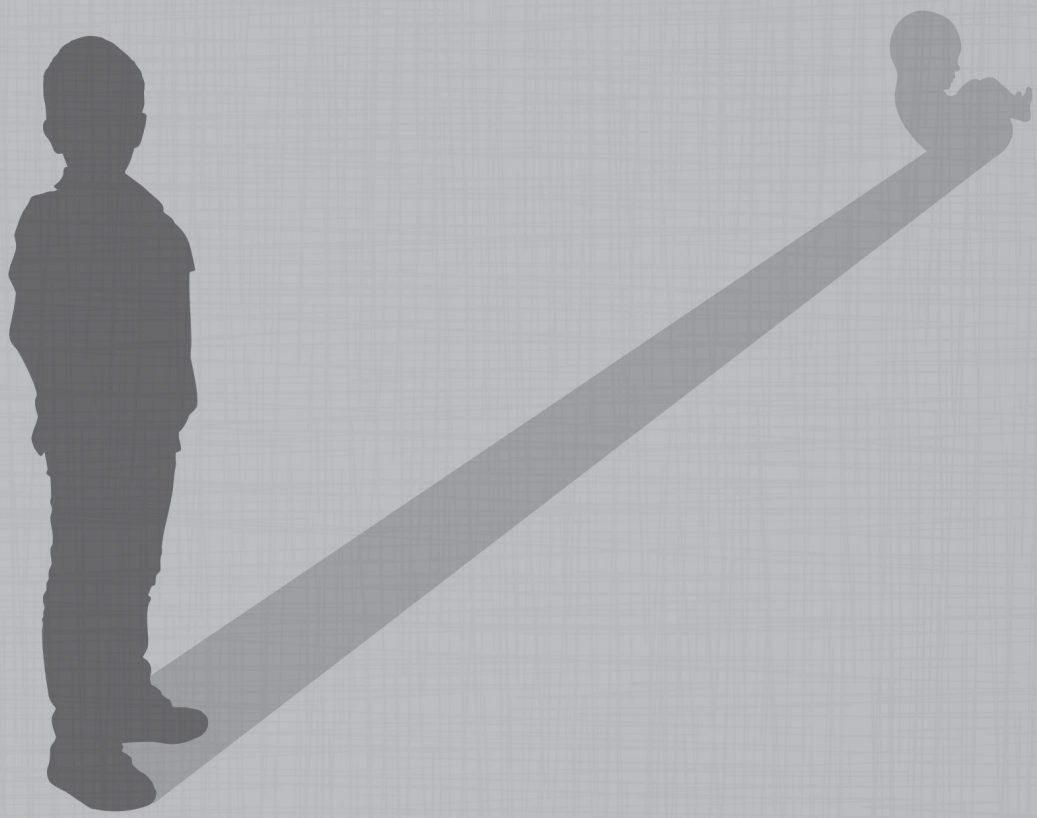
PART I

NEUROPSYCHOLOGICAL DEVELOPMENT

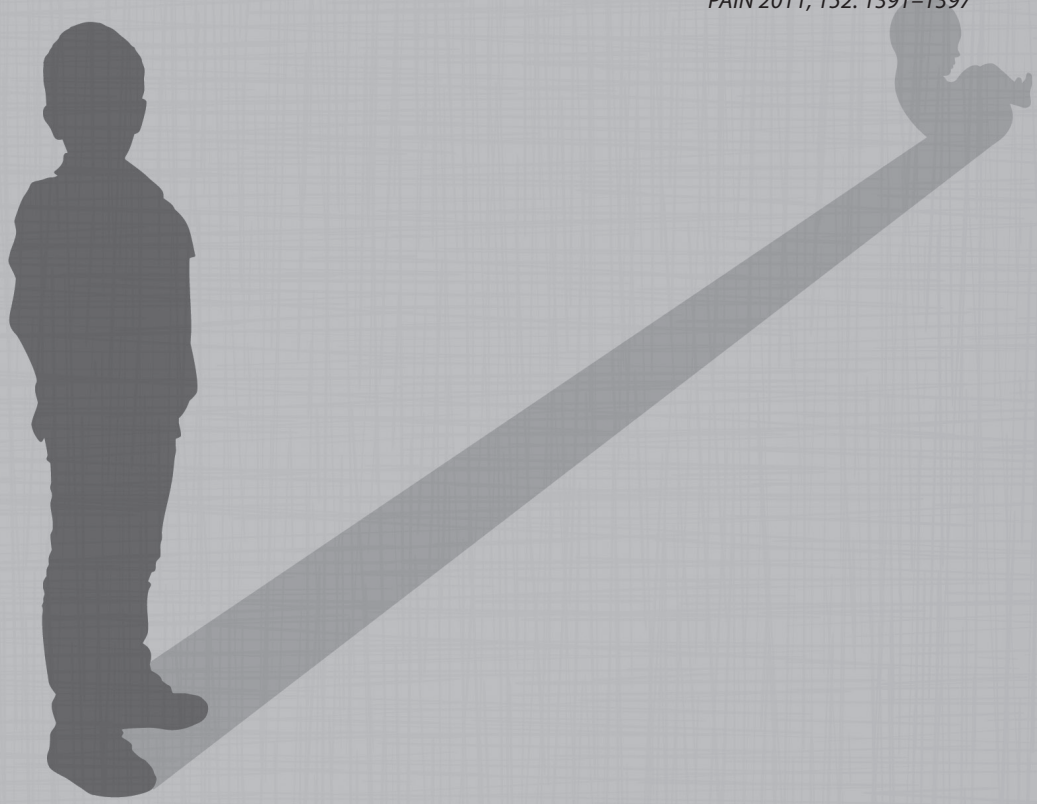


Chapter 2

Long-term effects of routine morphine infusion in mechanically ventilated neonates on children's functioning: Five-year follow-up of a randomized controlled trial

Joke de Graaf, Richard A. van Lingen, Sinno H.P. Simons, Kanwaljeet J.S. Anand, Hugo J. Duivenvoorden, Nynke Weisglas-Kuperus, Daniella W.E. Roofthooft, Liesbeth J.M. Groot Jebbink, Ravian R. Veenstra, Dick Tibboel, Monique van Dijk

PAIN 2011; 152: 1391–1397



ABSTRACT

Newborns on ventilatory support often receive morphine to induce analgesia. Animal experiments suggest that this may impair subsequent cognitive and behavioral development. There are sparse human data on long-term effects of neonatal morphine. We aimed to investigate the effects of continuous morphine administered in the neonatal period on the child's functioning. We conducted a follow-up study among 5-year-olds who, as mechanically ventilated neonates, had participated in a placebo-controlled trial on effects of morphine administration on pain and neurologic outcome. They were now tested on intelligence, visual motor integration, behavior, chronic pain, and health-related quality of life. Univariate analyses showed significantly lower overall intelligence quotient (IQ) scores for children who earlier had received morphine, that is, mean 94 (SD 14.5) versus 100 (SD 12.9) for those who received placebo (P = 0.049). Other between-group differences in outcomes were not found. The statistical difference disappeared after correction for treatment condition, open-label morphine consumption over the first 28 days, and a propensity score for clinically relevant co-variables in multiple regression analyses. However, scores on one IQ subtest, "visual analysis," were significantly negatively related to having received morphine and to open-label morphine consumption the first 28 days. The finding of a significant effect of morphine on the "visual analysis" IQ subtest calls for follow-up at a later age focusing on the higher-order neurocognitive functions.

INTRODUCTION

As recently as 2 decades ago, anesthetists, for fear of respiratory depression, were reluctant to prescribe pain-relieving perioperative opioids to neonates[34]. Then in 1987, Anand et al.[3] published a landmark article showing dramatically worse outcomes after patent ductus arteriosus surgery for preterm neonates who did not receive perioperative opioids compared to those who did. In further research on human neonatal pain, it proved to be associated with changes in pain responses[17,26,47-49], behavior[16,20], somatosensory perception[42,51], modulation of pain[15], and stress responses[19]. Later studies brought evidence that morphine analgesia has positive short-term effects, for example, diminished stress response[33,36,54].

We studied the administration of continuous morphine in a randomized, placebo-controlled trial (RCT) in 150 mechanically ventilated preterm neonates. These neonates showed no differences in pain intensities and no beneficial effect on neurologic outcomes following continuous morphine infusions[43-45]. The incidence of intraventricular hemorrhage (IVH) was significantly reduced in the morphine-treated neonates. A larger RCT, using higher doses of morphine, showed that morphine decreased clinical signs of pain, but no differences occurred in the rates of neonatal mortality, severe IVH, and periventricular leukomalacia[4]. Both RCTs as well as a meta-analysis[7] concluded that routine morphine use is not recommended for ventilated neonates.

Studies in animals suggest potential adverse long-term effects of morphine. Morphine administration to neonatal rats produces long-term changes in behavior and brain function[22] and impairs the adult cognitive functioning[29], in particular, spatial recognition memory[27]. Basic science has shown that the opioid system modulates neural proliferation in vivo[41]. Extrapolating from these data suggests a harmful role of morphine treatment in disturbing neurogenesis of newborn babies. Mechanistically, morphine increases apoptosis of human microglial cells[25] and increases red neuron degeneration in the rat brain, which may lead to cerebral dysfunction[5].

Boasen et al.[10] recently showed in rodents that both neonatal pain and morphine treatment produced long-lasting behavioral effects to a degree sufficient to alter learning, while the combined impact of the treatments did not.

In just one published human study, neonates were randomized to receive morphine or placebo[28]. The researchers evaluated intelligence, motor abilities, and behavior at 5–6 years. The follow-up tests showed no beneficial effect of morphine administration. In a cohort follow-up study, Grunau et al.[21] found that poorer cognition and motor function were associated with higher number of skin-breaking procedures, but independent of early illness severity, total

morphine dose, or treatment with postnatal steroids. To resolve the discrepancy between findings of animal studies and the sparse human data of the long-term effect of morphine administration, we undertook a 5-year follow-up study of patients previously enrolled in the RCT of Simons et al.[43]. Our aim was to investigate the effects of morphine received in the neonatal period on the child's functioning at the age of 5 years in terms of intelligence, visual motor integration, behavior, chronic pain, and health-related quality of life.

METHODS

The original study

The original study was performed in 2 level III neonatal intensive care units (NICUs) in the Netherlands in 2000–2002[43-45]. Neonates younger than 3 days on ventilatory support were randomly allocated to receive a loading dose (100 mcg/kg) followed by a continuous infusion of 10 mcg/kg/h of either morphine (n = 73) or placebo (n = 77). Children judged to be in pain or distress received open-label morphine of 50 mcg/kg followed by 5-10 mcg/kg/h.

Overview of the follow-up-study

Eighteen of the 150 participants in the original study had died at the time we started the present study. Seven of the 132 survivors were being followed elsewhere and were not invited. Two of them had severe physical limitations; one had severe developmental delay, and 4 had both. Three of these 7 children were diagnosed with mild IVH in the neonatal period and one with periventricular leukomalacia. Three had been randomized to receive continuous morphine, and 4 to receive placebo.

Parents of the 125 eligible children were invited to participate between December 2005 and January 2008. Written informed consents were obtained before follow-up testing. Parents also provided consent for the child's teacher's contribution to testing.

The medical ethical review boards at Erasmus MC-Sophia Children's Hospital (Rotterdam) and Isala Clinics (Zwolle) approved this study.

The follow-up assessment started with measurements of height and weight and medical evaluation by pediatricians (R.vL. and N.W.). Subsequently, child psychologists (J.dG. and R.V.), blinded to the neonatal treatment condition, administered an intelligence test and a visual motor integration test. These assessments required approximately 2 hours. Two children were seen at home. The parents and the child's teacher completed different questionnaires about the child's behavior, chronic pain, and quality of life.

ASSESSMENTS AND VARIABLES

Background characteristics

Baseline characteristics were available from the original study[43-45]. Data on total open-label morphine intravenous infusion in the first 7 and 28 days, and other cumulative analgesic/ sedatives/ anti-epileptics given in the first 28 days were retrieved from the medical charts of all participants and nonparticipants of the follow-up study. Total hospitalization, including the NICU stay or admission to other hospitals, was obtained from the referral centers.

The Growth Analyzer, version 3.5 (Dutch Growth Foundation, Rotterdam, The Netherlands) served to evaluate height to age and weight to height, expressed in z scores, on the basis of Dutch and Turkish reference values.

Parents completed a questionnaire asking about profession and education, predominantly spoken language at home, and details about the child's surgeries in the past 5 years, including opioids use before, during, or after surgery. Surgeries were classified as minor or major based on opioids use or clinical indications.

Family socioeconomic status (SES) was derived from the highest occupational or educational level of either one of the parents, using a computer program, Occupation Classification[46]. The score could range from 1 to 9 and was reduced to 3 levels: scores 1 to 3 corresponding with low SES, 4 and 5 with middle SES, and 6 to 9 with high SES.

Intelligence

Intelligence was measured with the short version of the Revision Amsterdam Child Intelligence Test (RAKIT) [8]. This test produces a mean intelligence quotient (IQ) score of 100 and standard deviation (SD) of 15, based on a Dutch norm group. This short version consists of 6 subtests, to measure reasoning, passive verbal learning, spatial orientation, active learning, visual analysis, and verbal fluency[39].The full version of the RAKIT correlates well with the Wechsler Intelligence Scale for Children – Revised ($r = 0.86$)[9].

Visual motor integration

Visual motor integration was assessed with the Beery-Buktenica Developmental Test of Visual-Motor Integration (Beery VMI)[6]. This test helps to identify difficulties that children may have with integrating, or coordinating, their visual-perceptual and motor (finger and hand movement) abilities. Children are asked to copy geometric figures in order of increased difficulty. The Beery VMI produces a mean score of 100 and SD of 15.

Behavior

Parents completed the Dutch-language version of the Child Behavior Checklist 1½-5 years (CBCL)[2]. Three scales are produced: the Total Problem Scale (100 items) and subscales Internalizing and Externalizing problems. For the 3 scales, alike T scores of 60 or higher are considered to be consistent with a problem in the subclinical or clinical range[2]. Teachers completed the Dutch-language version of the Teacher Report Form for ages 1½-5 years. This tool evaluates the child's behavior in the school setting. Structure and scoring system are similar to those of the CBCL[2].

Chronic pain

Prevalence of chronic pain was assessed from the Dutch Chronic Pain Questionnaire[32], completed by the parents. The questionnaire defines chronic pain as pain with duration longer than 3 months.

Health-related quality of life

Parents completed the Dutch-language version of the 15-item Health Utility Index (HUI-15)[12] to evaluate their child's health-related quality of life. HUI-15 scores were transformed to the Health Utility Index 3 (HUI3), which consists of 8 attributes of health status: vision, hearing, speech, ambulation, dexterity, emotion, cognition, and pain. Each attribute comprises 5 or 6 levels, varying from highly impaired to normal[11,13]. Using the given formula of the HUI3, the theoretically possible score ranges from 0.0 (dead) to 1.0 (perfect health). Scores between 0.89 and 0.99 reflect mild disability, scores between 0.70 and 0.88 reflect moderate disability, and scores <0.70 reflect severe disability[14].

Statistical analyses

Background characteristics and continuous outcome variables were compared with Student t test for continuous and normally distributed data, displayed as means and standard deviation. The Mann-Whitney test was used in case of skewed continuous data distribution; the medians and interquartile range (IQR) were given. The Pearson chi-square test or Fisher's exact test were used to evaluate categorical data.

To take relevant neonatal and other background characteristics into account, multiple linear regression analyses were performed for the normally distributed outcome variables. A large number of neonatal and background characteristics described in the literature were combined into a propensity score to correct for baseline imbalance[55]. This score was derived from logistic regression analysis using a forced-entry model. In this analysis, the treatment condition (morphine or placebo) was the criterion variable; the co-variables were: center, sex, gestational age (days), Clinical Risk Index for Babies [1], medium SES and high SES, with low SES as the reference variable.

Birth weight was compared to national standards and expressed in z scores, depending on sex and age and corrected for prematurity.

We performed multiple regression analyses, with and without the propensity score to adjust for the relevant background variables and with the amount of open-label morphine in the first 28 days after birth and treatment condition as explanatory variables. Because primary ventilation is known to be associated with morphine treatment, we will perform analyses with and without this explanatory variable. To determine if outliers influenced the regression model, the Cook's distances were calculated. Multicollinearity was tested with the Variance Inflation Factor, which should not exceed 10 for any of the variables.

The critical level of significance was set at 0.05 (2-sided) for all analyses. Data were analyzed using the statistical software package SPSS 15.0 for Windows (SPSS Inc, Chicago, IL, USA).

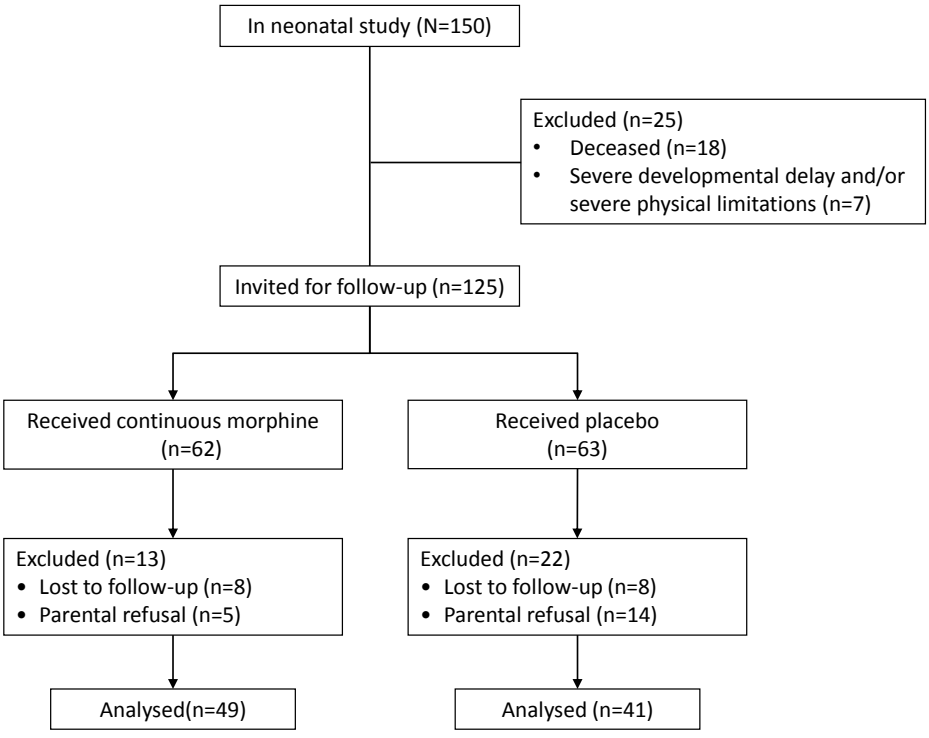


Figure 1. Flow chart of participation

RESULTS

Background characteristics

Parents of 90 of the 125 eligible children (72%) consented to participate. Forty-nine children had earlier received continuous morphine and 41 children received placebo (Figure 1).

Parents of 16 children (13%) could not be traced and parents of 19 children (15%) refused participation. Neonatal characteristics of the nonparticipants did not differ from those of the participating children (Table 1).

Neonatal characteristics of the children participating in the follow-up evaluation did not differ between the morphine and placebo groups (Table 2). At the age of 5 years, children who had received morphine were significantly shorter (height for age) than those who had received placebo (P = 0.02). One part of the explanation could be that the correlation of z-score height with small for gestational age (SGA) at birth was 0.49, and the incidence of SGA was 26.5% in the morphine group and 12.2% in the placebo group. When the SGA children were excluded, the difference between the morphine and placebo group was no longer significant.

Six children in each group had undergone minor surgery after the neonatal period (Table 3). One child in the morphine group had undergone major surgery, that is, bowel resection, resulting in short bowel syndrome.

Intelligence

IQ scores were evaluated for 89 children, as one test result was unreliable. Ten of the 48 children (20.8%) in the morphine group had an IQ score of 1 SD below average (IQ score <85), versus 5 of 41 (12.2%) in the placebo group (odds ratio 1.90, 95% confidence interval [CI] .59–6.08). Univariate analyses showed that IQ significantly differed between groups, that is, mean 94.0 (SD 14.5) for the morphine group versus mean 99.8 (SD 12.9) for the placebo group (P = 0.049) (Table 4). Table 5 gives 3 multiple regression analyses with IQ as outcome variable. Model 1 includes treatment group and open-label morphine consumption in the .rst 28 days of life as predictor variables with a statistically significant worse outcome for the morphine group (P = 0.046), along with a trend towards significance for the open label morphine (P = 0.058) (Table 5). When the analysis was adjusted for the propensity score, treatment group and open label morphine consumption in the first 28 days were no longer significant. The further addition of primary ventilation as explanatory variable in model 3 reversed the sign of the regression coefficient of open label morphine and showed a trend for longer primary ventilation predicting IQ (P = 0.073). The 95% CI Variance Inflation Factors remained below 2.5.

Table 1. Demographic and Clinical Characteristics in Neonatal period from participating children versus non-participating children (n=125)

		Participating children n=90	Non-participating children n=35	P
Center, n(%)	Rotterdam	54 (60.0)	23 (65.7)	.56
	Zwolle	36 (40.0)	12 (34.3)	
Sex, n(%)	Male	51 (56.7)	19 (54.3)	.81
	Female	39 (43.3)	16 (45.7)	
Gestational age (weeks)	Median (IQR)	30.0 (27.5 to 31.6)	29.1 (28.1 to 31.2)	.62
Birth weight (grams)	Median (IQR)	1313 (975 to 1765)	1300 (1050 to 1560)	.84
Duration primary mechanical ventilation (hrs)	Median (IQR)	71 (29 to 159)	62 (25 to 89)	.23
Duration total first hospital admission (days)	Median (IQR)	58 (34 to 79)	61 (36 to 84)	.37
CRIB	Median (IQR)	3.0 (1.0 to 5.0)	2.0 (1.0 to 5.0)	.83
IVH, n(%)	No	62 (68.9)	25 (71.4)	
	Mild	25 (27.8)	9 (25.7)	.96
	Severe	3 (3.3)	1 (2.9)	
PVL, n(%)	All grades	2 (2.2)	-	-
Number of painful procedures first 14 days	Median (IQR)	124 (73 to 219) [n=82]	117 (69 to 196) [n=30]	.26
Cumulative dose of open-label morphine (mcg/kg) administration 1st 28 days	Median (IQR)	106 (15 to 446)	109 (0 to 324)	.84
Cumulative dose other analgesics/ sedatives/ anti-epileptic (mcg/kg) administration 1st 28 days	Fentanyl n (%) Median (IQR)	 5 (5.6) 6 (5 to 34)	 4 (11.4) 16 (11 to 261)	-
	Midazolam n (%) Median (IQR)	 3 (3.3) 130 (-)	 3 (8.6) 47 (-)	-
	Phenobarbital n (%) Median (IQR)	 3 (3.3) 28347 (20179 to 40404)	 3 (8.6) 28846 (20790 to 45145)	-
	Nalaxone n (%) Median (IQR)	 7 (7.8) 14 (10 to 20)	 - 	-

IQR, interquartile range; CRIB, Clinical Risk Index for Babies; IVH, intraventricular hemorrhage; PVL, periventricular leucomalacia. Data are expressed for sample size; participant, n=90; participants, n=35, unless otherwise indicated between brackets

Table 2. Neonatal demographic and Clinical Characteristics from children who participated in the follow-up; morphine versus placebo group (N=90)

		Morphine group n=49	Placebo group n=41	P
Center, n(%)	Rotterdam	32 (65.3)	22 (53.7)	.26
	Zwolle	17 (34.7)	19 (46.3)	
Sex, n(%)	Male	28 (57.1)	23 (56.1)	.92
	Female	21 (42.9)	18 (43.9)	
Gestational age (weeks), n (%)	<27	3 (6.1)	4 (9.8)	
	27-29.9	21 (42.9)	14 (34.1)	
	30-33.9	16 (32.7)	18 (43.9)	.71
	34-36.9	3 (6.1)	2 (4.9)	
	>37	6 (12.2)	3 (7.3)	
Birth weight (z-score)	Median (IQR)	-.68 (-2.36 to .37)	-.30 (-1.25 to .44)	.36
Duration primary mechanical ventilation (hrs)	Median (IQR)	87 (35 to 172)	66 (26 to 153)	.60
Duration total hospital admission (days)	Median (IQR)	58 (33 to 80)	58 (33 to 76)	.84
CRIB	Median (IQR)	3 (1 to 6)	3 (1 to 5)	.60
IVH, n(%)	No	38 (77.6)	24 (58.5)	
	Mild	10 (20.4)	15 (36.6)	.15
	Severe	1 (2.0)	2 (4.9)	
PVL, n(%)	All grades	2 (4.1)	-	-
Number of painful procedures first 14 days	Median (IQR)	139 (91 to 221) [n=43]	106 (67 to 218) [n=39]	.23
Cumulative dose of open-label morphine (mcg/kg) administration 1st 28 days	Median (IQR)	114 (20 to 334)	104 (0 to 667)	1.0
Cumulative dose other analgesics/ sedatives/ anti-epileptic (mcg/kg) administration 1st 28 days	Fentanyl n (%) Median (IQR)	 5 (10.2) 10 (5 to 190)	 4 (9.6) 16 (7 28)	-
	Midazolam n (%) Median (IQR)	 4 (8.2) 159 (107 – 3889)	 2 (4.9) 37 (-)	-
	Phenobarbital n (%) Median (IQR)	 2 (4.1) 30597 (20790 to 40404)	 4 (9.6) 28596 (22221 to 41070)	-
	Naloxone n (%) Median (IQR)	 3 (6.1) 14 (13 to 20)	 4 (9.6) 13 (9 to 68)	-

Abbreviations: IQR, interquartile range; CRIB Clinical Risk Index for Babies; IVH, intraventricular hemorrhage; PVL, periventricular leucomalacia. Data are expressed for sample size; Morphine group, n=49; Placebo group, n=41, unless otherwise indicated between brackets.

Table 3. Demographic and clinical characteristics at age 5 from children who participated in the follow-up; morphine group versus placebo group (N=90)

		Morphine group n=49	Placebo group n=41	P
SES, n(%)	Low	13 (26.5)	7 (17.1)	.50
	Medium	16 (32.7)	17 (41.5)	
	High	20 (40.8)	17 (41.5)	
Language, N (%)	Dutch	40 (81.6)	38 (92.7)	.21
	Other	9 (18.4)	3 (7.3)	
		[n=42]	[n=36]	
Surgery, n(%)	None	35 (83.3)	30 (83.3)	.63
	Minor surgery	6 (14.3)	6 (16.7)	
	Short Bowel	1 (2.4)	-	
Age of assessment (months)	Median (IQR)	63 (62 to 65)	63 (63 to 64)	.70
Height for age (z-score)	Mean (sd)	-.75 (1.35) [n=41]	-.11 (.95) [n=33]	**.02**
Weight for height (z-score)	Mean (sd)	-.54 (1.20) [n=41]	-.55 (.95) [n=33]	.96

Data are expressed for sample size; morphine group, n=49; placebo group, n=41 unless otherwise indicated between brackets. Details on 'Surgery' were provided by 78 parents, twelve parents did not document these details. Due to practical limitations in the outpatient clinic and two home visits, weight and length was not measured in sixteen children.

Evaluation of Cook's distances revealed no outliers among the cases. Entering all co-variables in the model separately gave the same results as the analyses corrected for the propensity score. Only SES contributed significantly to the prediction of IQ.

Regression analysis on separate subtests of the intelligence test, again adjusted for the propensity score, revealed a significant negative effect of the morphine condition (P = 0.021) and a significant negative effect of open-label morphine consumption (P = 0.029) on the "visual analysis" RAKIT subtest performance.

Visual motor integration

Fifteen of the 49 children (30.6%) in the morphine group had a Beery VMI score of <1 SD below average (VMI score <85) versus 5 of the 41 children (12.2%) in the placebo group (odds ratio 3.18, 95% CI 1.04–9.70). The mean VMI score for the morphine group tended to be lower than that for the placebo group, that is, 93.7 (SD 12.3) versus 98.0 (SD 12.7) (P = 0.104) (Table 4).

Behavior

As 6 of the 90 parents did not complete the Child Behavior Checklist, analysis included 84 children. Proportions of children in the (sub)clinical range varied from 10.3% (for externalizing problems in the placebo group) to 15.6% (for internalizing problems in the morphine group). These proportions are comparable to those found for normal

Table 4. Outcome variables for the morphine group and placebo group (N=90)

			Morphine Group n=49	Placebo Group n=41	P
IQ		Mean (SD)	94.0 (14.5) [n=48]	99.8 (12.9) [n=41]	**.049**
Visual Motor Integration		Mean (SD)	93.7 (12.3)	98.0 (12.7)	.104
Chronic Pain, n (%)		Yes	6 (14.6) [n=41]	5 (13.9) [n=36]	.93
		Daily to once a week	2 (4.9)	1 (2.8)	
		2 to 3 times per months	3 (7.3)	2 (5.6)	
		Once a month or less	1 (2.4)	2 (5.6)	
Heath Utility Index			[n=38]	[n=35]	
		Median (IQR)	1.0 (.9 to 1.0)	1.0 (.9 to 1.0)	.66
		No disability	24 (63.2)	23 (65.7)	
		Mild disability	7 (18.4)	7 (20.0)	.95
		Moderate disability	5 (13.2)	4 (11.4)	
		Severe disability	2 (5.3)	1 (2.9)	
Child Behavior Checklist			[n=45]	[n=39]	
Internalizing problems	T-score	Mean (sd)	47.1 (11.9)	46.5 (11.0)	.81
(Sub)clinical range		n (%)	7 (15.6)	5 (12.8)	.72
Externalizing problems	T-score	Mean (sd)	46.7 (10.2)	46.9 (9.6)	.93
(Sub)clinical range		n (%)	5 (11.1)	4 (10.3)	.90
Total problems	T-score	Mean (sd)	46.6 (11.1)	45.8 (9.9)	.72
(Sub)clinical range		n (%)	5 (11.1)	5 (12.8)	.81
Teacher Report Form			[n=44]	[n=37]	
Internalizing problems	T-score	Mean (sd)	50.1 (10.3)	48.8 (10.7)	.59
(Sub)clinical range		n (%)	10 (22.7)	6 (16.2)	.46
Externalizing problems	T-score	Mean (sd)	52.6 (7.9)	49.0 (9.7)	.07
(Sub)clinical range		n (%)	7 (15.9)	4 (10.8)	.51
Total problems	T-score	Mean (sd)	52.1 (8.8)	48.7 (11.7)	.15
(Sub)clinical range		n (%)	9 (20.5)	7 (18.9)	.86

IQ intelligence quotient; IQR, interquartile range.

American children. The CBCL total score and subscale scores did not differ between the morphine and placebo groups (Table 4).

Teachers completed the Teacher Report Form for 81 of the 90 children. Proportions of children in the (sub)clinical range varied from 10.8% for externalizing problems in the placebo group to 22.7% for internalizing problems in the morphine group (Table 4). There were no significant differences between the 2 groups.

Table 5. Results of Multiple regression analyses of IQ at 5 years (n=89)

	Model 1			Model 2 Adjusted for the propensity score			Model 3 Adjusted for the propensity score		
	B	**95% CI**	***P***	**B**	**95% CI**	***P***	**B**	**95% CI**	***P***
Constant	101.2	96.8 to 105.7	<0.001	101.6	97.4 to 105.8	<0.001	103.2	98.2 to 105.7	<0.001
Treatment group	-5.8	-11.6 to -0.1	**0.046**	-3.95	-9.4 to 1.5.1	0.155	-3.73	-9.1 to 1.7	0.174
Open label morphine	-0.003	-0.01 to 0.001	0.058	-0.002	-0.006 to 0.001	0.141	0.001	-0.004 to 0.005	0.77
Primary ventilation							-0.028	-0.058 to 0.003	0.073
R^2	0.083			0.039			0.069		

IQ intelligence quotient; CI, confidence interval. B values are unstandardized regression coefficients. R^2 excluding the variance explained by the propensity score in model 2 and 3

* propensity score including: center, sex, gestational age (days), birth weight (SDS), Clinical Risk Index for Babies (CRIB), socioeconomic status

Chronic pain

Parents reported chronic pain for 6/41 (14.6%) and 5/36 (13.9%) children in the morphine and placebo groups, respectively (Table 4). These 11 children with chronic pain had 1–4 different pain locations, including the abdomen (n = 5), limbs (n = 5), head (n = 2), throat (n = 2), back (n = 1), neck (n = 1), ear (n = 2), and chest (n = 1). Pain frequency varied between daily and once a month. Information on number of painful procedures in the first 14 days of life was available for 72 children. This number did not significantly (P = 0.57) differ between children with chronic pain now (n = 10) and those without (n = 62), with medians (IQR) of 91 (69–181) versus 168 (79–227), respectively.

Health-related quality of life

Parents' scores on the Health Utility Index, completed for 73 children, did not differ between the 2 groups (median 1.0, IQR 0.9-1.0 for each group) (Table 4). The 3 children for whom the HUI score reflected severe disability (2 in the morphine group and 1 in the placebo group) showed mild pain restricting daily activities and were reported to feel irritated/angry/agitated, or unhappy, not well understood by others, and to have difficulty with problem-solving, eyesight, and memory.

DISCUSSION

Follow-up at 5 years showed no significant differences in intelligence, visual-motor integration, behavior, chronic pain, or health-related quality of life between children who as neonates had received either morphine or placebo. However, there was a trend

towards a more negative outcome in subjects who received more morphine during the neonatal period. We cannot rule out that neonatal morphine consumption does clinically significant harm, as the 95% CI for treatment condition included a lower boundary of 9.4 IQ points.

Also, worse performance on "visual analysis," a subtest of the IQ test, was statistically significantly related to neonatal morphine consumption even after adjustment for baseline characteristics with the propensity score. The one comparable previous study did not find adverse effects of neonatal morphine on the intelligence, motor function, and behavior of 5-to 6-year-old children[28]. This study differed from the present study with regard to the 5-to 10-fold higher morphine dosage, the induction of neuromuscular paralysis, and lack of an observational and validated pain assessment instrument[35,36]. A limitation of the MacGregor[28] study was that only overall IQ, overall motor ability, and overall behavior were analyzed. The results of the present study show that it is extremely important to investigate effects of morphine on specific sub-domains of neurodevelopment. Children born preterm show relative vulnerability of visual processing and visual memory[38] that, in turn, contributes to their academic difficulties in mathematics[18,50]. Even when overall performance is good, adults born very preterm activate different neural networks to process visuospatial material[31]. If, based on the results of the present study, morphine adversely, selectively, further adds to effects on visual processing, this may have important long-term sequelae. For instance, children in the morphine group of our study obtained lower scores on "visual analysis," a subtest of the intelligence test, than the children in the placebo group. This test requires inhibitory skills, which is one of the domains of higher-order neurocognitive functions, the so-called executive functions. As skills like these are still developing at the age of 5 years, follow-up at later age is called for and may show more robust differences.

The multivariate regression analyses showed that only socioeconomic status was a significant predictor of IQ, consistent with our earlier findings[52]. Surprisingly, gestational age did not predict IQ at age 5 years, even though the study population consisted of children born at gestational ages from 25 to 40 weeks. A possible explanation could be that also, the term-born children were critically ill and needed mechanical ventilation, which may have moderated the effects of preterm birth on cognitive outcomes.

We found that duration of primary ventilation was a relevant contributing factor, although the subjects were randomized and duration of ventilation did not differ significantly between the 2 groups. We evaluated morphine consumption during the first 28 days of life, while the original RCT took place in the first 7 days of life.

Apart from the psychological assessments, chronic pain and health-related quality of life were other focal points of this study. We found an incidence of chronic pain comparable to the 19.3% incidence in healthy 4-to 7-year-old children in the Netherlands[32]. Also, the number of painful procedures in the neonatal period in this study did not seem to influence the development of chronic pain later in life, consistent with previous studies[40,51]. In addition, continuous morphine therapy in the NICU did not appear to mitigate or accentuate the risk of developing chronic pain in preschool-age children.

A surprising result was the difference in height between the 2 groups. One part of the explanation could be that the correlation of z-score height with SGA at birth was 0.49, and the incidence of SGA was 26.5% in the morphine group and 12.2% in the placebo group. When the SGA children are excluded, the difference between the morphine and placebo group is no longer significant.

A limitation of this study is the relatively small sample size, which, however, inevitably resulted from death of the children in the original sample, loss to follow-up, and parental refusal to participate. Another limitation is the potential for selection bias due to the exclusion of 7 children with severe mental or physical disabilities. Although they were equally divided over the 2 treatment groups, their exclusion may have influenced the outcomes. Third, considering that pain during the neonatal period has been associated with decreased thermal sensitivity later in life[23,42,51],it would have been interesting to investigate the relation between pain sensitivity and chronic pain conditions. Assessment of pain sensitivity was not feasible, however, because participants were too young. QST is feasible from the age of 6–7 years[24,30]. Follow-up at older ages should include QST as an objective method to determine pain sensitivity. Fourth, another limitation was that we did not assess gross motor development with, for instance, the Movement Assessment Battery for Children. Children born pre-term are at increased risk of motor impairment[53]. In addition, it would have been interesting because a short-term follow-up at 36 weeks postconceptional age of the NEOPAIN study revealed a significant association between morphine and poplietal angle (a subtest of the Neurobehavioral Assessment of the Preterm Infant)[37]. Furthermore, Grunau et al[21] found that larger total morphine dose correlated with poorer motor development at 8 months, but not at 18 months. However, because our tests took the larger part of a day, we had to make a selection of tests because otherwise, the 5-year-old subjects would not have been able to complete all tests in a reliable manner.

The results of the present study suggest that morphine does not have major long-term effects at the low doses used. However, the differences on visual analysis of the

intelligence test warrant follow-up at later age. The long-term consequences of larger morphine doses during the neonatal period would be highly relevant to study as well.

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Chapter 3

Does neonatal morphine use affect neuropsychological outcomes at 8 to 9 years of age?

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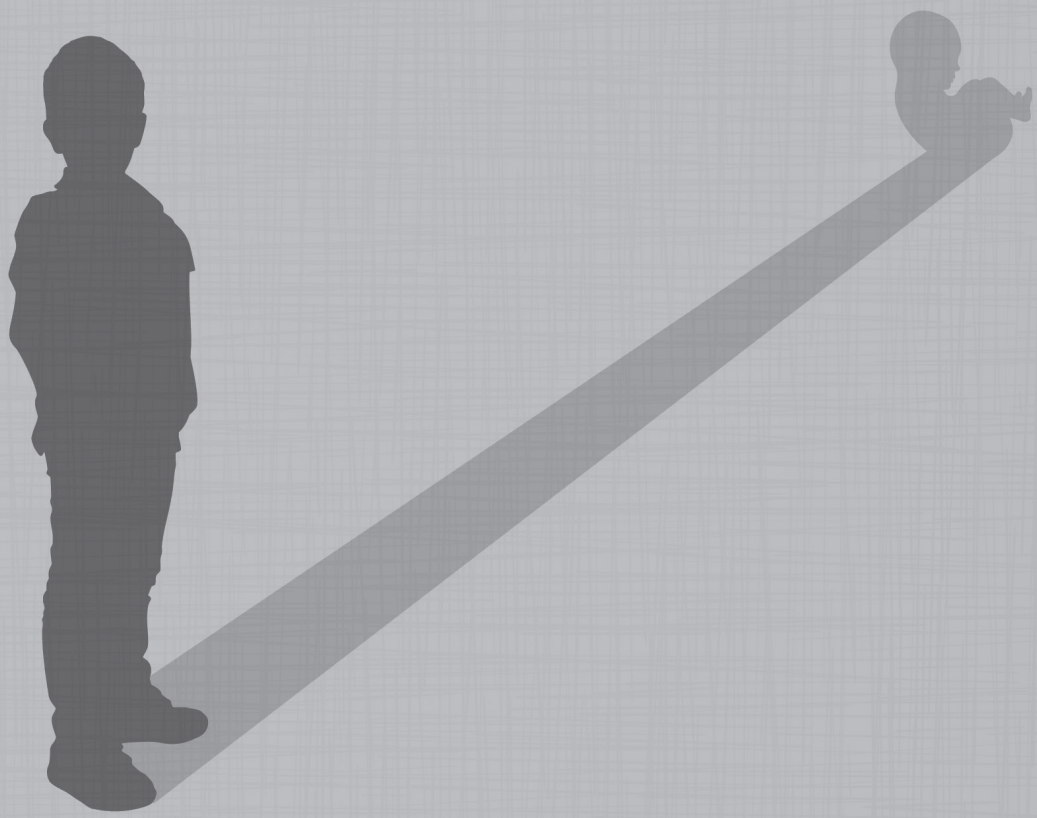

ABSTRACT

Morphine is widely used to treat severe pain in neonatal intensive care unit patients. Animal studies suggest adverse long-term side effects of neonatal morphine, but a follow-up study of 5-year-old children who participated in a morphine-placebo controlled trial as newborns found no such effects on the child's general functioning. This study indicated that morphine may negatively affect response inhibition, a domain of executive functions. Therefore, we performed a second follow-up study in the same population at the age of 8 to 9 years, focused on the child's general functioning in terms of intelligence, visual motor integration, and behavior and on executive functions. Children in the morphine group showed significantly less externalizing problems according to the parents but more internalizing behavior according to the teachers, but only after adjustment for intelligence quotient (IQ), potential confounders using a propensity score, and additional open-label morphine. Morphine-treated children showed significantly fewer problems with executive functions in daily life as rated by parents for the subscales inhibition and organization of materials and for planning/organizing as rated by the teachers. After adjustment for IQ and the propensity score, executive functioning as rated by the parents remained statistically significantly better in the morphine-treated group. The influence of the additional morphine given was not of a significant influence for any of the outcome variables. Overall, the present study demonstrates that continuous morphine infusion of 10 lg/kg/h during the neonatal period does not harm general functioning and may even have a positive influence on executive functions at 8 to 9 years.

INTRODUCTION

Morphine analgesia is widely used in neonatal intensive care, particularly to provide analgesia following major surgery[13,32,68]. Animal studies have shown, however, that neonatal morphine administration increased hypersensitivity[55], impaired learning[49] and altered the neurochemical profile and decreases cell division within the hippocampus – which may explain neurobehavioral deficits in adulthood[21]. Moreover, a number of studies suggested increased neuro-apoptosis in rodents treated with morphine, affecting both neurons and microglia[7,36]. In humans, possible associations were found between greater morphine exposure and poorer motor development at 8 months, but this could not be confirmed at 18 months of age[30]. Causality between neonatal morphine intake and long-term effects is difficult to evaluate because critical illness and prematurity play a major role in determining neurodevelopmental outcomes[56]. Follow-up of randomized controlled trials (RCT) helps to evaluate the effect of morphine on long term outcomes. More than a decade ago, the first follow-up study[46] of two RCTs[53,54] in preterm infants on morphine, pancuronium, and placebo found no effect of neonatal morphine use on intelligence, visual motor integration or behavior 5-6 years later – . In a small pilot follow-up in 5-7 year-old children who had been mechanically ventilated after birth, those who had received morphine (n=14) had comparable IQ scores and academic achievements to those who had received placebo (n=5). However, the morphine treated children had significantly longer choice response latencies and more social problems[24]. In another follow-up study (n=90), we found no significant effects of neonatal morphine use on children's intelligence, visual motor integration, behavior, chronic pain, and health-related quality of life at 5 years of age[20]. We did find a small but significant negative effect of morphine use on one subtest of the intelligence test, which requires response inhibition[20].

Response inhibition is a component of the executive functions, mediated by the prefrontal brain areas and involve those cognitive processes that are necessary for goal-oriented, adaptive behavior, which includes the capacity to think ahead, to suppress impulses and to hold information in mind[37]. These processes are important mechanisms underlying academic and behavioral skills[3,8,16]. Preterm born children often develop problems with executive functions[2,45]. In our 5-year follow-up study, however, we did not include measures of executive functions because we considered children of that age too young for these tests. The rationale for the current study therefore was to examine these functions at the age of 8 or 9 years, when more complex testing is possible.

Therefore, we set up a second follow-up study of our initial RCT, testing children at the age of 8 or 9 years. Two questions were addressed: (1) does neonatal morphine use affect general functioning at the age of 8/9 years in terms of intelligence, visual motor

integration and behavior in terms of internalizing and externalizing problems; and (2) does neonatal morphine use affect executive functions at this age?

METHOD

Participants

Participants were recruited from a RCT performed in two level III neonatal intensive care units (NICUs) in the Netherlands in 2000-2002[61-63]. In this RCT, 150 ventilated children younger than 3 days were randomly allocated to receive continuous morphine (n=73; loading dose of 100µg/kg + continuous infusion of 10µg/kg/h) or placebo (n=77). All children received additional open-label morphine (loading dose of 50µg/kg + continuous infusion of 5-10µg/kg/h) when they were judged to be in pain. Eighteen of the 150 children of the RCT died, all in the first year of life. At the age of 5 years the children were invited to a follow-up study of whom 90 participated (continuous morphine n=49; placebo n=41)[20].

For the present follow-up study all 132 survivors, including children with severe disabilities (n=7) and children who did not participate in the 5-year follow-up (n=42), were found eligible.

Procedure

Children were assessed within the framework of a larger study, designed to examine the long-term effects of morphine on neuropsychological development (present study) and pain sensitivity evaluated with quantitative sensory testing. Between November 2009 and September 2011, children were seen at the outpatient clinic of either hospital or at home if the parents preferred. The total assessment took 4 hours. Written informed consent was obtained from all parents. Parents and, when the parents consented, the child's teacher completed several questionnaires about background characteristics, behavior and executive functions of the child. The study was approved by the medical ethics review boards at Erasmus MC-Sophia Children's Hospital (Rotterdam) and the Isala Clinics (Zwolle).

Assessments

Background variables

The original RCT[61-63] and the 5-year follow-up[20] provided baseline characteristics, including additional open-label morphine received in the first 28 days. A general physical examination, including screening on minor neurological dysfunctions was performed by experienced pediatricians. Socio-economic status (SES; low, middle and high) of parents

was determined with the use of the Occupation Classification software, which takes into account the highest level of occupation and education level of either parent[66]. Parents provided updated details about the child's health status, special services at school (extra support from teacher, remedial teacher or speech therapist) and grade repetition. When parents consented, teachers provided details of the child's academic achievements with the Dutch National Pupil Monitoring System[3,19], used by up to 95% of Dutch mainstream primary schools. This system monitors a child's development in relation to both individual and peer development at given times during a school year. The following 4 tests are used; Spelling test (writing down verbally presented words), Reading Comprehension Test (multiple-choice questions about text), The Three Minute Test (reading of complex words) and Mathematics test (general knowledge of mathematics and arithmetic).

Intelligence

A short version of the Wechsler Intelligence Scale for Children-III[70] was administered. The subtests Vocabulary, Similarities, Block Design and Picture Completion served to estimate the full-scale intelligence-quotient (IQ). Subtest scores were converted to a composite score that was used to calculate an estimated full-scale IQ[58]. The estimated full-scale IQ correlates highly (>0.9) with full-scale IQ[58]. IQ test norms show a mean score of 100 with a SD of 15

Visual Motor Integration

Visual motor integration was assessed with the Beery-Buktenica Developmental Test of Visual Motor Integration (Beery VMI)[9]. Children are asked to copy geometric figures in order of increased difficulty. The Beery VMI test norms show a mean score of 100 and SD of 15.

Behavior

Parents and teachers completed the Dutch-language version of the Child Behavior Checklist 6-18 years (CBCL) and Teacher Report Form (TRF) 6-18 years[4]. Scores on three scales are calculated: The Total Problem Scale (n=120 problem behavior items) and subscales for Internalizing and Externalizing problems. T-scores of 60 (84th percentile) or higher are considered to indicate a problem in the subclinical or clinical range.

Executive function skills

Executive functions were tested with six subtests of the Cambridge Neuropsychological Test Automated Battery (CANTAB)[1]. All tests of the CANTAB are computerized and were presented on a touch screen.

Reaction time 1- and 5-choice (RTI-1 and RTI-5)
This test measures speed of response and movement in 1- and 5-choice paradigms measured in milliseconds (ms)[1].

Spatial span forward and backward (SSP-f and SSP-b)
The test measures spatial short-term (working) memory capacity. A pattern of boxes that change color is presented on the screen. First, the child has to touch the boxes in the same order as they were originally presented (SSP-f). Second, the child has to touch each of the boxes in the reverse order (SSP-b). Measurement reports the longest sequence successfully recalled by the child[1].

Rapid Visual Information Processing (RVP)
Digits from 2 to 9 rapidly appear in the centre of the computer screen. To assess the processing of visual information, the child is requested to detect target sequences of the numbers 3-5-7. From this test the 'RVP A'[1] measures how good the participant is at detecting target sequences using the probability of 'hit' (responding correctly) and the probability of false alarm (responding inappropriately), within a range of 0.00 (bad) to 1.00 (good)[1].

Stockings of Cambridge (SOC)
To assess spatial planning and spatial working memory, the child is shown two displays containing three colored balls. The child has to copy a pattern given by the computer. The number of occasions upon which the subject has successfully completed a test problem in the minimum possible number of moves is reported[1].

Intra/Extradimensional set shifting (IED)
The IED subtest assesses the ability to acquire rules and adjust to set shifting. The task begins with a simple visual-discrimination task and then gradually increases in complexity. Subjects progress through the test by reaching a set criterion of learning at each stage by 6 consecutive correct responses. If at any stage the subject fails to reach this criterion after 50 trials, the test terminates. The following measures were derived from this test: number of stages completed (1–9) and total adjusted trials (the adjustment adds 50 trials for each stage not attempted due to failure)[1].

Stop Signal Test (SST)
The SST measures an individual's ability to inhibit a response. The child is requested to press the left button when they see a left-pointing arrow and the right hand button when they see a right-pointing arrow. If they hear 'a beep' they should withhold their response. Outcome measures were: stop signal delay (SSD), at which the participant was able to stop 50% of the time, and the stop-signal reaction time (SSRT), which is an

estimate of the length of time between the go-stimulus and the stop-stimulus at which the participant is able to successfully inhibit their response on 50% of the trials. The SSD and SSRT are calculated using the last half of the assessed sub-blocks in the test[1].

Executive function behavior in daily life

The Dutch version[37,64] of the BRIEF (Behavior Rating Inventory of Executive Functioning) questionnaire[25] was completed by parents and teachers. The questionnaire requests information about executive functions in daily life and contains 86 items constituting two major index scales: behavioral regulation index and metacognition index, and the Global Executive Composite. The behavioral regulation index consists of three subscales: inhibition (ability to suppress impulses), shifting (ability to adjust behavior flexibly to changing demands) and emotional control (the capacity to modulate emotional responses)[37]. The metacognition index is composed of five subscales: initiation (initiation of tasks or activities and independent generation of ideas), working memory (hold information in mind), planning/organization (capacity to manage current and future-oriented task demands), organization of materials (orderliness of work, play, and storage spaces), and monitoring (ability to check work and performance). T-scores of 65 and higher (≥1 ½ standard deviation) and percentile scores of 91 and higher indicate poor executive functions[64].

Statistical analyses

Background characteristics and outcome variables were compared between the two groups using the Students t test or the Mann-Whitney test dependent of distribution of normality. The Fisher exact 2-sided test was used to evaluate categorical data.

We adjusted for the additional open-label morphine and potential confounders using IQ, a propensity score[20,72] consisting of: center (Zwolle/ Rotterdam), sex, primary ventilation (hours), gestational age (days), Clinical Risk Index for Babies (CRIB) and Socioeconomic Status (middle and high versus low). Because assumptions of normal distribution and equal variances for the outcome variables were violated and possibly affected by outlying observations, we used the method of robust regression analysis (SAS 9.2 for Windows). Robust regression is a method to analyse non-normal data that include (influential) outliers. An outlier is an observation whose outcome variable value is extreme. An observation with an extreme value of a predictor variable (e.g. some subjects in the neonatal RCT received a high dose of morphine across the first 28 days of life) is a point with high leverage. Leverage is a measure of how far an independent variable deviates from its mean. An outlier is influential when an observation has both a high leverage and an extreme value on the outcome variable. An observation is said to be influential if removing the observation substantially modifies the estimate of the regres-

sion coefficients. The output of robust regression analyses summarizes which and how many observations are of high leverage and/or are an outlier. It is undesirable to have influential observations, therefore, their influences should be down weighted; we have used bi-square weighing function. Robust regression is performed by the procedure of iteratively re-weighted least squares (IWLS). As estimation method we have used the Multiple Model (MM)-estimator, which both minimizes the variance of the estimator and downweighs observations with large residuals by using the least trimmed squares estimator[18,73].

To evaluate differences in IQ, CBCL and TRF levels between 5 and 8 years we applied a repeated ANOVA with condition (morphine vs placebo) and the interaction between time x condition.

The critical level of significance was set at 0.05 (two-tailed) for all analyses. Data were analyzed using the statistical software packages SPSS 17.0 and SAS 9.2 for Windows.

RESULTS

Background characteristics

Parents of ten of the 132 eligible children (8%) refused participation and 33 (25%) could not be traced. Thus, parents of 89 children (67%) consented to participate. Two children were seen at a home visit. Parents of nine children only completed questionnaires. Three of these nine children had severe physical and cognitive disabilities making testing impossible, also CBCL and BRIEF were not filled in by the parents. Parents of the other six children only consented to complete questionnaires and refused consent for testing. In total, 80 children were tested. Seventy-four of these children were also participants in the 5-year follow up study[20].

The non-participants had a lower birth weight and their first hospital stay was longer compared to the participants. Of all 89 participants, 43 had received morphine in the original RCT and 46 received placebo (Figure 1).

Neonatal background characteristics did not differ between the morphine and the placebo group (Table 1). Total morphine exposure in the first 28 days of life was statistically significantly higher in the morphine group ($p<0.001$). Twelve children (26.1%) in the placebo group did not receive any opioids. Also, background characteristics and minor neurological dysfunctions at the age of 8/9 years did not differ (Table 2). Fifty children attended mainstream education without any additional support, and proportions of

Table 1. Neonatal background characteristics from children who participated in the follow-up; morphine cohort versus placebo cohort (N=89)

		Morphine cohort n=43	**Placebo cohort n=46**	**P**
Center, n(%)	Rotterdam	25 (58.1)	25 (54.3)	.83
	Zwolle	18 (41.9)	21 (45.7)	
Sex, n(%)	Male	28 (65.1)	28 (60.9)	.83
	Female	15 (34.9)	18 (39.1)	
Gestational age (weeks),	Median (IQR)	31 (28 to 32)	30 (29 to 32)	.39
Birth weight (grams)	Median (IQR)	1520 (995 to 2145)	1400 (1024 to 1788)	.70
Primary mechanical ventilation (hrs)	Median (IQR)	77 (36 to 176)	73 (33 to 183)	.75
Total hospital admission (days)	Median (IQR)	54 (33 to 79)	48 (28 to 76)	.57
CRIB	Median (IQR)			.74
IVH, n(%)	No	34 (79.1)	29 (63.0)	
	Mild	9 (20.9)	15 (32.6)	.15
	Severe	0 (0.0)	2 (4.3)	
PVL, n(%)	All grades	1 (2.3)	1 (2.2)	1.00
Number of painful procedures first 14 days	Median (IQR)	[n=37] 116 (72 to 218)	[n=43] 111 (67 to 218)	.54
Cumulative dose total morphine exposure (mcg/kg) 1st 28 days	Median (IQR)	751 (485 to 1185)	109 (0 to 719)	<.001
Cumulative dose other medication administration 1st 28 days	Fentanyl (mcg/kg) n (%) Median (IQR)	3 (7.0) 23 (5 to 37)	4 (8.7) 14 (7 to 28)	-
	Midazolam (mcg/kg) n (%) Median (IQR)	3 (7.0) 130 (100 to 5123)	2 (4.3) 6333 (145 to 12520)	
	Phenobarbital (mg/kg) n (%) Median (IQR)	*1 (2.3)* *21*	*4 (8.9)* *25 (13 to 41)*	-
	Naloxone (mcg/kg) n (%) Median (IQR)	*2 (4.7)* 13 (13 to 14)	*3 (6.5)* 17 (10 to 85)	

Abbreviations: IQR, interquartile range; CRIB Clinical Risk Index for Babies; IVH, intraventricular hemorrhage; PVL, periventricular leucomalacia. Data are expressed for sample size; Morphine group, n=43; Placebo group, n=46, unless otherwise indicated between brackets.

children attending this type of education did not differ between the two groups (53.5 % versus 58.7% respectively, *P*=0.65). Teachers of 59 of the 71 children receiving mainstream education (83%) sent a copy of the Dutch National Pupil Monitoring System, which showed no differences between the two groups in their academic skills related to spelling, reading comprehension, complex word reading, and mathematics (Table 2).

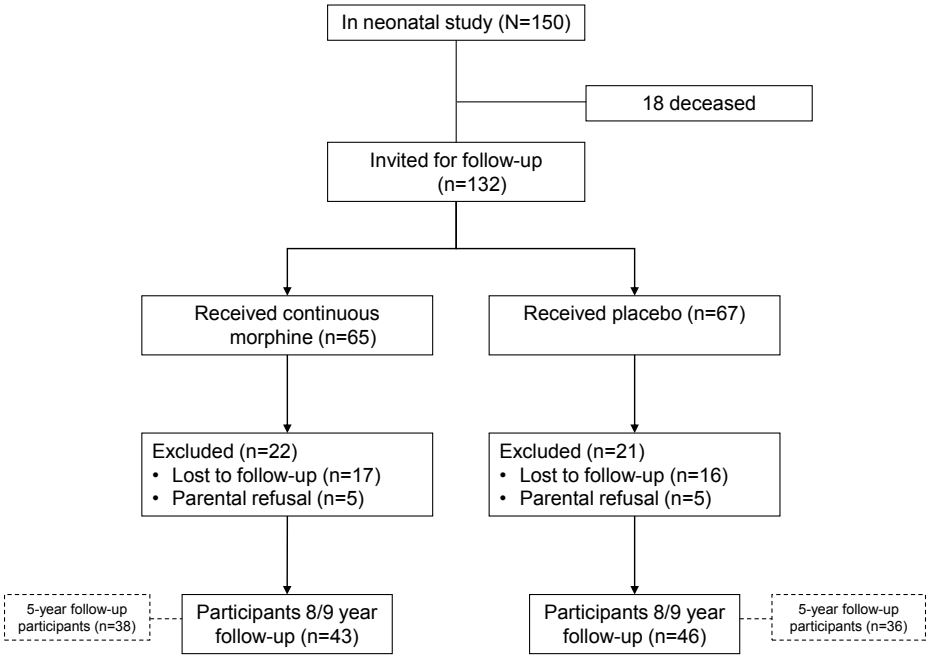

Figure 1. Flow chart of participation

Intelligence

IQ scores were obtained for 79 of the 80 participants available for testing, because one child did not want to cooperate. Three of the 79 children had already been tested with the WISC-III elsewhere within the previous six months and in the analyses we used these scores.

IQ scores were normally distributed and mean scores did not differ between cohorts: 99 (SD 19) in the morphine cohort versus 101 (SD 18) in the placebo cohort (P=0.63) (Table 3). Two children, one in each study cohort, had an IQ below 70 (<2SDS).

Sixty-nine of these 79 children (87%) had also been tested at age 5 years. IQ scores obtained at 5 years were moderately correlated with scores at 8/9 years (r=0.54, P=<0.001). There was not a statistically significant difference in IQ at 5 years and 8 years within (p=0.26) and between groups (p=0.34). The change in IQ over time was also not statistically significantly different for the two groups (p=0.68).

Table 2. Background characteristics at age 8/9 years from children who participated in the follow-up; morphine cohort versus placebo cohort (N=89)

		Morphine cohort n=43	Placebo cohort n=46	P
Age of assessment (years)	Median (IQR)	8 (8 to 9)	8 (8 to 9)	.15
Height for age (SDS)		n=38	n=40	.69
	Mean (sd)	.07 (1.14)	.16 (.94)	
Weight for height (SDS)		n=39	n=41	.82
	Mean (sd)	.03 (1.62)	.11 (1.10)	
		n=39	n=41	.51
Head circumference for age (SDS)	Mean (sd)	-.19 (1.27)	-.02 (1.03)	
Minor Neurological Dysfunctions	n(%)	9/38 (24%)	16/41 (39%)	0.14
SES, n(%)	Low	8 (18.6)	14 (30.4)	
	Medium	19 (44.2)	15 (32.6)	.36
	High	16 (37.2)	17 (37.0)	
Language spoken most often at home, n (%)	Dutch	41 (95.3)	42 (91.3)	.68
	Other	2 (4.7)	4 (8.7)	
Surgery, n(%)	None	34 (79.1)	32 (69.6)	
	Minor surgery	8 (18.6)	10 (21.7)	.48
	Major surgery	1 (2.3)	4 (8.7)	
Glasses, n(%)	No	35 (89.7)	30 (73.2)	.09
	Yes	4 (10.3)	11 (26.8)	
Education	Mainstream school	23 (53.5)	27 (58.7)	
	Mainstream extra	12 (27.9)	9 (19.6)	.65
	Special education	8 (18.6)	10 (21.7)	
grade repetition in mainstream education		[n=35]	[n=36]	
	No	27 (77.1)	28 (77.8)	
	Yes	8 (18.6)	8 (22.2)	1.00
Academic skills of children in mainstream school				
Spelling		[n=28]	[n=31]	
	Sufficient	12 (42.9)	15 (48.4)	
	Insufficient	16 (57.1)	16 (51.6)	.67
Reading comprehension		[n=28]	[n=26]	
	Sufficient	14 (50.0)	17 (65.4)	
	Insufficient	14 (50.0)	9 (34.6)	.25
Complex word reading		[n=25]	[n=25]	
	Sufficient	11 (44.0)	12 (48.0)	
	Insufficient	14 (56.0)	13 (52.0)	.78
Mathematics/artithmetic		[n=28]	[n=31]	
	Sufficient	17 (60.7)	15 (48.4)	
	Insufficient	11 (39.3)	16 (51.6)	.34

Abbreviations: SES, socio economic status; Mainstream extra=mainstream school with extra support
Data are expressed for sample size; Morphine group, n=43; Placebo group, n=46, unless otherwise indicated between brackets.

Table 3 . Neuro-psychological assessments

		Morphine cohort	Placebo cohort	P
WISC-III		[n=38]	[n=41]	
Intelligence - IQ score	Mean (SD)	99 (19)	101 (18)	.63
VMI		[n=38]	[n=41]	
Visual Motor Integration	Mean (SD)	95 (12)	98 (12)	.18
CANTAB				
Reaction time (ms)		[n=34]	[n=38]	
RTI-1	Median (IQR)	357 (302 to 458)	345 (291 to 401)	.32
RTI-5	Median (IQR)	406 (340 to 470)	396 (321 to 465)	.60
Spatial Span (SSP)		[n=36]	[n=40]	
SSP-f	Median (IQR)	5 (4 to 5)	5 (4 to 5)	.60
		[n=35]	[n=40]	
SSP-b	Median (IQR)	4 (4 to 5)	4 (4 to 5)	.36
Rapid Visual Information Processing (RVP)		[n=37]	[n=41]	
RVP 'A	Median (IQR)	.95 (.92 to .96)	.93 (.90 to .97)	.77
Stockings of Cambridge (SOC)		[n=36]	[n=40]	
Problems solved in minimum moves	Median (SD)	7 (6 to 8)	7 (5 to 8)	.74
Intra-Extra Dimensional Set Shift (IED)		[n=35]	[n=40]	
Stages completed	Median (IQR)	9 (9 to 9)	9 (8 to 9)	.63
Total trials (adjusted)	Median (IQR)	86 (77 to 122)	96 (84 to 127)	.35
Stop Signal test (SST)		[n=32]	[n=38]	
SSD	Median (IQR)	428 (320 to 498)	380 (290 to 467)	.40
SSRT	Median (IQR)	246 (199 to 313)	226 (188 to 287)	.31

Visual Motor Integration

The Beery VMI was assessed in again 79 of 80 participants, because one child did not want to cooperate. None of the children scored < 2 SDS, with no differences between the morphine group (95 (SD 12)) and the placebo group (98 (SD 12), *P*=0.18) (Table 3).

Seventy children (89%) had been tested with the Beery VMI at the age of 5 years as well; Beery VMI scores obtained at 5 years were moderately correlated with the scores at 8/9 years (*r*=0.52, *P*=<0.001).

Behavior

Median overall and subscale scores of the CBCL (n=84) and TRF (n=78) did not differ significantly between the morphine and placebo groups (Table 4). Table 6 gives the results of robust regression analyses of the CBCL and TRF subscales comparing the groups

Table 4. Behavior rated by parents and teacher

		CBCL -parents			TRF-teacher		
		Morphine cohort [n=40]	**Placebo cohort [n=44]**	**P**	**Morphine cohort [n=38]**	**Placebo cohort [n=40]**	**P**
Internalizing problems	Median (IQR)	51 (45 to 60)	52 (42 to 63)	.78	54 (48 to 60)	52 (44 to 58)	.10
	n (%)	10 (25.0)	13 (29.5)	.	10 (26.3)	5 (12.5)	
Externalizing problems	Median (IQR)	49 (40 to 56)	52 (44 to 60)	.08	48 (43 to 57)	52 (43 to 59)	.30
	n (%)	4 (10.0)	11 (25.0)	.	3 (7.9)	9 (22.5)	.
Total problems	Median (IQR)	51 (44 to 57)	55 (42 to 61)	.30	52 (49 to 56)	53 (50 to 59)	.75
	n (%)	4 (10.0)	15 (34.1)		6 (15.8)	8 (20.0)	

Abbreviations: n(%), number of children in (sub)clinical range T≥60

controlling for propensity score and IQ. Children in the placebo group had significantly higher CBCL Externalizing problems scores (p=0.02) adjusting for propensity score and IQ and this was irrespective of the extra morphine given (p=0.72). Teachers identified more Internalizing problems (p=0.05) in the morphine group adjusting for propensity score and IQ, irrespective of extra morphine given (p=0.48).

CBCL scores for 70 children out of 84 (83%) were also obtained at the age of 5 years; these scores were moderately correlated for the internalizing (*r*=0.40, *P*=0.001), externalizing (*r*=0.59, *P*=<0.001) and total problem scales (*r*=0.60, *P*=<0.001). Internalizing (p=0.004) and total scores were statistically significantly worse for the 8 years olds (p<0.001) but not for the Externalizing score (p=0.06). Between groups differences were not statistically significant for Internalizing (p=0.60), Externalizing (p=0.15) and Total CBCL score (p=0.48) respectively. The changes in CBCL scores over time were also not statistically significantly different for the two groups (p-values 0.39, 0.31 and 0.17 for Internalizing, Externalizing and Total scores respectively).

TRF scores for 63 out of 78 children (81%) were obtained at the age of 5 years as well; these scores were marginally correlated for the internalizing (*r*=0.29, *P*=0.02), externalizing (*r*=0.27, *P*=0.04) and total problem scales (*r*=0.40, *P*=0.001). The Total score was statistically significantly worse for the 8 years olds for (p=0.05), but not for the Internalizing (p=0.10) and Externalizing scores (p=0.84). Between groups differences were not statistically significant for TRF Internalizing (p=0.29), TRF Externalizing (p=0.74) and Total TRF score (p=0.44) respectively. The changes in TRF scores over time were also not statistically significantly different for the two groups (p-values 0.15, 0.10 and 0.58 for Internalizing, Externalizing and Total scores respectively).

Executive function skills

Sixty-eight children completed all CANTAB tests; the individual tests were completed by between 70 to 78 children. Five of the 12 others lost concentration, technical failure occurred in 5 cases, and in 2 there was not enough time to finish the CANTAB tasks. None of the scores on the six tested CANTAB subtests differed significantly between the morphine and placebo groups (Table 3).

Executive function behavior in daily life

The results of the BRIEF questionnaires completed by parents and teachers are shown in Table 5. Children of the morphine cohort were rated as having significantly fewer problems on the subscales *inhibition*, *organization of materials* and *monitoring* by parents

Table 5. Executive functions in daily life rated by parents and teacher

		BRIEF -parents			BRIEF-teacher		
		Morphine cohort [n=40]	**Placebo cohort [n=44]**		**Morphine cohort [n=37]**	**Placebo cohort [n=40]**	
Inhibition	Median (IQR)	40 (36 to 48)	46 (40 to 58)	**.02**	45 (43 to 53)	51 (43 to 61)	.09
	n (%)	1 (2.5)	6 (13.6)		5 (13.5)	6 (15)	
Shifting	Median (IQR)	42 (39 to 51)	45 (39 to 54)	.39	48 (44 to 52)	51 (44 to 61)	.21
	n (%)	1 (2.5)	2 (4.5)		4 (10.8)	7 (17.5)	
Emotional control	Median (IQR)	41 (35 to 50)	42 (38 to 55)	.10	47 (43 to 51)	49 (44 to 59)	.12
	n (%)	1 (2.5)	8 (18.2)		2 (5.4)	9 (22.5)	
Behavioral Regulation Index	Median (IQR)	41 (35 to 48)	43 (39 to 59)	.08	47 (43 to 54)	50 (44 to 63)	.11
	n (%),	1 (2.5)	5 (11.4)		3 (8.1)	8 (20.0)	
Initiation	Median (IQR)	42 (35 to 50)	46 (39 to 51)	.12	54 (43 to 63)	55 (45 to 66)	.58
	n (%)	2 (5.0)	2 (4.5)		9 (24.3)	10 (25.0)	
Working memory	Median (IQR)	46 (37 to 55)	46 (39 to 57)	.48	49 (45 to 67)	53 (44 to 65)	.91
	n (%)	3 (7.5)	6 (13.6)		10 (27)	11 (27.5)	
Planning/organize	Median (IQR)	41 (35 to 49)	43 (38 to 53)	.12	44 (40 to 55)	49 (44 to 59)	**.05**
	n (%)	1 (2.5)	2 (4.5)		5 (13.5)	7 (17.5)	
Organization of materials	Median (IQR)	37 (30 to 44)	41 (34 to 50)	**.04**	44 (41 to 53)	48 (44 to 58)	.10
	n (%)	0 (0)	2 (4.5)		3 (8.1)	7 (17.5)	
Monitoring	Median (IQR)	38 (33 to 48)	42 (38 to 54)	**.01**	48 (42 to 56)	52 (45 to 64)	.06
	n (%)	2 (5.0)	3 (6.8)		5 (13.5)	10 (25)	
Metacognition Index	Median (IQR)	39 (34 to 49)	43 (37 to 51)	**.09**	47 (43 to 60)	52 (45 to 64)	.32
	n (%)	2 (5.0)	4 (9.1)		5 (13.5)	9 (22.5)	
Global Executive Composite	Median (IQR)	40 (33 to 49)	44 (37 to 50)	.13	46 (43 to 58)	51 (44 to 62)	.08
	n (%)	1 (2.5)	5 (11.4)		5 (13.5)	9 (22.5)	

Abbreviations: n(%), number of children in (sub)clinical range T≥65

Table 6. Results of robust regression analysis adjusted for propensity score and IQ showing unstandardized regression coefficients with 95% CI

	Intercept	Treatment group* B (95% CI)	P	Extra morphine B (95% CI)	P
CBCL					
Internalizing problems	51.6	-2.6 (-7.6 to 2.5)	0.32	-0.7 (-3.7 to 2.4)	0.67
Externalizing problems	59.7	-5.7 (-10.2 to -1.3)	**0.02**	0.5 (-2.2 to 3.2)	0.72
Total Problem Scale	64.3	-4.0 (-8.5 to 0.5)	0.09	0.08 (-2.6 to 2.8)	0.95
TRF					
Internalizing problems***	48.5	4.1 (0.1 to 8.1)	**0.043**	0.9 (-1.5 to 3.3)	0.48
Externalizing problems	51.6	-2.0 (-5.8 to 1.8)	0.30	1.3 (-1.0 to 3.5)	0.28
Total Problem Scale	51.5	0.4 (-2.9 to 3.6)	0.81	1.5 (-0.4 to 3.5)	0.13
BRIEF parents					
Behavioral Regulation Index	55.1	-6.6 (-11.3 to -2.0)	**0.006**	-0.9 (-3.7 to 1.9)	0.54
Metacognition Index	52.9	-5.5 (-10.3 to -0.6)	**0.03**	0.5 (-2.4 to 3.4)	0.73
Global Executive Composite	54.0	-6.7 (-11.8 to -1.7)	**0.009**	0.06 (-3.0 to 3.1)	0.97
BRIEF teacher					
Behavioral Regulation Index	59.2	-3.5 (-8.5 to 1.4)	0.16	2.1 (-0.8 to 5.0)	0.16
Metacognition Index	67.5	-2.2 (-8.4 to 4.0)	0.49	1.6 (-2.1 to 5.2)	0.40
Global Executive Composite	64.8	-2.5 (-7.6 to 2.7)	0.35	1.5 (-1.5 to 4.6)	0.33

All regression estimates were adjusted for the propensity score including: location, sex, primary ventilation, gestational age, Clinical Risk Index for Babies (CRIB) and Socioeconomic Status (middle and high versus low).

* Treatment group: 0=placebo and 1=morphine ** R^2 = R^2 of model including treatment group + extra morphine + propensity - R^2 model including only propensity score ***n=77

and on the subscale *planning/organization* by teachers (P=.02, P=.04, P=.01 and P=.05, respectively). After adjusting for the additional open-label morphine, the propensity score and IQ, children in the morphine cohort had significantly fewer problems on the Behavioural regulation index (P=0.006), Metacognition Index (p=0.03) and the Global Executive Composite (p=0.009) as rated by parents. Although the results for the teacher ratings were in the same direction, these did not reach statistical significance (Table 6). The additional open-label morphine had no effect on these outcomes.

DISCUSSION

Our results show that continuous morphine infusions in the neonatal period have no significant negative consequences on the child's general functioning in terms of intelligence, visual motor integration and behavior at the age of 8/9 years. A similar

conclusion was drawn from our previous follow-up study at 5 years of age in the same population[20]. At that time we suggested that continuous morphine administration may have an adverse effect on executive functions, but this was not confirmed in the 8/9 year olds. Rather the reverse may be true, judging from the results of the BRIEF questionnaire completed by parents pointing at fewer problems in these children. Our previous work examined the effect of morphine on endotracheal suctioning, which may cause discomfort or transient air hunger but does not cause acute pain like skin-breaking procedures, or prolonged pain like postoperative or inflammatory pain. Therefore, our previous trial tested the analgesic efficacy of morphine somewhat inappropriately using a potentially non-painful but stressful procedure. However, our current results do suggest a potential neuroprotective effect of morphine on the painful and non-painful experiences to which ventilated newborns are routinely exposed. This is seen from the differences in BRIEF parental subscales as well as less Externalizing problems in the CBCL.

Animal experiments investigating long term effects of perinatal morphine were designed to give very large doses, over prolonged developmental periods and in the absence of any painful stimulation. Therefore, their findings are not clinically relevant and do not apply to the findings of this study. There are only two published animal studies which used clinically relevant doses of morphine and administered morphine in the setting of inflammatory pain. Both studies showed clearly beneficial long-term effects of morphine exposure[12,41].

We did not find an additional effect of the extra morphine doses given by clinicians if they thought that newborns were experiencing pain/stress. We propose that continuous morphine infusions will avoid the peaks and valleys in morphine exposure associated with intermittent doses. Following a bolus dose, peak plasma levels may be associated with neurotoxicity because of over-sedation or tolerance, whereas the painful episodes occurring in the intervals between morphine boluses may also cause excitotoxic cell death of the immature neurons. Clinical practices should consider the steady state concentrations maintained by continuous low-dose infusions, taking care to avoid over-sedation and to match the amount and timing of morphine analgesia with the degree of pain being experienced.

Overall, this follow-up study at 8/9 years demonstrates that infusion of 10 mcg/kg/hr of continuous morphine, adjusted for additional open-label morphine, in ventilated neonates does not harm the general functioning of the child and may have a positive effect on executive functions in daily life.

The conclusion that continuous morphine administered in the neonatal period shows no negative effect on general functioning later in life is also supported by the fact that the proportion of children with minor neurological dysfunctions and children attending special education or receiving extra support in mainstream education did not differ between the morphine and placebo groups. In addition, both groups show comparable academic achievements, such as spelling, reading comprehension, complex word reading and mathematics. Nonetheless, the percentages of children in both cohorts attending special education (18% and 22%, respectively) are higher than the corresponding 4.9% in the general Dutch population[50], which exemplifies the long-term learning problems of children who received neonatal intensive care.

Our results are in contrast with the outcome of a recently published pilot-study. Although it included only 19 children, these children had participated in a RCT - the NEOPAIN trial -, with a comparable study design. This pilot-study reported adverse effects of continuous morphine administration on head circumference, body weight, social problems and altered response latencies at the age of 5-7 years. Our study did not find such differences. This can possibly be explained by the substantial differences in the amounts of morphine administered. In our initial RCT a loading dose of 100 mcg/kg was administered followed by 10 mcg/kg/h morphine infusion independent of gestational age[61]. In the NEOPAIN trial children in the morphine cohort received a loading dose of 100 mcg/kg followed by infusions of 10 to 30 mcg/kg/h depending on gestational age[5]. In the NEOPAIN pilot follow-up study, 8 of the 14 (57%) participants of the morphine cohort were born > 27 weeks of gestation and thus received a morphine dosage of 20 mcg/kg/h or more. New model-based simulations of morphine requirements in newborns below 10 days postnatal age after major non-cardiac surgery show that a dosage of 2.5 mcg/kg$^{1.5}$/h[39,40] is sufficient to reach adequate morphine plasma levels. In this view, the morphine dosage given to neonates in NEOPAIN, especially to those born at 27-32 week of gestation, can be considered to be very high. A possible dosing effect on later brain development could therefore not be ruled out and a larger follow-up of the NEOPAIN trial would be warranted to provide more definitive answers.

In the present study, children in the morphine group exhibited fewer problems than the placebo group based on the BRIEF questionnaire, but not on the CANTAB tasks. This discrepancy occurred in previous studies as well[6,47]. The CANTAB tasks make it possible to identify specific problems in executive functioning. These tasks are performed in a well-structured laboratory setting, they require little of the child's own behavior regulation skills and therefore lack ecological validity[60]. In contrast, the questionnaires rely less on specific skills but more on everyday demands in the child's natural setting[60]. As our cohorts did not differ in the specific executive function skills, but showed differences in

the executive functions in daily life, to the effect that the morphine-treatment group had fewer problems monitoring their behavior, planning a task, organizing their materials and suppressing their impulses, it seems that neonatal treatment with continuous morphine has a positive effect on subsequent behavioral regulation.

From the present study it is not possible to conclude whether this positive effect of continuous morphine administration occurred because it adequately prevented pain, or it provided sedation, or it adequately prevented stress. Research has shown that neonatal pain may be related to adverse long-term effects on subsequent development[22,26,30,33-35,59,67,69,71]. A recent MRI brain study found evidence that repetitive pain experienced by preterm born children in the neonatal period is related to reduced white matter and reduced subcortical gray matter[15]. They did not, however, find any evidence that morphine ameliorated these effects. In general, one can question whether morphine administration is effective in the treatment of procedural pain in preterm neonates[10,17].

In our initial RCT, severe pain was mostly absent and both groups had comparable pain scores[61]. Our initial RCT, however, also showed that morphine administration had a positive effect on the stress system by lowering noradrenaline levels[62]. In the preterm born population, neonatal pain and stress have been associated with increased cortisol levels up to 18 months of age[27-29,31]. Brummelte et al. (2011) showed that cortisol levels in the neonatal period were correlated with internalizing behaviors at 18 months in children born preterm, which suggests an early dysregulation in the hypothalamic-pituitary-adrenal axis (HPA-axis)[14]. In view of associations between cortisol levels on the one hand, and psychopathology[42,51,52], problem behavior[48,57,65] and cognitive problems[23,43,44] on the other hand, prevention of neonatal stress seems very important. Because of the serious short-term side-effects of morphine such as respiratory depression[11] and withdrawal symptoms[38], most clinicians do not promote the use of morphine in neonates.

A number of limitations of our study should be addressed. First, the entire testing program took 4 hours and therefore some children could not perform all the tests due to loss of concentration and fatigue. Second, for nine children, only the questionnaires were completed; they were not tested. As these children were equally divided over both groups, we assume this had no consequential effect on the outcomes. Thirdly, a comparable visual analysis subtest suitable for age 8-9 years similar to that found to be lower in the morphine group at 5 years was not re-assessed. Fourth limitation is that only a subset of children completed the full CANTAB battery; twelve children lost their concentration and completed just one or several subtests. This may have biased the results.

In conclusion, in this cohort, neonatal morphine use does not seem to affect cognition and behavior 8 or 9 years later and may even have a positive effect on everyday executive functions. Based on the short-term outcome of previous RCTs[5,61] and the Cochrane review of Bellu et al. (2010)[10], the use of continuous morphine is not considered as 'standard of care' in ventilated neonates. Yet, the present study demonstrates that, in view of the long-term sequelae, continuous morphine given in dosages up to 10 mcg/kg/hr can be safely administered to neonates and may improve their subsequent behavior.

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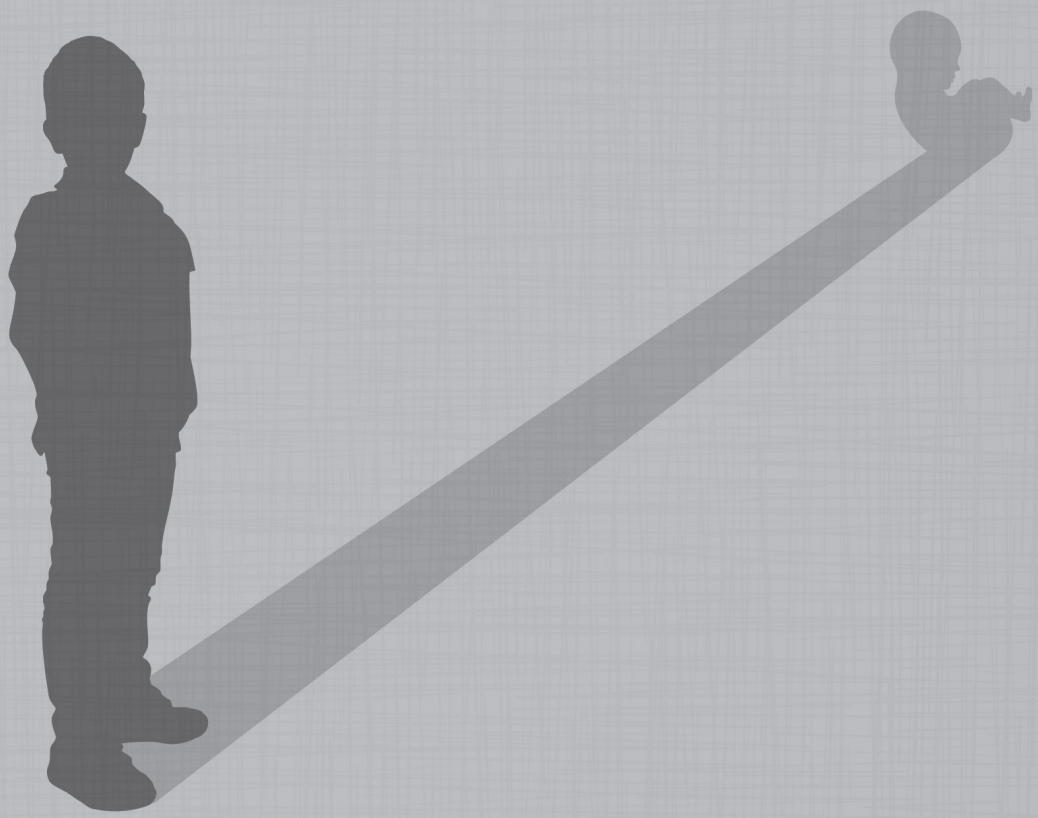
PART II

Stress system



Chapter 4

Long-term effects of neonatal intensive care and morphine infusion during mechanical ventilation on diurnal cortisol rhythm: Five-year follow-up study

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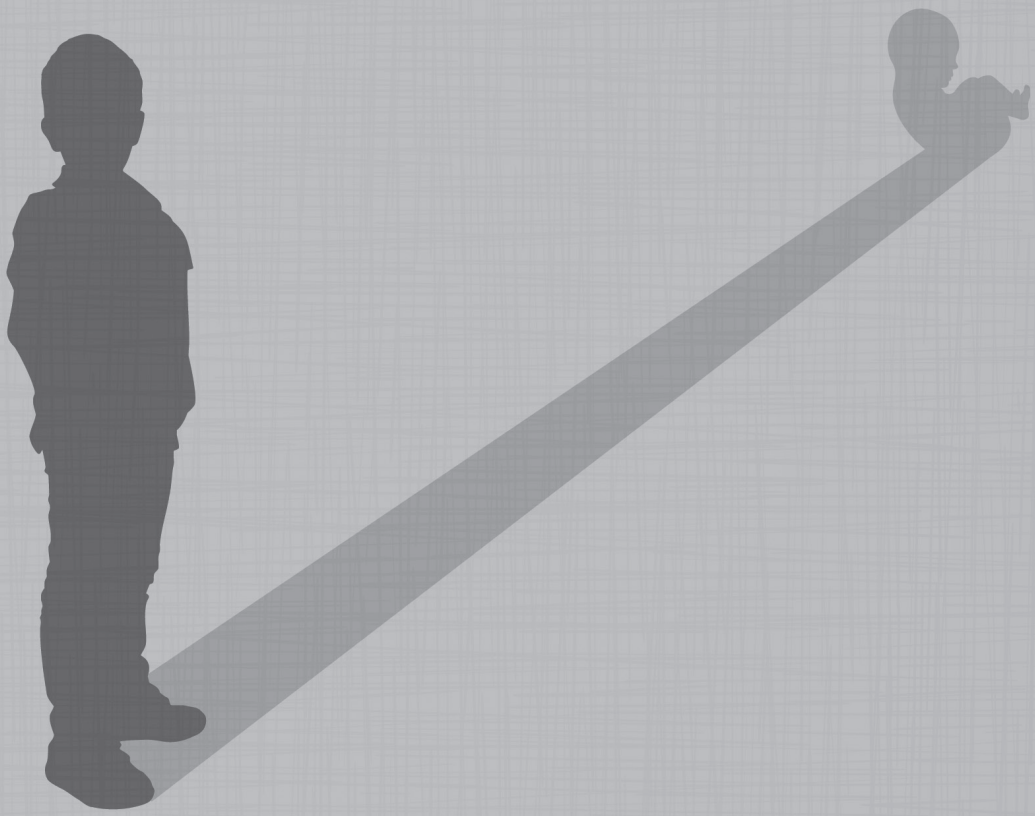
Submitted



SUMMARY

Context

Mechanically ventilated neonates undergo procedures that often induce stress and pain. It may well be that morphine treatment or pain experience in the first weeks of life affects the stress system, in terms of the hypothalamic-pituitary-adrenal (HPA) axis, later in life.

Objective

This study aimed to examine whether (1) the diurnal cortisol secretion rhythm of children who as neonates had been hospitalized differs from that of children without a history of neonatal hospital admission; and (2) this rhythm differs between these hospitalized children treated with continuous morphine infusion and those treated with placebo.

Design and procedure

A follow-up cohort study was performed of five-year-old children who as neonates participated in a randomized controlled trial of continuous morphine infusion (born 24-42 weeks gestation), and a control group of healthy term born (≥37 weeks gestation) children. Five saliva samples over a school day were assayed for cortisol concentrations. The diurnal cortisol rhythm was analyzed with random regression analysis for repeated measurements (PROC MIXED in SAS).

Results

Compared to the healthy controls, the former trial participants (both morphine and placebo treated children) had higher cortisol levels (P=.002), adjusted for sex and socioeconomic status. Morphine administration did not affect the cortisol concentrations (P=.66), adjusted for sex, socioeconomic status and gestational age at birth.

Conclusion

The fact that former trial participants had higher cortisol levels at five years of age supports the concept of long lasting programming of the hypothalamic-pituitary-adrenal axis. Morphine infusion in the neonatal period did neither ameliorate nor exacerbate the altered cortisol secretion at five years of age.

INTRODUCTION

Stress and pain are common findings in mechanically ventilated neonates[7,39]. They are exposed to stress not only from intubation and other painful medical procedures in the neonatal intensive care unit (NICU), but also from separation from their mothers[6,29,39]. Procedural pain-related stress increases levels of the stress hormone cortisol. In response to stress, the hypothalamo-pituitary-adrenal (HPA) axis is activated and cortisol is produced by the adrenal glands.

Sustained increases of glucocorticoids during development can permanently modify brain structure and function[14,26]. The developing limbic system, the hippocampus, hypothalamus and anterior pituitary synthesize high levels of glucocorticoid receptors, and are sensitive to glucocorticoid levels. Exposure to increased levels of glucocorticoids at this time alters the development and subsequent function of both the limbic system and the HPA axis. Prenatal and postnatal exposure to elevated levels of glucocorticoids can influence programming of adult HPA function and behavior. Prenatal exposure to elevated glucocorticoid levels by maternal stress, exposure to synthetic glucocorticoids and nutrient restriction have been linked to increased HPA axis activity at long term[27,37]. This also holds true for postnatal exposure to elevated glucocorticoid levels by neonatal handling, modified maternal behavior[13], exposure to synthetic glucocorticoids[2] and infection[30]. Most studies have been undertaken in animals. There is considerable evidence that a similar process can also occur in humans. Few studies are available on premature children exposed to postnatal stress by medical procedures at NICUs. Cortisol levels in children born very preterm or born small for gestational age who often stay in the NICU for many weeks, are low during the first 3 months of life[17,18,31]. Then, however, from the age of 8 months up to 14 years, they are higher than in term born children[5,18]. This phenomenon, reflecting alterations of the HPA axis, supports the 'early-life programming' concept which proposes that adverse early life experiences can permanently affect physiological systems[27,37,49].

Most studies of NICU populations have evaluated cortisol levels during baseline[18], or in relation to specific potential stressors, such as heel lance[31], immunization[19], exposure to a visual novelty[16] or developmental assessment[4]. Production of cortisol follows a diurnal rhythm, with highest concentrations in the morning and lowest in the evening[38] and is an important index of HPA axis regulation[34]. This rhythm establishes in the first year of life[45] and may even be present, although with variation between individuals, as early as the age of two or three months[12,32,45]. To our knowledge, only one long-term follow-up study of children born preterm has examined the diurnal cortisol profile; this did not statistically significantly differ between preterm born children age 8-14 years and

age-matched full-term born children[5]. However, cortisol levels after awakening were significantly higher in the former preterm born children[5].

Given the potential for adverse experiences in the neonatal period to affect regulation of stress later in life, it is important to evaluate whether pain management ameliorates or exacerbates patterns of cortisol expression. Continuous morphine administration used to be 'standard of care' for ventilated children without real evidence for its safety, until the results of two randomized controlled trials (RCTs) suggested against it[1,40]. These RCTs showed that continuous morphine was not effective to decrease pain and prevent poor neurological outcome. However, one of these RCTs also showed that continuous morphine decreased noradrenaline, which is a hormone produced in acute stress or pain, in the neonatal period[41]. It is not yet known whether this beneficial effect on the stress system is maintained on the longer term. Sparse data show no effect of morphine on later cortisol levels up to 18 months discharge[16-18]. However, a drawback of these studies is that morphine was not the main outcome variable, but 'only' reported as a co-variable. A correlation between the administered morphine and severity of illness can therefore not be excluded. To examine solely the effect of the administered morphine, follow-up of a randomized controlled trial is inevitable.

In the present study, we evaluated the diurnal pattern of cortisol release in five-year-old children who had participated in the RCT of morphine administration during mechanical ventilation[40] and in the five-year follow-up study[9]. We hypothesized that the levels of cortisol secretion during the day of children who underwent mechanical ventilation in a NICU will be higher from that of healthy term born children without a history of neonatal hospital admission. In addition, as continuous morphine administration was associated with decreased noradrenaline levels in the neonatal period[41] we hypothesize that receiving morphine would have led to a less activated HPA axis during NICU period, leading to less HPA axis activation on long term and thus lower cortisol levels.

METHODS

Participants

We included five-year-old children who as neonates participated in the morphine RCT briefly described below and described in detail previously[40-42]. We also included a cohort of term born children of the same age without NICU admission. The study was approved by the medical ethics review boards at Erasmus MC-Sophia Children's Hospital, Rotterdam, and the Isala Clinics, Zwolle, both the Netherlands.

The original RCT included 150 neonates born 25-42 weeks gestational age, admitted to two level III Neonatal Intensive Care Units (NICU) in the Netherlands; Erasmus MC-Sophia Children's Hospital in Rotterdam and the Isala Clinics in Zwolle. They all received mechanical ventilation and were randomly allocated to receive either a loading dose of 100 mcg/kg of morphine followed by a continuous infusion of 10 mcg/kg/h (morphine condition) or placebo (placebo condition). Children in both conditions who were judged to be in pain or distress, were allowed to receive additional doses of 'open-label' morphine of 50 mcg/kg followed by 5-10 mcg/kg/h.

At five years of age, these children were invited to participate in a larger follow-up program to evaluate functioning in terms of intelligence, behavior, quality of life and sensory thresholds[9,10]. Eighteen of the 150 children had died, all in the first year of life. Seven children were known to have severe cognitive and/or physical disabilities and were therefore not invited. At the time the medical ethics review boards approved the present study, thirty-seven children had already attended the five-year follow-up visit. These children were not invited to participate in the present study. As children were invited in order of date of birth, selection bias could be considered minimal. Parents or caregivers of the remaining 88 children were sent a letter explaining the purpose of the larger five-year follow-up study and explaining that we would like to collect saliva to determine cortisol levels. Signed informed consent for the psychological assessment[9], sensory thresholds[10], and saliva collection (present study) was sought.

In addition, a control cohort of healthy, term born (≥37 weeks) children were recruited from nine mainstream elementary schools (n=536 children), thus schools in which pupils do not have special educational needs. All these children were term born and had not a history of hospitalization. These schools were located in the same geographical area as the NICU population was recruited from. This cohort can be considered as a representative norm cohort. Teachers in these schools distributed an information letter on the study to the parents, which requested them to collect saliva for cortisol level determination. If parents agreed they were asked to sign the informed consent form, which they could hand to the teacher or send by post to the investigators.

Procedure

Background characteristics

Parents completed a questionnaire related to background information, including the child's sex, gestational age, birth weight and their own profession and education. The latter information served to determine socioeconomic status (SES) with the use of the Occupation Classification system[44]. For the NICU cohort, additional detailed information

was available from the original RCT[40] and the larger five-year follow-up[9]: additional morphine received in the first 28 days, duration of mechanical ventilation, type and number of surgeries, pain medication, Clinical Risk Index for Babies (CRIB), IQ and behavior. Surgeries were classified as minor or major based on opioid use or clinical indications.

Saliva collection – diurnal rhythm

Parents received an instruction letter and five (+ one substitute) cotton rolls within labeled tubes. Saliva was to be collected five times during a school day: directly after the child's awakening, 30 minutes after awakening, lunch time (1200h) at home or at school (by the teacher), directly on return home from school (1530h), and at bedtime. In the Netherlands, the school day ends at approximately the same time, resulting in small sampling-time variation. Parents were asked to collect samples before meals or 30 minutes after meals. Both children and parents were asked not to use hormonal cream (i.e. for eczema) on the day of saliva collection. If this was not possible, parents were to wash hands twice before touching the cotton role to avoid contamination. Parents were instructed to store the collected samples in a freezer. Parents of the NICU cohort were asked to bring the samples to the five-year follow-up visit. Parents of the control cohort were asked to send the samples by post. Parents completed a checklist informing after the exact times of saliva collections, times of meal/ snacks, use of milk products, use of any kind of hormone cream, and the child's sleep pattern. Also information about sickness on the day of sample collection, special events or stressful circumstances was collected.

Cortisol assay

Saliva was collected in Salivette tubes (Sarstedt, Nümbrecht, Germany) and stored in the department of Internal Medicine, Section of Endocrinology, Erasmus Medical Center, Rotterdam, the Netherlands at –20 ˚C until further analyses. Salivary cortisol concentration was determined with an enzyme-linked immunosorbent assay (ELISA – DRG Salivary Cortisol ELISA KIT) based on the competition principle and microplate separation. An unknown amount of cortisol present in the sample and a fixed amount of cortisol conjugated with horseradish peroxidase compete for the binding sites of mouse monoclonal cortisol antibodies. After one hour incubation the microplate is washed to stop the competition reaction. After addition of the substrate solution and 30 min of incubation the concentration of cortisol is calculated on basis of the optical density measured, compared to that measured in a series of samples with known amounts of standard cortisol on the same microtiter plate. Cortisol concentration is described in nmol/L. The Lower Limit of Quantitation of this assay is 0.60 nmol/L. Inter-assay coefficients of variation (n=23) between 22.8% and 16.4% were found for mean cortisol concentrations between 2.8 and 41.05 nmol/L respectively.

Statistical analyses

Background characteristics of the two groups were compared using Student t test (normally distributed continuous data) or Mann-Whitney test (non-normally distributed data). Categorical data were evaluated with Fisher's exact test.

The effect of NICU admission and morphine exposure on the diurnal cortisol rhythm was investigated with random regression analyses for repeated measurements (PROC MIXED in SAS 9.2[33,43,47]. This procedure allows for unequally spaced time intervals of measurements in individuals and missing data. Random regression models enable to include the unequally spaced time intervals expressed by the variable 'time'.

The missing data were considered to be 'Missing At Random' (MAR). The error covariance structure was considered to be unstructured. To test our first hypothesis, the diurnal rhythm of cortisol concentration was compared between the total NICU cohort (both morphine and placebo treated children) and the control cohort. We adjusted for sex and SES. The treatment cohorts, NICU and control, highly correlated with gestational age (r=.91, P=.001), as the control cohort consisted only of term born children and the study cohort mostly of preterm born children (97.5% of children with gestational <37 weeks). To avoid multicollinearity gestational age was excluded from the analysis.

To test our second hypothesis, only the data of the NICU cohort were included. Diurnal rhythm of cortisol concentration was compared between children treated with continuous morphine and placebo. We adjusted for the confounders sex, gestational age and SES (classified as low, medium or high).

The critical level of significance was set at 0.05 (two-tailed) for all analyses. Data were analyzed using the statistical software packages SPSS 19.0 and SAS 9.2 for Windows.

RESULTS

Background characteristics

Of the 88 eligible children in the original RCT, 16 children (18%) were lost to follow-up and parents of 19 children (22%) refused participation in the entire five-year follow-up examination, so that 53 parents were invited to collect saliva (Figure 1). Parents of one child (2%) did not consent to saliva collection. The sample taking failed in nine cases (17%); either because parents were not able to do it or the child did not cooperate. For one child (2%) only a single sample contained sufficient saliva; for another child (2%) none of the samples contained enough saliva to determine the cortisol concentration,

and for yet another child (2%) the sampling times were unknown. Of the remaining 40 children (75%) (placebo n=20; morphine n=20) at least two suitable samples were obtained. Neonatal characteristics (treatment group (P=.22), center (P=.28), gestational age (P=.97), birth weight (P=.80), cumulative dose of open-label morphine administration 1st 28 days (P=.73) did not differ significantly between non-participants and participants.

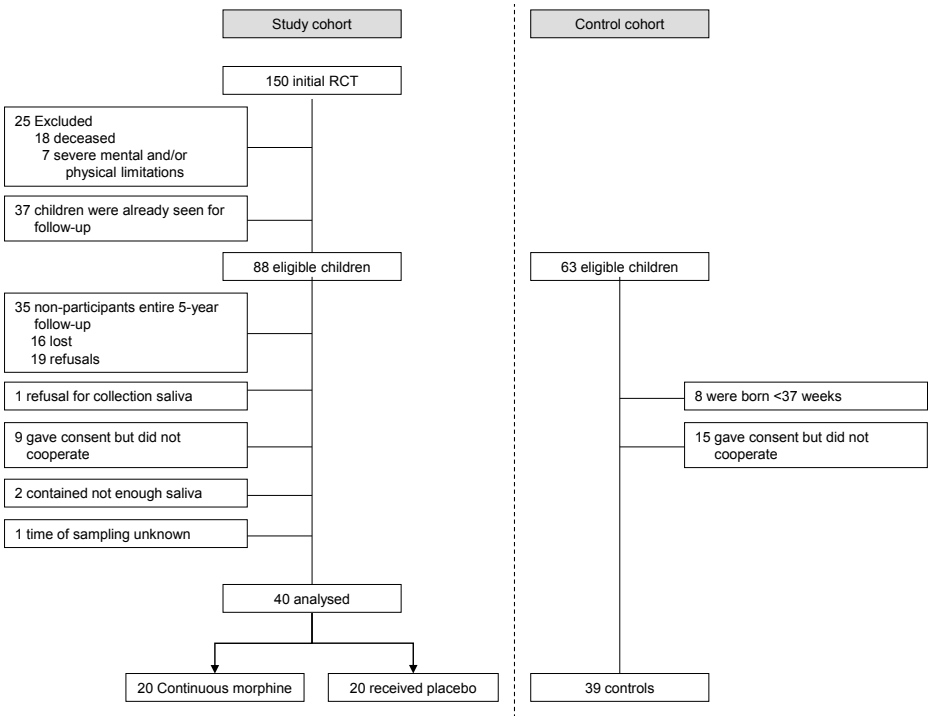


Figure 1. Flowchart of participation

Eight of the 62 children (13%) in the control group whose parents consented to collecting saliva were not eligible for the study due to gestational age <37 weeks. Parents of 14 of the eligible 54 children (26%) did not send the samples. Gestational age (P=.21) and birth weight (P=.72) of these 14 nonparticipants did not differ from those of the participating children. Data of one child (2%) known to have asthma were excluded from the analyses because corticosteroid use was strongly suspected from the extreme outliers (awakening, 29.7 nmol/ml; 30 min after awakening, 28.8 nmol/ml; 1200h, 38.2 nmol/ml; 1430h, 82.6 nmol/ml; bedtime, 23.5 nmol/ml). Of the remaining 39 control children (63%) at least two samples were obtained.

Table 1 presents background characteristics for the NICU and control cohorts. As was expected, gestational age (P<0.001) and birth weight (P<0.001) were significantly lower in the placebo and morphine groups compared to the control children. Other characteristics did not differ significantly. Children in all cohorts did not use hormonal cream and were not sick on the day of testing. The morphine and placebo cohorts did not differ in background characteristics (Table 2).

Salivary cortisol

Table 3 shows that saliva was collected reasonably close to the suggested sampling times. All five samples were obtained in 68 of the total sample of 79 (86%); four samples in seven children (9%); three samples in one child (1%), two samples in three children (4%). Mean (SD) and median (IQR) values are also given in Table 3. Mean (±1 SEM) salivary cortisol concentrations for the placebo and morphine cohorts are displayed in Figure 2. Eight of the 377 saliva samples (2%) were collected within 30 minutes after a meal, and in two of these cases the meals included a milk product. Cortisol levels seemed not to be influenced by it. Cortisol concentrations changed significantly during the day (P<0.001) following a quadratic trend with a peak at approximately 30 minutes after awakening.

The possible interaction between variables and the unequally spaced time intervals was evaluated using the interaction terms 'linear Time*Treatment condition' and 'quadratic Time*Treatment condition'. It appeared that neither interaction term was significant;

Table 1. Neonatal and five-year background characteristics NICU (morphine and placebo cohorts) versus Controls (N=79)

		NICU (n=40)	Control (n=39)	P
Sex, n(%)	Male	21 (53)	25 (64)	.36
	Female	19 (48)	14 (36)	
Gestational age (weeks)	Mean (SD)	30 (3.1)	40.0 (1.1)	**<.001**
Gestational age in weeks, n(%)	< 28	13 (33)	-	
	28 - 31	18 (45)	-	-
	32 – 36	8 (20)	-	
	≥37	1 (3)	39 (100)	
Birth weight (grams)	Mean (SD)	1384 (634)	3572 (589)	**<.001**
Socioeconomic status, n(%)	low	3 (8)	0 (0)	
	middle	12 (30)	17 (46)	.12
	high	25 (63)	20 (54)	
Age cortisol assessment (months)	Median (IQR)	63 (62-64)	64 (54 to 71)	.98
Average sleep hours at night (hours)	Median (IQR)	11 (11 to 11)	11 (11 to 12)	.34

Abbreviations: IQR, interquartile range; CRIB Clinical Risk Index for Babies

T**able 2.** Neonatal and five-year background characteristics morphine cohort versus placebo cohorts (N=40)

		Placebo (n=20)	Morphine (n=20)	P
Sex, n(%)	Male	10 (50)	11 (55)	.75
	Female	10 (50)	9 (45)	
Gestational age (weeks)	Mean (SD)	30.2 (3.4)	29.8 (2.9)	.64
Gestational age in weeks, n(%)	< 28	5 (25)	8 (40)	
	28 – 31.6	11 (55)	7 (35)	.38
	32 – 36.6	3 (15)	5 (25)	
	≥37	1 (5)	0 (0)	
Birth weight (grams)	Mean (SD)	1427 (678)	1341 (603)	.68
Socioeconomic status, n(%)	low	2 (10)	1 (5)	
	middle	6 (30)	6 (30)	.83
	high	12 (60)	13 (65)	
Age cortisol assessment (months)	Median (IQR)	63 (62 to 64)	63 (62 to 64)	.66
Average sleep hours at night (hours)	Median (IQR)	11 (11 to 11)	11 (11 to 11)	.81
Center	Rotterdam	9 (45)	11 (55)	.75
	Zwolle	11 (55)	9 (45)	
Surgery, n(%)	None	17 (85)	15 (68)	
	Minor	3 (15)	4 (27)	.70
	Short bowel	-	1 (5)	
Primary mechanical ventilation (hrs)	Median (IQR)	65 (29 to 176)	99 (46 to 147)	.43
CRIB	Median (IQR)	3 (1 to 4)	2 (1 to 5)	.97
Number of painful procedures first 14 days	Mean (SD)	134 (78) [n=20]	179 (74) [n=18]	.08
Cumulative dose of open-label morphine administration 1st 28 days (mcg/kg)	Median (IQR)	107 (0 to 780)	110 (68 to 269)	.88
IQ 5 years	Mean (SD)	104 (12)	102 (10)	.51
CBCL: Internalizing problems	Mean (SD)	48 (12)	46 (14) [n=19]	.70
CBCL: Externalizing problems	Mean (SD)	46 (9)	47 (9) [n=19]	.64
CBCL: Total problems	Mean (SD)	46 [10]	46 (12) [n=19]	.94
TRF: Internalizing problems	Mean (SD)	47 (11) [n=19]	47 (10) [n=18]	.97
TRF: Externalizing problems	Mean (SD)	50 (11) [n=19]	52 (9) [n=18]	.40
TRF: Total problems	Mean (SD)	48 (12) [n=19]	50 (10) [n=18]	.57

Abbreviations: IQR, interquartile range; CRIB, Clinical Risk Index for Babies; CBCL, Child Behavior Checklist; TRF, Teacher report form. Data are expressed for sample size; morphine group, n=20; placebo group, n=20, unless otherwise indicated between brackets.

consequently, in further analyses all interaction terms were left out. PROC MIXED repeated-measures regression analysis revealed that children who had undergone mechanical ventilation in a NICU (both morphine and placebo groups) had higher cortisol levels (P=.002) at five years of age than term born children without a history of hospitalization

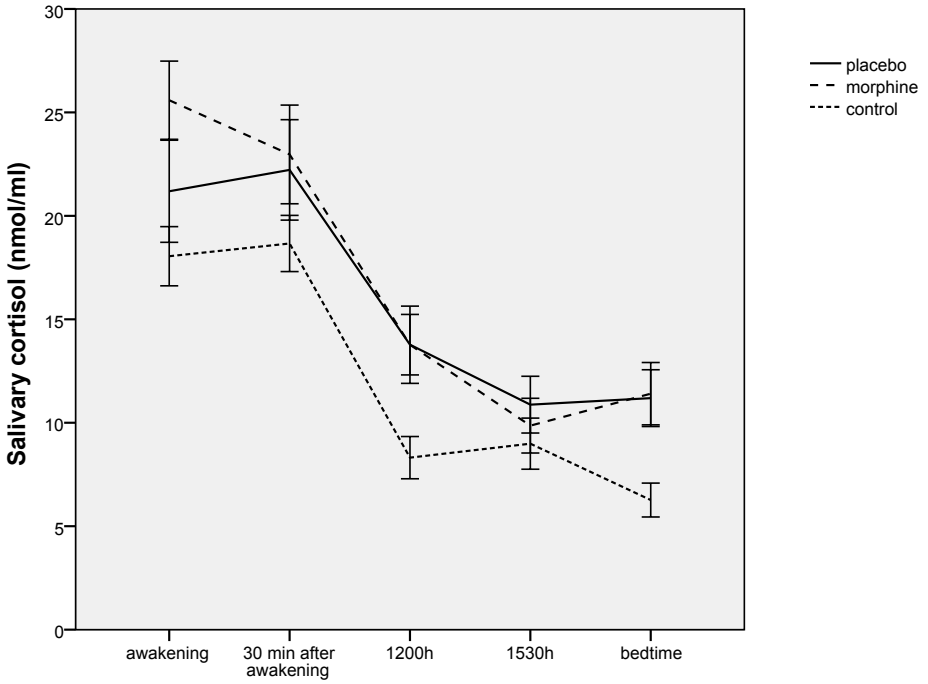


Figure 2. Salivary cortisol concentrations (mean, ± 1 SEM) at five time points during the day; directly after awakening (awakening), 30 minutes after awakening, before lunch (1200h), directly after school (1530h) and bedtime in NICU patients who received continuous morphine or placebo and children without a hospital history (n=79). Abbreviations: SEM, standard error of the mean

(Table 4, model 2). Sex (P=.17) and SES (medium P=0.53, high P=.11) did not have a significant association with the cortisol concentrations.

Salivary cortisol concentration of children treated with continuous morphine did not differ significantly from children treated with placebo (P=.66) (Table 5, model 2). Additional morphine, sex (P=.08), SES (medium SES P=0.44, high SES P=.07) and gestational age (P.94) did not have a significant effect on the cortisol concentrations.

DISCUSSION

We found that cortisol levels across the day of children admitted at neonatal age to a NICU were during the day higher than those in full-term control children, which supports the 'early-life programming' concept of the HPA axis. The diurnal pattern of cortisol secretion did not significantly differ between children treated with continuous morphine five years earlier and children treated with placebo.

Table 3. Salivary cortisol (nmol/ml) for placebo and morphine cohorts, and healthy controls, over the day (N=79)

		Awakening	30 min after awakening	1200h	1530h	Bedtime
Time of sampling	N (%)	73 (92)	77 (97)	72 (91)	78 (99)	77 (97)
	Median (IQR)	0710h (0659h to 0724h)	0743h (0730h to 0800h)	1210h (1200h to 1222h)	1530h (1527h to 1545h)	1930h (1910h to 1945h)
Salivary Cortisol						
Placebo	N	17	19	19	20	19
	Mean (CI 95%)	21.2 (16.0 to 26.4)	22.2 (17.1 to 27.3)	13.8 (10.7 to 16.8)	10.9 (8.0 to 13.7)	11.2 (8.3 to 14.1)
	Median (IQR)	18.4 (14.6 to 24.1)	18.1 (13.4 to 36.5)	12.0 (9.2 to 15.5)	9.3 (6.4 to 14.4)	8.3 (6.8 to 17.1)
Morphine	N	19	19	17	20	20
	Mean (CI 95%)	25.9 (21.6 to 29.6)	23.0 (18.0 to 28.0)	13.8 (9.8 to 17.7)	9.9 (7.1 to 12.6)	11.4 (8.3 to 14.6)
	Median (IQR)	23.5 (18.8 to 33.5)	19.6 (14.6 to 27.4)	10.7 (8.2 to 20.1)	8.4 (6.4 to 11.4)	7.8 (6.5 to 17.7)
Control	N	37	39	36	38	38
	Mean (CI 95%)	18.0 (15.1 to 21.0)	18.7 (15.9 to 21.4)	8.3 (6.2 to 10.4)	9.0 (6.5 to 11.5)	6.3 (4.6 to 7.9)
	Median (IQR)	15.7 (12.0 to 23.8)	18.3 (12.4 to 23.7)	7.8 (4.4 to 9.6)	7.5 (4.6 to 11.0)	4.7 (2.9 to 8.7)

Table 4. Results random regression analysis with salivary cortisol at five years of age as outcome variable; NICU population versus controls cohort

	Model 1*			Model 2**		
	Estimate	**SE**	**P**	**Estimate**	**SE**	**P**
Intercept	23.8	1.0	**<.001**	27.3	3.5	**<.001**
Time	-2.5	0.2	**<.001**	-2.5	0.2	**<.001**
Time²	0.1	0.02	**<.001**	0.1	0.02	**<.001**
Treatment group: NICU vs control	-4.7	1.4	**<.001**	-4.5	1.4	**.002**

SE, standard error of regression estimate; Treatment group: control=0 and NICU=1
Model 1: unadjusted for covariables, Model 2: adjusted for sex and SES

One of the objectives of the original RCT was to examine if continuous morphine would reduce pain levels. This was not the case, although it proved to be associated with lower noradrenaline levels during NICU admission[41]. As noradrenaline is produced by acute stress, we hypothesized that receiving morphine would have led to a less activated HPA axis during NICU period, leading to less HPA axis activation on long term and thus lower cortisol levels. The present study, however, did not find any evidence for effects of morphine administration on long-term cortisol levels five years later. In studies of Grunau and colleagues (2004, 2005), in which morphine administration was only reported as a

Table 5. Results random regression analysis with salivary cortisol at five years of age as outcome variable; morphine versus placebo cohort

	Model 1*			Model 2**		
	Estimate	**SE**	**P**	**Estimate**	**SE**	**P**
Intercept	24.2	1.7	**<.001**	26.8	10.8	**.02**
Time	-2.9	0.3	**<.001**	-2.9	0.3	**<.001**
Time²	0.2	0.02	**<.001**	0.2	0.02	**<.001**
Treatment group: morphine vs placebo	0.3	2.1	.88	0.9	2.0	.66
Additional morphine	0.6	1.2	.61	1.0	1.2	.38

SE, standard error of regression estimate; treatment group: placebo=0 and morphine=1
Model 1: unadjusted for covariables, Model 2: adjusted for sex and SES

'covariable', also had no effect on cortisol levels at 32 weeks post-conceptional age while children were still in the NICU, or at three, eight or 18 months corrected age, long after hospital discharge[16-18].

The present study provides further evidence that former NICU-children have higher cortisol levels later in life, as several other studies have reported[5,16,18]. A possible explanation for higher long term cortisol levels in premature born children compared to term born controls is the vulnerability of the immature neuro-endocrine system. However, in contrast to a previous study (2007) in former preterm born children by Buske-Kirschbaum and colleagues[5], the present study found no association between cortisol levels and gestational age. This may be due to the fact that the numbers of term born (>37 weeks of gestation) and late preterm born (gestational age: 32-37 weeks) were low, 1 (2.5%) and 8 (20%) respectively. An effect of gestational age could have been missed. Another explanation could be that it is not prematurity as such but rather the adverse early life experiences such as medication or painful experiences that affect the physiological systems in the long term[27,37,49]. Grunau and colleagues showed that greater cumulative exposure to painful (skin breaking) procedures was associated with altered cortisol level in infants born at extremely low gestational ages (≤28 weeks), independent of illness severity or cumulative exposure to intravenous morphine[16,18]. Furthermore, Grunau and colleagues found that their baseline cortisol levels exceeded those in more mature preterm infants and full-term controls, suggesting that the timing of immaturity of physiological systems may be crucial for the impact of prolonged stress[16,18]. Importantly, our findings in the present study indicate that not only do higher cortisol levels persist to school-age, but that this effect was found in a broader range of gestational ages. Other possible explanations, for the increased long term cortisol levels we found in our NICU sample, that cannot be excluded in our study are for example medication use or altered interactions between the child and its parents and its peers.

The strength of the present study is that we evaluated the diurnal cortisol secretion rhythm of children who underwent mechanical ventilation in a NICU. However, the diurnal cortisol secretion rhythm is just one of many aspects of HPA axis function. Other aspects worth examining are activation of the HPA axis relative to stressors and personal experiences[22,23,28], or cumulative stress as indexed by hair cortisol[46]. The present study was part of a larger study, and involved a 4-hour hospital visit. Therefore, an additional stress-test was not considered feasible[9]. A second limitation of our study was that we were only able to include control children of parents of middle and high socioeconomic status as they were the only ones willing to participate. Of relevance is that children with high socioeconomic status have lower cortisol levels than children with low socioeconomic status[20,25,36]. Nevertheless, it seems unlikely that this affected our findings, since we adjusted statistically for SES. Third limitation is that we did not record children's physical and mental health at the day of testing, which may have influenced the diurnal cortisol secretion. This may have created biased statistical inferences. Fourth limitation is that we did not collect behavioral and cognitive data of the control cohort and therefore were not able to adjust for these covariables. Both outcome at 5 years[9] and 8/9 years[11] of this same NICU sample are consistent with intelligence and behaviour not differing from the general population. Nonetheless, at 8/9 years a higher percentage of children in this NICU sample attended special education than the general Dutch population. It is unknown whether these academic problems affects cortisol levels.

The long term clinical implications of higher cortisol levels in our NICU sample are unknown. Alterations in HPA activity throughout life will impact on adult health because of altered tissue exposure to endogenous glucocorticoids. Elevated plasma cortisol has been associated with dyslipidemia, diabetes mellitus, atherosclerosis, immunosuppression, depression and cognitive impairment[8,35] as well as with psychiatric disorders such as social fear, anxiety disorders and depression[3,4,15,21,24,48]. Understanding the mechanisms that underlying developmental programming of the HPA axis could hold the key to therapeutic interventions aimed at reversing the impact of an adverse intrauterine and neonatal environment.

In summary, admission to a NICU appears to be associated with higher levels of diurnal cortisol secretion rhythm at age 5 years, suggesting early programming of the hypothalamic-pituitary-adrenal axis. In our cohort, gestational age did not contribute significantly to later cortisol levels and should be further evaluated. Importantly, morphine infusion in the neonatal period did not ameliorate or exacerbate the association with upregulation of cortisol secretion at school entry age.

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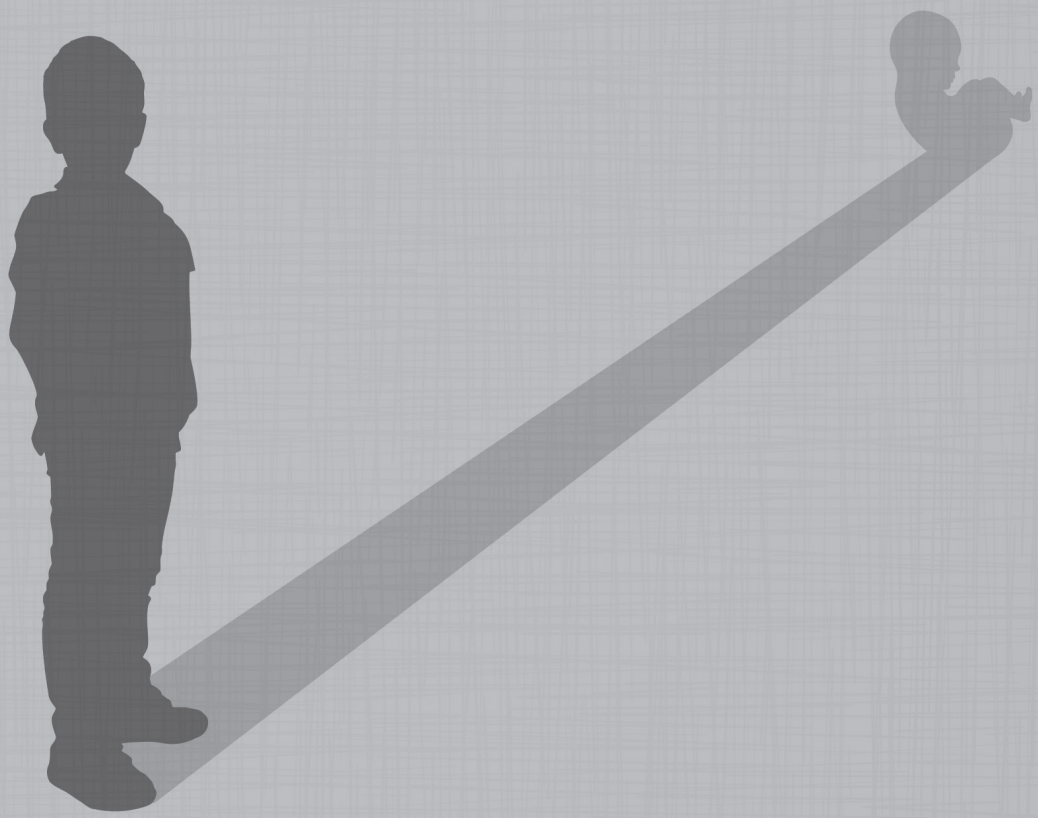
PART III

PAIN PERCEPTION

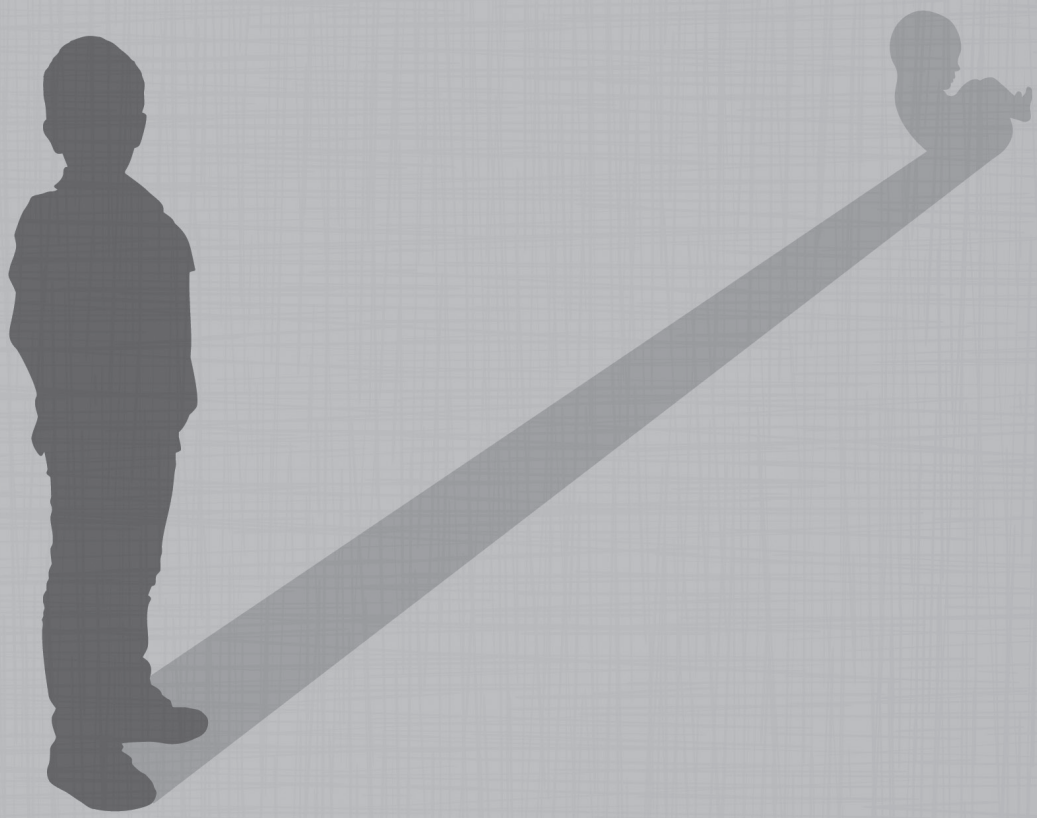


Chapter 5

Thermal detection thresholds in 5-year-old preterm born children; IQ does matter

Joke de Graaf, Abraham J. Valkenburg, Dick Tibboel, Monique van Dijk

Early Hum Dev. Jul 2012;88(7):487-491



ABSTRACT

Background

Experiencing pain at newborn age may have consequences on one's somatosensory perception later in life. Children's perception for cold and warm stimuli may be determined with the Thermal Sensory Analyzer (TSA) device by two different methods.

Aim

This pilot study in 5-year-old children born preterm aimed at establishing whether the TSA method of limits, which is dependent of reaction time, and the method of levels, which is independent of reaction time, would yield different cold and warm detection thresholds. The second aim was to establish possible associations between intellectual ability and the detection thresholds obtained with either method.

Study design

A convenience sample was drawn from the participants in an ongoing 5-year follow-up study of a randomized controlled trial on effects of morphine during mechanical ventilation.

Methods

Thresholds were assessed using both methods and statistically compared. Possible associations between the child's intelligence quotient (IQ) and threshold levels were analyzed.

Results

The method of levels yielded more sensitive thresholds than did the method of limits, i.e. mean (SD) cold detection thresholds: 30.3 (1.4) versus 28.4 (1.7) (Cohen's d=1.2, P=0.001) and warm detection thresholds; 33.9 (1.9) versus 35.6 (2.1) (Cohen's d=0.8, P=0.04). IQ was statistically significantly associated only with the detection thresholds obtained with the method of limits (cold: r 0.64, warm: r=-0.52).

Discussion

The TSA method of levels, is to be preferred over the method of limits in 5-year-old preterm born children, as it establishes more sensitive detection thresholds and is independent of IQ.

INTRODUCTION

Preterm born children have to endure painful treatment in neonatal period[5,21], which may have consequences on sensitivity to pain[5,11,16,25-27], responses to pain[10,12] and somatosensory perception[20,28] later in life. For example, 8 to 12-year-old preterm born children were found to be less sensitive in detecting a thermal stimulus[13,28] and showed higher pain sensitization after prolonged thermal stimulation[13] than their term born counterparts. Somatosensory perception can be objectively measured with quantitative sensory testing using the Thermal Sensory Analyzer (TSA)[2,14,15,18]. This device produces thermal stimuli to determine cold and warm detection thresholds as well as pain thresholds. The TSA has been validated for use in children from the age of 4 years[14]. We suspected, however, that application at this young age could present problems, especially for preterm born children, who have a greater risk of intellectual disabilities[17,19]. The two studies in preterm born children[13,28] applied thermal stimulation with different equipment, but both with the method of limits, in which the subject presses a button as soon as the stimulus is felt. The results, therefore, are dependent on reaction time. In adults with a mild intellectual disability this dependency resulted in elevated pain thresholds compared to the controls, probably due to a slower reaction time[9]. An alternative method – not dependent on reaction time – is the method of levels, in which the subject receives computer-set thermal stimuli and is asked whether they were felt.

We conducted a pilot study in 5-year-old preterm born children who as neonates had participated in a randomized controlled trial (RCT) on pain-reducing effects of morphine during mechanical ventilation[22-24]. The first aim was to find out whether detection thresholds obtained with either method would differ. The second aim was to establish possible associations between intellectual ability and the detection thresholds obtained with either method.

METHODS

Participants

A convenience sample was drawn from the participants in an ongoing 5-year follow-up study (n=90) of the above-mentioned RCT (n=150) conducted in the Erasmus MC-Sophia Children's Hospital, Rotterdam and Isala Clinics, Zwolle; both in The Netherlands. For more details see Simons et al[22] and de Graaf et al[7]. Eligibility for the present study was restricted to children seen in the Erasmus MC-Sophia Children's Hospital (n=54). At the time the Erasmus MC medical ethical review board approved this study, thirteen children had already undergone the 5-year follow-up program. These children were

not invited to participate again. Parents or caregivers of 41 children who had not yet undergone the 5-year follow-up were sent a letter explaining the purpose of the follow-up program, the TSA assessment and the methodology used. Consent for a medical visitation, psychological assessment and the additional TSA assessment was sought. The Erasmus MC medical ethical review board approved this study.

Background characteristics

The original RCT[22-24] provided details on: sex, gestational age, birth weight, total duration of first hospital admission (NICU admission + transfer), duration of primary mechanical ventilation, Clinical Risk Index for Babies (CRIB) and total morphine consumption during first admission. At 5-year follow-up, the child's intelligence quotient (IQ) was measured with the Revision Amsterdam Child Intelligence Test (RAKIT)[3,4]. This test produces a mean intelligence quotient (IQ) score of 100 and standard deviation (SD) of 15, based on a Dutch norm group.

Test procedure

Cold and warm 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). A standardized protocol, comparable with other studies[2,9,14,15,18], was used for the test procedure. The test took place in a quiet room at constant room temperature (approximately 21^{0}C), in the presence of a parent but without physical contact. Parents were asked not to interact with their child during the assessment. First, the procedure was explained and the child was invited to touch the thermode at baseline level of 32^{0}C. Actual testing did not start until we were sure that the child fully understood it. To prevent a painful sensation, the minimum temperature was set at 20^{0}C; the maximum at 40^{0}C. Set at 32^{0}C baseline temperature, the thermode was attached on the thenar eminence of the non-dominant hand.

First, the cold detection threshold was assessed using the method of limits. Starting from 32^{0}C, the temperature was steadily decreased at a rate of 1.0^{0}C/sec. The child was asked to press a button as soon as a cold sensation was felt, upon which the temperature directly reversed to baseline with a return rate of 10.0^{0}C/sec. Next, the warm detection threshold was determined likewise, but now with steadily increasing temperature. Reliability was enhanced by administering series of four stimuli, of which the first served as a rehearsal stimulus. Either detection threshold was calculated as the mean value of the temperatures obtained with the second, third and fourth stimulus.

Second, the method of levels was applied to determine the cold and warm detection thresholds, in this order. The thermal stimuli were presented in a series set by the

computer. The first stimulus was 2.0^{0}C below baseline (32.0^{0}C). Following the thermal stimulus the researcher asked the child "did you feel something or did I make a joke?", which is an adaptation of the recommended test question "did you feel something yes or no". The adaption was made because 5-year-olds are inclined to automatically answer the first question with 'yes'. Dependent on the child's response, the following stimulus was increased or decreased by one half of the initial temperature change. The test terminated when the step size of the stimulus had decreased to a level of 0.1^{0}C. The warm detection threshold started with a stimulus 2.0^{0}C above baseline.

Data analysis

Participants and non-participants background characteristics were compared using the Mann-Whitney test in case of non normally distributed variables. Mean detection thresholds obtained with the method of limits and method of levels were compared using the paired-samples t-test. Pearson product moment correlation coefficients served to identify linear associations between detection thresholds and between IQ and detection thresholds. Outcome differences between the two methods were visualized using Bland-Altman plots [1]. Mean scores and standard deviations (SD) were compared with the mean scores (SD) of a norm population [14] by the independent groups t-test. Cohen's *d* (standardized mean difference) was used to estimate the magnitude of differences between mean scores. Differences were regarded as small at Cohen's *d* of 0.20, medium at 0.50, and large at 0.80 [6]. All statistical tests took place at 0.05 level of significance (two-sided). Data were analyzed using the statistical software package SPSS 17.0 for Windows.

RESULTS

Background characteristics

Of the 41 eligible children, 21 (51%) were actually tested. Five children (12%) were lost to follow-up, parents of 10 children (24%) did not give consent for the entire 5-year follow-up examination, and in 5 other cases (12%), either the child or its parents did not consent to TSA testing. None of the tested children was sick or used pain medication on the day of the assessment. Four of the 21 children (17%) failed to complete all TSA tests because they lost concentration. Consequently, 17 out of 21 children (81%) completed all tests. The participants included statistically significantly more boys than the non-participants (P=0.05) (Table 1). Participants experienced more painful procedures during the first 14 days of life than non-participants (P=.008). The median age of the 17 children who completed TSA testing was 5.4 (range 5.2 to 5.8) years.

Table 1. Demographic and clinical characteristics of participating children (n=17)

		Participating children completing TSA	Non-participating children	P
		n=17	n=24	
Sex, n(%)	Boy/ Girl	13 (77)/ 4 (24)	11 (46)/ 13 (54)	.05
Gestational age (weeks)	Median (range)	28 (25 to 33)	28 (25 to 41)	.95
Birth weight (grams)	Median (range)	920 (675 to 1630)	1062 (590 to 3575)	.40
total duration of first hospital admission (days)	Median (range)	79 (28 to 149)	79 (31 to 137)	.88
Duration primary mechanical ventilation (hrs)	Median (range)	156 (21 to 600)	75 (10 to 1032)	.11
CRIB	Median (range)	3 (0 to 9)	4 (0 to 10)	.50
Study condition, n(%)	Morphine/ Placebo	10 (59)/ 7 (41)	7 (29)/ 17(71)	.06
Total morphine consumption first admission (mcg/kg)	Median (range)	1153 (0 to 4873)	363 (0 to 4746)	.14
Number of painful procedures first 14 days	Median (IQR)	17 (2 to 21)	14 (2 to 19) [n=22]	**<.008**
Age at assessment (years)	Median (range)	5.4 (5.2 to 5.8)	-	-
socioeconomic status, n(%)	Low/ Middle/ High	2 (12)/ 4 (24)/ 11 (65)	-	-
IQ	Median (range)	97 (79 to 121)	105 (66 to 115)	.57

Abbreviation: CRIB, Clinical Risk Index for Babies; IQ, Intelligence quotient

Cold and warm detection thresholds

Detection thresholds obtained with the two methods were not significantly correlated; i.e: r= 0.26 (95% CI -0.25 to 0.66; P=0.32) for cold sensation and r =-0.18 (95% CI -0.61 to; 0.33, P=0.49) for warm sensation. The mean (SD) cold detection thresholds differed significantly: 28.4 (1.7) for the Method of Limits versus 30.3 (1.4) for the method of levels (Cohen's d=-1.2, P=0.001), and so did the mean (SD) warm detection thresholds; 35.6 (2.1) for the method of limits versus 33.9 (1.9) for the method of levels (Cohen's d=0.8, P=0.04) (Table 2).

Table 2. Mean detection thresholds expressed as degrees Celsius (^{0}C)

	n=17	P[a]
Cold sensation		
Method of limits, mean (SD)	28.4 (1.7)	0.001
Method of levels , mean (SD)	30.3 (1.4)	
Warm sensation		
Method of limits, mean (SD)	35.6 (2.1)	0.04
Method of levels, mean (SD)	33.9 (1.9)	

[a]paired T-test

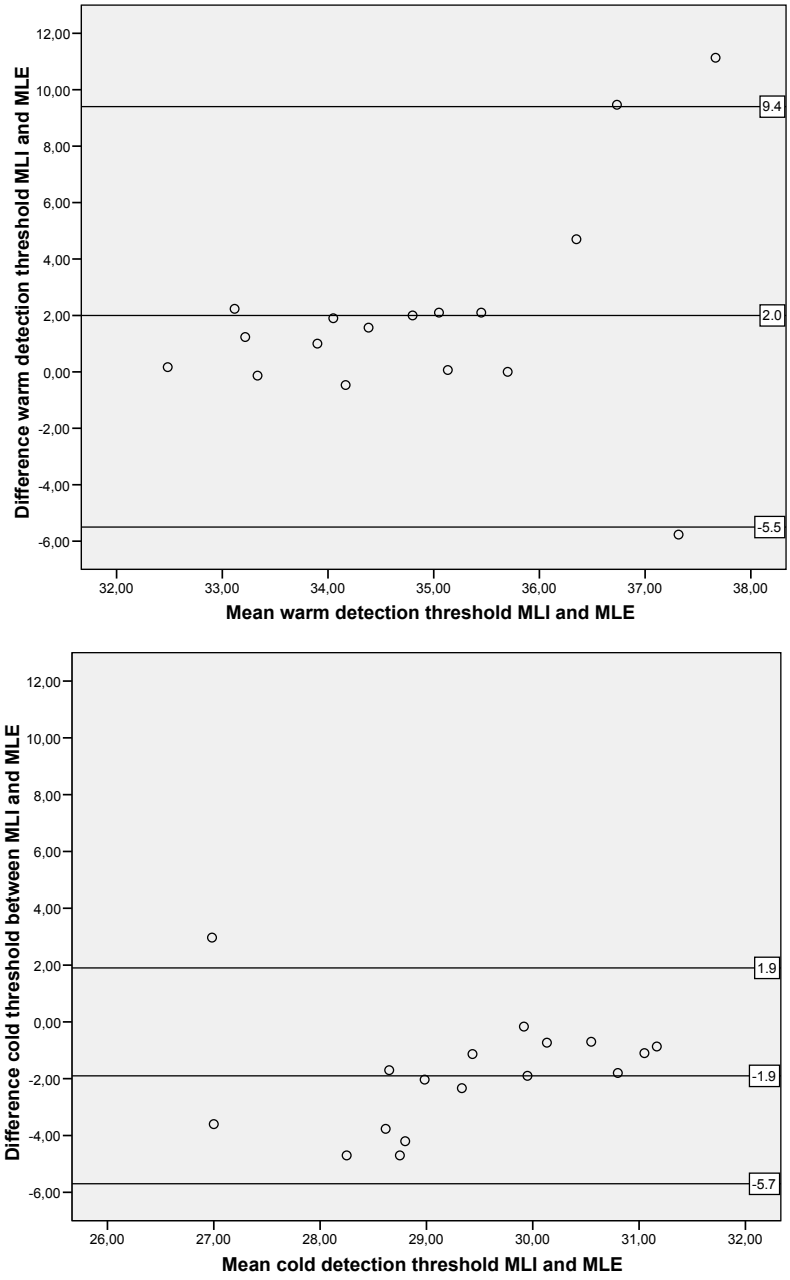


Figure 1. Bland-Altman plots
(A) Relation between the mean cold detection thresholds of both methods and the difference in cold detection thresholds between both methods, including the mean difference and limits of agreement
(B) Relation between the mean warm detection thresholds of both methods and the difference in warm detection thresholds between both methods, including the mean difference and limits of agreement

Differences between the two methods were more profound when children had lower cold detection thresholds and higher warm detection thresholds, as shown in the Bland-Altman-plots (Figure 1A and 1B). Both plots show one outlier, representing a more sensitive threshold in the method of limits rather than the method of levels. These two outliers were not deviant with respect to background characteristics, IQ or neonatal pain exposure and opioid use. The mean (SD) cold and warm detection thresholds obtained with the method of limits differed significantly from the norm values[14], i.e. 28.4 (1.7) versus 29.3 (1.2)[14] for cold sensation (Cohen's d=-0.61, P=0.01) and 35.6 (2.1) versus 34.0 (1.0)[14] for warm sensation (Cohen's d=0.97, P<0.001).

The Pearson correlation coefficient between IQ and the cold detection threshold measured with method of limits was r=0.64 (95% CI 0.23 to 0.86; p=0.006); that between IQ and the warm detection threshold was r=-0.52 (95% CI -0.80 to -0.06; p=0.03). Cold and warm detection thresholds established with the method of levels were not significantly correlated with IQ scores, i.e. r=0.24 (95% CI -0.27 to 0.64; p=0.36) and r= 0.03 (95% CI -0.46 to 0.50; p=0.91).

Assessment

The detection thresholds of 4 out of the 17 participants (24%) reached the temperature limit of the TSA. For the warm detection threshold, one child reached the maximum temperature of 40^{0}C with the method of limits and another child reached this maximum temperature with the method of levels. During warm detection threshold testing with the method of levels, two other children responded to the baseline temperature of 32^{0}C with 'yes, I felt something'. After another 5 stimuli of 32^{0}C, which all were felt, the procedure was manually stopped. Their warm detection threshold was then established at 32^{0}C.

The Method of Levels required a median number of 8 stimuli (range 5 to 14) to obtain the cold detection threshold and 8 stimuli (range 5 to 16, n=15) to obtain the warm detection threshold. Including instruction, the method of limits took approximately 4 minutes to assess and the method of levels 8 minutes.

DISCUSSION

This pilot study in 5-year-old preterm born children, TSA testing proved challenging but feasible. Detection thresholds obtained by the method of limits significantly differed from thresholds obtained with the method of levels. One explanation could be

that there was a strong correlation between IQ and the detection threshold measured with the method of limits. Therefore the method of levels is to be preferred over the method of limits as it was not correlated with IQ and yielded more sensitive thresholds. The discrepancy between the method of limits and the method of levels is in line with previous studies in individuals with[9] and without[18,29] an intellectual disability. Yarnitsky et al (1991) concluded that reaction time may be the source of an elevated threshold for the method of limits, and advised to use the method of levels instead[29]. Remarkably, most TSA-studies use the method of limits regardless of this possible bias. To add, the findings from the present study point at another possible source of bias i.e., children with a lower IQ were less sensitive in detecting the cold or warm stimulus with the use of the method of limits than were children with a higher IQ. Such correlation was not found between IQ and the method of levels. Probably, this IQ effect can be explained from the correlation between IQ and reaction time[8]. Therefore, the significant difference between thresholds of our population and those of the norm population could be the result of slower response time in our population rather than diminished sensitivity.

TSA testing presented some practical problems. Four children lost concentration during the method of levels testing and failed to complete all TSA tests. Loss of concentration was not seen during the method of limits testing. As a possible reason, the method of levels was always administered after the method of limits. It also takes longer because it requires on average twice the number of stimuli applied with the method of limits. Furthermore, two children said they felt the baseline temperature already or even reached the maximum temperature of 40^0C with one of the two methods, which normally is quite unlikely. This could be an indication that they did not understand the instruction after all.

Several limitations of this study need to be addressed. First, the small sample size; only children who had not yet undergone the 5-year follow-up program were eligible for the present study. Second, it is not possible to substantiate our findings to the general population because a control cohort was lacking. Third, we did not randomize for order of methods. Fourth, we could not establish pain thresholds: the local medical ethical review board does not allow this in children under the age of 6 years. As a fifth limitation, TSA testing took place after a psychological assessment on the same day, on account of which the children may have been tired.

TSA assessment in 5-year-old preterm born children is feasible in the majority of children. Loss of concentration could be prevented by ensuring that children are not too tired. We recommend the method of levels to establish warm and cold detection thresholds. When it comes to the assessment of pain thresholds in older children, the method of

levels is less suitable as this requires applying thermal stimuli above the pain threshold. Presumably, this was a consideration in the previous studies in preterm born children[13,28] which used the method of limits instead. However, it is unknown if IQ and reaction time also influence the results of the method of limits applied to establish pain thresholds rather than detection thresholds. Future research on the long-term effects of neonatal pain should therefore adjust for reaction time and IQ.

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Chapter 6

Long-term effects of neonatal continuous morphine infusion on pain sensitivity: Follow-up of a randomized controlled trial

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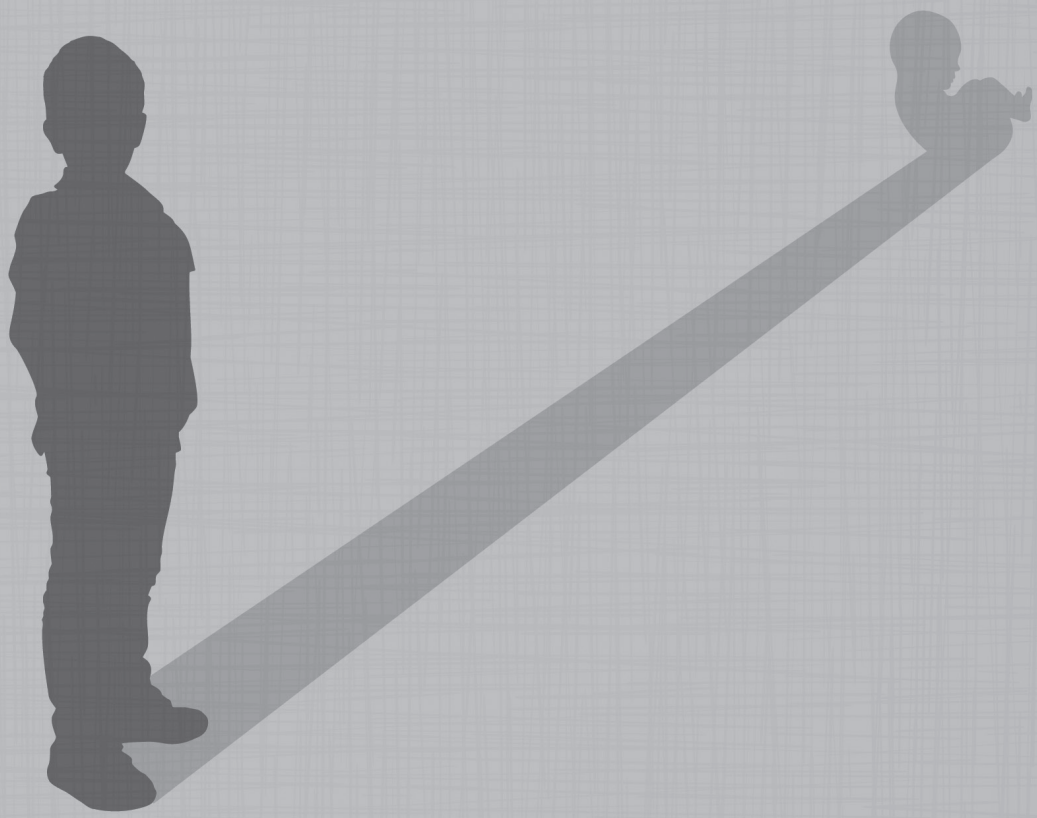
Submitted



ABSTRACT

Short-term and long-term effects of neonatal pain and its analgesic treatment have been topics of translational research over the years. The present study aimed to identify possible long-term effects of continuous morphine infusion on thermal pain sensitivity, incidence of chronic pain and neurological functioning. Eighty-nine of the 150 participants of a neonatal RCT on continuous morphine infusion versus placebo during mechanical ventilation underwent quantitative sensory testing and neurological examination at the age of 8 or 9 years. Forty-three children from the morphine group and 46 children from the placebo group participated. Thermal detection and pain thresholds were compared to data of 139 historical controls. Multivariate analyses revealed no statistically significant differences in thermal detection thresholds and pain thresholds between the morphine and placebo group. More children in the morphine group experienced an episode of pain in the three months before the follow-up visit compared to the placebo group, but the incidence of chronic pain (>3 months) was comparable. Neurological examination was normal in the majority of the children; mild deviations in coordination and balance were present in 7/9 (78%) of the morphine group versus 5/16 (31%) of the placebo group ($p=0.04$). We found in the present study that neonatal continuous morphine infusion (10 mcg/kg/hr) has no adverse effects on thermal detection and pain thresholds or overall neurological functioning eight to nine years later.

INTRODUCTION

Providing adequate and evidence-based analgesia and sedation to newborns receiving intensive care is an ongoing challenge; one has to account for developmental changes in drug pharmacokinetics and pharmacodynamics as well as in the human nervous system[12,23]. The short-term and long-term effects of neonatal pain and its analgesic treatment have become topics of translational research. The repeated painful procedures in intensive care can lead to short-term hyperalgesia[22]. Long-term follow-up of the extremely preterm born showed a generalized decrease of thermal sensitivity, probably due to tissue injury and modulation of nociceptor pathways[26]. Opioids have both beneficial and adverse short-term effects. Morphine is an effective analgesic agent for newborns' postoperative pain but not acute procedural pain[1,3,5,20]. However, continuous morphine infusion does not lower the risk of poor neurological outcome after intensive care[20]. Animal studies have shown negative long-term effects of neonatal morphine on cognitive functioning and proliferation of damaged astrocytes[13,21,25]. Participants of two RCTs on neonatal morphine administration were studied again five years after the original RCT[1,20]. The one, a small-scale follow-up of the NEOPAIN trial, showed that children who had received morphine (n=14) had a smaller head circumference, weighed less, and had more social problems than children who had received placebo (n=5)[8]. The other, performed in our institution, showed that children who had received morphine (n=49) performed more poorly on one subtest of the intelligence scale than did the children who had received placebo (n=41); other neurobehavioral outcomes and the incidences of chronic pain were comparable between the two groups[6]. This unique cohort is being followed and at the age of 8 years, participants were old enough for quantitative sensory testing[7].

Morphine is used worldwide for opioid analgesia in neonates, infants and children.

In the present study we aimed to identify any adverse effects of continuous morphine infusion on thermal detection thresholds and pain thresholds, incidence of chronic pain, and neurological functioning at 8 to 9 years of age.

METHODS

Original study

Between 2000 and 2002, 150 neonates who received mechanical ventilation in two level III neonatal intensive care units participated in a multi-center randomized controlled trial. Seventy-three neonates were randomly assigned to the continuous morphine

group (loading dose of 100 mcg/kg followed by infusion of 10 mcg/kg/hr) and 77 to the placebo group (normal saline). If pain or distress was noted, children in both groups received open-label morphine bolus of 50 mcg/kg and, if indicated, open-label morphine infusion (5-10 mcg/kg/hr) as rescue medication. Open-label morphine was administered to 27% of the children in the morphine group versus 40% of the placebo group (P=0.10). Further details, including background characteristics of the participants, can be found in the original article[20].

Follow-up study (8 to 9 year)

The institutional ethics review boards of the two study sites (Erasmus University Medical Center - Sophia Children's Hospital, Rotterdam, the Netherlands) and Isala Clinics, Zwolle, the Netherlands) approved the study plan. Parents of the 132 survivors were informed of the study and were asked for written informed consent. Seventeen participants from the morphine group were lost to follow-up and 5 parents refused informed consent for the follow-up study. Sixteen patients of the placebo group were lost to follow-up and 5 parents refused informed consent for the follow-up study. The remaining 89 children and their parents were then invited for a follow-up visit in their hospital (either Rotterdam or Zwolle). (See Figure 1)

Parents were asked to complete several questionnaires (see below) before the visit. The visit consisted of three parts: Quantitative sensory testing by a trained researcher, medical examination by a pediatrician and neuropsychological testing by a psychologist. These health professionals were blind to the participants' study condition (continuous morphine infusion versus placebo) in the original RCT.

Quantitative sensory testing

Participants underwent quantitative sensory testing in a quiet hospital room, with a stable room temperature (20 to 22 degrees Celsius). Parents were present in the room and were instructed 'not to interfere during the test'. Reaction time was measured using the baseline speed task for the dominant hand (Amsterdam Neuropsychological Tasks, Version 3.1, Boom test publishers, Amsterdam, the Netherlands). This computerized visual-motor task includes 32 repetitions. Skin temperature was measured at the thenar eminence of the non-dominant hand to confirm it was within the range of 27 to 37 degrees Celsius[9].

Thermal thresholds were measured at the thenar eminence of the non-dominant hand, using the Thermal Sensory Analyzer-II (Medoc Ltd, Ramat Yishai, Israel) with the 30 by 30 mm thermode. Baseline temperature for all measurements was 32 degrees Celsius, the minimum temperature was 0.0 °C and the maximum temperature was 50.0 °C. Detection thresholds were measured using both the method of limits (reaction time dependent) and

the method of levels (reaction time independent). The standardized instructions were in accordance with other quantitative sensory testing studies in children[4,7,17]. Six modalities were tested in the following order. 1) Detection threshold for cold (method of limits): Six repetitions, temperature decreased by 1.0 °C/s. Children were asked to press the button as soon as the cold stimulus was perceived. 2) Detection threshold for warmth (method of limits): Six repetitions, temperature increased by 1.0 °C/s. Children were asked to press the button as soon as the warm stimulus was perceived. 3) Pain threshold for cold (method of limits). Five repetitions, temperature decreased by 1.5 °C/s. Children were asked to press the button as soon as the stimulus was so cold it became painful. A note was made when the minimum temperature (0.0 °C) was reached. 4) Pain threshold for heat (method of limits): Five repetitions, temperature increased by 1.5 °C/s. Children were asked to press the button as soon as the stimulus was so hot it became painful. A note was made when the maximum temperature (50.0 °C) was reached. 5) Detection threshold for cold (method of levels): Children were asked per step if the cold stimulus was perceived, until the difference between two steps was less than 0.1 °C/s. The number of steps needed was recorded. 6) Detection threshold for warmth (method of levels): Children were asked per step if the warm stimulus was perceived, until the difference between two steps was less than 0.1 °C/s. The number of steps needed was recorded.

Detection thresholds established by the method of limits were calculated as the mean value of the final four of the six measurements. The first two measurements were used to assure that the child understood the test correctly. Pain thresholds established by the method of limits were calculated as the mean value of the final four of five measurements. The first measurement was used to assure that the child understood the test correctly. Detection thresholds established by the method of levels were measured once. In case the participant did not establish a pain threshold before the minimum or maximum temperature was reached, the device recorded the minimum temperature (0.0 °C) / maximum temperature (50.0 °C) as the result.

Reference data Quantitative Sensory Testing (historical controls)

Reference values for quantitative sensory testing in 139 children between 7 and 11 years have been collected by our group.(http://repub.eur.nl/res/pub/8210/Early%20Pain.pdf). Subjects (ages 7 through 11 years) were recruited at two elementary schools in the reference area of the Erasmus University Medical Center – Sophia Children's Hospital, Rotterdam, the Netherlands. The local ethics committee and the Dutch Central Committee on Research involving Human Subjects approved the study. Parents provided written informed consent for the study. Exclusion criteria were a history of surgery or admission to a neonatal intensive care unit.

The cold/warm detection and cold/heat pain thresholds were obtained using the method of limits. Instructions and methods were the same as in the present study. The only difference was that the lower limit for the cold pain threshold was -10 °C, where nowadays 0.0°C is the lower limit.

All 139 subjects were tested between July 2004 and August 2005. Eighty-one (58%) of them were male. The median [IQR] age was 8 [7 to 9] years.

Questionnaires

The participants' parents completed the Vineland Adaptive Behavior Scale - Screener (Dutch version, PITS B.V., Leiden, the Netherlands)[24]; the four domains of this scale are communication, daily living, socialization and motor skills. Parents also completed the Chronic Pain Questionnaire (Dutch version)[18]; this questionnaire asks for the incidence of pain in the 3 months before the visit and additional information about the pain (location, frequency, duration and intensity).

Medical examination

All children were examined by a pediatrician and were, if indicated, referred for further diagnosis or treatment. Weight, height and head circumference were measured and plotted against sex-matched reference values for the Netherlands (4th nation-wide growth study 1997). The neurological examination was based on the Touwen assessment of minor neurological dysfunctions[14]. We assessed 41 items in 5 domains (posture/muscle tone, reflexes, involuntary movements, coordination/balance and cranial nerve dysfunctions). Minor neurological dysfunctions were defined as the presence of 2 or more deviant items in at least one domain.

Neuropsychological testing

A trained psychologist tested the children's intelligence quotient (IQ) with the Wechsler Intelligence Scale for Children - III (Dutch version).

Statistical analysis

Summary statistics of continuous variables are presented as median [interquartile range] and as percentage for ordinal and categorical variables. Data were compared between the continuous morphine and the placebo group using the Mann-Whitney test for continuous, non-normal data and the chi-square test (or Fisher exact test in case of low predicted cell counts) for nominal data. The quantitative sensory testing data is compared to reference values of 139 historical controls using analysis of variance with post-hoc Bonferroni correction for multiple comparisons.

For the multivariate analysis of the quantitative sensory testing data, we built stepwise robust regression models for each of the six modalities, using robust regression procedure with MM estimation[27]. We applied this method for the very reason that the outcome variables were non-normally distributed. Tukey bisquare estimator was the weight function. In the first model, we added the treatment condition (continuous morphine versus placebo) and the amount of additional morphine in the first 28 days after birth as covariables. In the second model, we added sex and study site (Rotterdam versus Zwolle) as additional covariables. In the third model, we added the intelligence quotient as additional covariable. For the model on the cold pain threshold we added floor (pain threshold was not reached at minimum) and for the heat pain threshold ceiling (pain threshold was not reached at maximum) as covariable in all three models. For each model, we present the unstandardized regression estimates, including the 95% confidence intervals and P values (chi-square test).

Logistic regression analyses were applied with prevalence of pain and chronic pain as dichotomous outcome variables and the treatment condition (continuous morphine versus placebo) and the amount of additional morphine in the first 28 days after birth as predictor variables.

P values (two-sided) of less than 0.05 are considered statistical significantly. Data were analyzed by SPSS software, version 19.1 (IBM, Armonk, NY, USA) and SAS, version 9.2 (SAS, Cary, NC, USA).

RESULTS

Background characteristics

Of the 132 survivors, 43 children from the morphine group and 46 children from the placebo group participated in this follow-up study (See Figure 1). Their mean age was now 8.9 years. Background characteristics were comparable between the two groups (see Table 1). Mean IQ was 101 (SD 18) for the placebo group versus 99 (SD 19) for the morphine group (P = 0.63).

Quantitative sensory testing

Quantitative Sensory Testing (QST) was performed in 41(89%) of the placebo group and in 37(86%) of the morphine group. Nine children did not attend the follow-up visit (but completed the questionnaires) and the equipment was not available in the remaining 2 occasions. QST was feasible in all subjects with regard to understanding the instructions and completing the test.

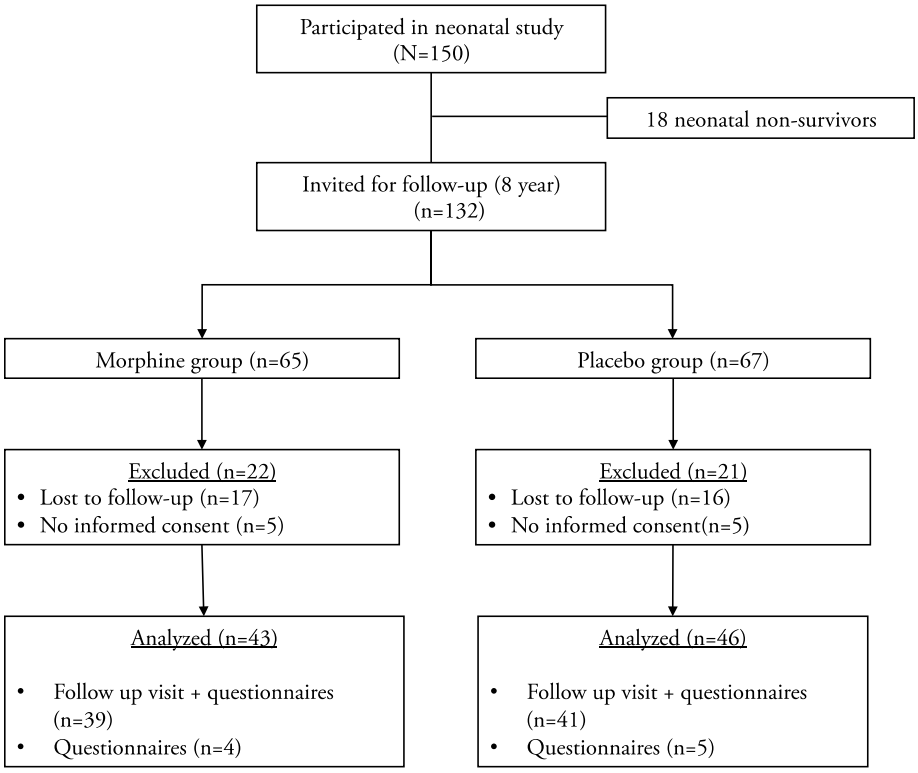


Figure 1. Flowchart based on the CONSORT flowchart

Univariate analysis showed that these children (both morphine and placebo group) were more sensitive for the detection of cold (method of limits), compared to the historical controls (P=0.002 and P=0.005 respectively). The children in the morphine group were more sensitive for the detection of cold than the children in the placebo group (method of levels, P=0.045) (see Table 2).

Ten (24%) of the children in the placebo group did not establish a pain threshold for cold before the minimum temperature (0.0 °C) was reached, versus 9 (25%) of the morphine group (P=0.82). Ten (24%) of the children in the placebo group did not establish a pain threshold for heat before the maximum temperature (50.0 °C) was reached, versus 8 (22%) of the children in the morphine group (P=0.95).

Regression estimates for the six QST modalities are presented in Tables 3 to 5. Neither treatment modality (morphine group versus placebo) nor the amount of additional morphine in the first 28 days was a statistically significant predictor in any of the models.

Table 1. Background Characteristics (distinguished by group)

	Placebo group (n= 46)	**Morphine group (n=43)**	**P value[a]**
	Median [IQR] or number (%)		
Sex, n(%) male	28 (61)	28 (65)	0.83
Birth characteristics			
Gestational age in weeks	31 (28 to 32)	30 (29 to 32)	0.39
Birthweight in grams	1520 (995 to 2145)	1400 (1024 to 1788)	0.70
Age in years	8.9 [8.7 to 9.3]	8.9 [8.8 to 9.1]	0.54
Height in cm	137 [131 to 140]	135 [131 to 140]	0.42
SDS height for age[b]	0.02 [-0.7 to 1.1]	-0.04 [-0.5 to 0.7]	0.72
Weight in kg	29.2 [26.5 to 32.8]	27.9 [24.6 to 31.6]	0.29
SDS weight for age[b]	0.05 [-0.6 to 0.7]	0.01 [-1.3 to 0.8]	0.53
Head circumference in cm	52 [51 to 53]	52 [51 to 54]	0.90
SDS head circumference for age[b]	-0.20 [-0.8 to 0.7]	-0.03 [-1.0 to 0.6]	0.92
IQ[c]	101 (18)	99 (19)	0.63
Developmental age in months[d]	72 [67 to 77]	73 [69 to 77]	0.41
Reaction time in ms[e]	391 [354 to 442]	390 [331 to 442]	0.82
Test location Rotterdam, n(%)	25 (54)	25 (58)	0.83

[a] Mann-Whitney test for continuous variables and Fisher exact test for categorical variables;
[b] According to reference values (the Netherlands 1997); [c] Intelligence Quotient (Wechsler Intelligence Scale for Children), mean (SD), *P* value from *t* test; [d] Vineland Adaptive Behavior Scale;
[e] Amsterdam Neuropsychological Tasks; IQR = Interquartile Range; cm = centimetre; kg = kilogram; ms = milliseconds.

For the cold and warmth detection thresholds (method of limits), IQ was a statistically significant predictor (P=0.024 and P=0.005 respectively). The higher the IQ, the more sensitive the children were for the detection of cold and warmth, see Table 3. Children who reached the minimum (0.0 °C) or maximum (50.0 °C) at one or more occasions, had statistically significantly higher mean pain thresholds. Children seen in the hospital in Zwolle (n=39) had statistically significant lower mean cold pain thresholds (P=0.0003), but we did not see an effect from the study site on the heat pain thresholds (P=0.26). (Table 4) For the warm detection thresholds (method of levels), IQ was a statistically significant covariable (P=0.029).

Chronic pain

Nine (20%) of the children in the placebo group versus 18 (43%) children in the morphine group experienced an episode of pain in the three months before the study visit, as reported by the parents in the Chronic Pain Questionnaire. This prevalence was entered in the logistic regression analysis as dichotomous outcome variable and treatment modality (morphine versus placebo group) was a significant predictor (P=0.02), but the amount of additional morphine in the first 28 days was not (P=0.98).

Table 2. Results of Quantitative Sensory Testing, distinguished by group

	Placebo group (n= 41)	Morphine group (n= 37)	Historical controls (n=139)	P value[a]
	Mean (standard deviation) or number (%)			
Method of Limits				
Cold detection threshold in °C	29.6 (2.4)	29.9 (1.4)	28.1 (3.1)	**<0.001[c]**
Warm detection threshold in °C	34.8 (1.4)	34.5 (1.4)	34.7 (2.2)	0.70
Cold pain threshold in °C	11.8 (8.4)	12.3 (8.9)	8.5 (9.7)	0.06
Threshold not reached[b]	10 (24%)	9 (25%)		0.82
Heat pain threshold in °C	45.0 (3.5)	44.9 (4.3)	44.8 (4.7)	0.97
Threshold not reached[b]	10 (24%)	8 (22%)		0.95
Method of Levels				
Cold detection threshold in °C	30.7 (1.2)	31.1 (0.7)		**0.045**
Number of stimuli	11 (3)	10 (2)		0.32
Warm detection threshold in °C	33.3 (1.1)	33.2 (1.1)		0.74
Number of stimuli	11 (4)	10 (2)		0.15

[a] ANOVA test for the comparison between the three groups, t-test for continuous variables and Fisher exact test for categorical variables; [b] Patients in whom 1 or more times the pain threshold was not reached (the child did not press the button before the temperature of the thermode reached its minimum/maximum (0.0 °C and 50.0 °C respectively); [c] Post-hoc Bonferroni correction: Placebo group versus Controls P=0.005 and Morphine group versus Controls P=0.002

Abdominal pain was the most common type of pain; it was present in 4 (44%) of the children in the placebo group versus in 10 (56%) of the children in the morphine group with pain (P=0.70). The second most common type of pain was headache; it was present in 6 (67%) of the children in the placebo group versus in 6 (33%) of the children in the morphine group with pain (P=0.13). Six (67%) children in the placebo group suffered from pain at more than one body site, versus 8 (44%) of the children in the morphine group with pain (P=0.42) The pain was chronic (duration longer than 3 months) in 4 (9%) children in the placebo group versus 5 (12%) in the morphine group (P=0.42), see Figure 2. There was a weak but significant correlation between a reported episode of pain in the last three months at the follow-up visit at 5 years versus a reported episode or pain at 8 to 9 years of age (64 subjects, r=0.32, P=0.01).

Neurological examination

Two children in the placebo group and two children in the morphine group were severe intellectually and developmentally disabled, and did therefore not attend the follow-up visit. Neurological examination was performed in 37 children of the morphine group and 41 of the placebo group, see Table 6. The neurological examination was normal in

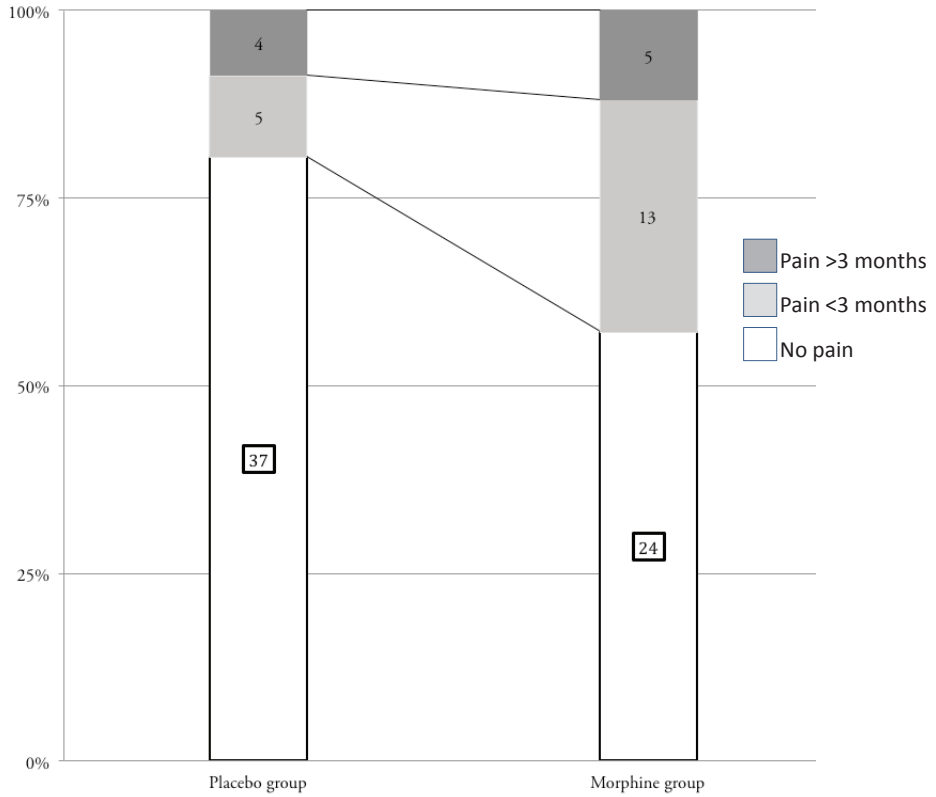


Figure 2. Prevalence of chronic pain, based on Chronic Pain Questionnaire, by group

28 (76%) of the children in the morphine and in 25 (61%) of the children in the control group. (P=0.14). In the children with minor neurological dysfunctions, mild deviations in coordination and muscle tone were more common in the morphine group (7/9 (78%)) than in the placebo group (5/16 (22%) (P=0.04). The presence of minor neurological dysfunctions at 8-9 years of age was not related to the presence of intraventricular hemorrhages in the neonatal period (*P*=0.26).

DISCUSSION

A cohort of children who as neonates participated in a RCT on continuous morphine infusion versus placebo for mechanical ventilation was seen at the age of 8-9 years. Univariate analysis revealed that children in the morphine group were more sensitive for the detection of cold (according to the method of levels). However, this was not confirmed in the multivariate analysis. The treatment condition (morphine versus placebo)

Table 3. Regression estimates of intervention, with and without adjustment for different covariables: Detection thresholds (method of limits)

		Estimate	95% CI limits	P value
Detection threshold for cold				
1	Intercept	30.13	29.85 to 30.41	<.0001
	Treatment condition[a]	0.21	-0.18 to 0.60	0.29
	Additional morphine[b]	0.00	-0.0002 to 0.0002	0.81
2	Intercept	30.21	29.79 to 30.63	<.0001
	Treatment condition[a]	0.22	-0.19 to 0.62	0.29
	Additional morphine[b]	0.00	-0.0002 to 0.0003	0.85
	Sex	-0.096	-0.51 to 0.32	0.65
	Study site	-0.047	-0.46 to 0.37	0.82
3	Intercept	28.95	27.80 to 20.09	<.0001
	Treatment condition[a]	0.25	-0.15 to 0.64	0.22
	Additional morphine[b]	0.0001	-0.0002 to 0.0003	0.57
	Sex	-0.15	-0.56 to 0.26	0.48
	Study site	-0.012	-0.42 to 0.39	0.95
	IQ[c]	0.012	0.0016 to 0.0228	**0.024**
Detection threshold for warmth				
1	Intercept	34.58	34.11 to 35.04	<0.0001
	Treatment condition[a]	-0.35	-0.98 to 0.29	0.29
	Additional morphine[b]	0.00	-0.0003 to 0.0004	0.87
2	Intercept	34.18	33.50 to 34.86	<0.0001
	Treatment condition[a]	-0.37	-1.006 to 0.26	0.25
	Additional morphine[b]	0.00	-0.0003 to 0.0004	0.82
	Sex	0.33	-0.33 to 0.99	0.33
	Study site	0.37	-0.27 to 1.01	0.26
3	Intercept	36.51	34.76 to 38.25	<0.0001
	Treatment condition[b]	-0.50	-1.09 to 0.10	0.10
	Additional morphine[c]	0.00	-0.0004 to 0.0003	0.88
	Sex	0.39	-0.23 to 1.01	0.21
	Study site	0.44	-0.16 to 1.04	0.15
	IQ[c]	-0.023	-0.039 to -0.0069	**0.005**

[a] Morphine group versus placebo group; [b] Additional morphine in first 28 days; [c] Intelligence Quotient (Wechsler Intelligence Scale for Children); CI = Confidence Interval

was not a significant covariable in any of the 6 quantitative sensory testing modalities. The multivariate analysis did show correlations between IQ and the detection thresholds, both with the reaction time dependent method of limits and the reaction time

Table 4. Regression estimates of intervention, with and without adjustment for different covariables: Pain thresholds (method of limits)

		Estimate	95% CI limits	P value
Pain threshold for cold				
1	Intercept	14.26	11.75 to 16.76	<0.0001
	Treatment condition[a]	1.67	-1.58 to 4.93	0.31
	Additional morphine[b]	-0.0013	-0.0032 to 0.0007	0.22
	Floor[c]	-4.49	-5.73 to -3.25	**<0.0001**
2	Intercept	16.35	13.31 to 19.40	<0.0001
	Treatment condition[a]	2.21	-0.66 to 5.08	0.13
	Additional morphine[b]	-0.0014	-0.0032 to 0.0004	0.12
	Sex	0.65	-2.34 to 3.65	0.70
	Study site	-5.38	-8.30 to -2.46	**0.0003**
	Floor[c]	-4.20	-5.31 to -3.09	**<0.0001**
3	Intercept	15.27	6.98 to 23.56	0.0003
	Treatment condition[a]	2.23	-0.67 to 5.13	0.13
	Additional morphine[b]	-0.0014	-0.0032 to 0.0004	0.14
	Sex	0.52	-2.53 to 3.57	0.74
	Study site	-5.42	-8.37 to -2.47	**0.0003**
	IQ[d]	0.012	-0.067 to 0.090	0.77
	Floor[c]	-4.19	-5.31 to -3.07	**<0.0001**
Pain threshold for heat				
1	Intercept	44.01	42.93 to 45.10	<0.0001
	Treatment condition[a]	0.42	-1.05 to 1.88	0.58
	Additional morphine[b]	-0.0004	-0.0031 to 0.0005	0.40
	Ceiling[c]	1.74	1.21 to 2.27	**<0.0001**
2	Intercept	44.02	42.48 to 45.57	<0.0001
	Treatment condition[a]	0.34	-1.13 to 1.81	0.65
	Additional morphine[b]	-0.0005	-0.0014 to 0.0004	0.31
	Sex	-0.61	-2.21 to 0.90	0.43
	Study site	0.86	-0.64 to 2.36	0.26
	Ceiling[c]	1.67	1.13 to 2.20	**<0.0001**
3	Intercept	45.50	41.07 to 49.93	<0.0001
	Treatment condition[a]	0.36	-1.10 to 1.82	0.63
	Additional morphine[b]	-0.0005	-0.0014 to 0.0004	0.27
	Sex	-0.55	-2.05 to 0.96	0.48
	Study site	0.92	-0.57 to 2.42	0.23
	IQ[d]	-0.015	-0.056 to 0.026	0.47
	Ceiling[c]	1.61	1.07 to 2.16	**<0.0001**

[a] Morphine group versus placebo group; [b] Additional morphine in first 28 days; [c] Pain threshold was not reached in one of more occasions; [d] Intelligence Quotient (Wechsler Intelligence Scale for Children); CI = Confidence Interval

Table 5. Regression estimates of intervention, with and without adjustment for different covariables: Detection thresholds (method of levels)

		Estimate	95% CI limits	P value
Detection threshold for cold				
1	Intercept	31.04	30.78 to 31.30	<0.0001
	Treatment condition[a]	0.21	-0.14 to 0.56	0.25
	Additional morphine[b]	-0.0001	-0.0003 to 0.0001	0.22
2	Intercept	31.05	30.66 to 31.44	<0.0001
	Treatment condition[a]	0.23	-0.14 to 0.59	0.23
	Additional morphine[b]	-0.0002	-0.0004 to 0.0001	0.17
	Sex	-0.14	-0.52 to 0.24	0.47
	Study site	0.12	-0.25 to 0.50	0.53
3	Intercept	30.74	29.66 to 31.82	<0.0001
	Treatment condition[a]	0.24	-0.13 to 0.61	0.20
	Additional morphine[b]	-0.0002	-0.0004 to 0.0001	0.18
	Sex	-0.16	-0.54 to 0.23	0.43
	Study site	0.13	-0.24 to 0.51	0.49
	IQ[c]	0.0030	-0.0069 to 0.013	0.55
Detection threshold for warmth				
1	Intercept	32.98	32.73 to 33.23	<.0001
	Treatment condition[a]	-0.079	-0.42 to 0.26	0.65
	Additional morphine[b]	0.0001	-0.0001 to 0.0003	0.27
2	Intercept	32.96	32.57 to 33.34	<.0001
	Treatment condition[a]	-0.090	-0.43 to 0.25	0.61
	Additional morphine[b]	0.0001	-0.0001 to 0.0003	0.17
	Sex	0.17	-0.18 to 0.53	0.34
	Study site	-0.14	-0.50 to 0.21	0.43
3	Intercept	33.95	32.99 to 34.92	<.0001
	Treatment condition[a]	-0.13	-0.46 to 0.19	0.42
	Additional morphine[b]	0.0001	-0.0001 to 0.0003	0.23
	Sex	0.22	-0.12 to 0.56	0.20
	Study site	-0.14	-0.48 to 0.19	0.39
	IQ[c]	-0.0098	-0.019 to -0.0010	**0.029**

[a] Morphine group versus placebo group; [b] Additional morphine in first 28 days; [c] Intelligence Quotient (Wechsler Intelligence Scale for Children); CI = Confidence Interval

independent method of levels. However, all correlations were weak (see Table 3 and Table 5) and clinically not significant.

Table 6. Minor Neurological Dysfunctions (distinguished by group)

	Placebo group (n=41)	Morphine group (n=38)	P value[a]
Minor Neurological Dysfunctions	16/41 (39%)	9/38 (24%)	0.14
Mild deviations in posture/muscle tone	6/16 (38%)	2/9 (22%)	0.66
Mild deviations in reflexes	4/16 (25%)	1/9 (11%)	0.62
Presence of involuntary movements	0/16 (0%)	0/9 (0%)	1.00
Mild deviations in coordination/balance	5/16 (31%)	7/9 (78%)	**0.04**
Mild deviations in cranial nerve function	1/16 (6%)	0/0 (0%)	1.00

[a] Fisher exact test

The children in both the placebo and the morphine group were more sensitive for the detection of cold (method of limits), compared to the reference data (1.5°C and 1.8°C, respectively). This might be due to methodological variations; cold detection threshold testing was the first test for the controls, so they might have needed to adapt to a test situation and setting, whereas the children in the present study already did several other tests before the quantitative sensory testing. Analysis of the other three method of limits modalities revealed no differences between the morphine or placebo group on the one hand and the reference data on the other hand.

Numbers of children who did not establish a pain threshold before reaching the minimum or maximum temperature were comparable between the morphine and placebo group. Blankenburg et al. provided an overview of QST studies in children that revealed a wide variation of pain thresholds, probably due to methodological variations (e.g. test site and instructions to participants)[4].The 95% confidence intervals in that study suggest that some children reached the minimum or maximum as well[4]. Our multivariate analyses showed that the floor/ceiling effect should be added as a covariable when analyzing QST data.

In two other follow-up studies[19,26], the lower limit of the thermal sensory analyzer was set at 10.0 °C, instead of the generally accepted 0.0 °C. International and multidisciplinary guidelines on quantitative sensory testing in children are needed to improve reproducibility of testing and reduce methodological variations[16].

Long-term effects of surgery and morphine

Hermann et al. reported long-term hypoalgesia for heat pain in both preterm and term born 9 to 14-year-old children who had received neonatal intensive care[11]. The preterm neonates in that study underwent a mean number of 172 invasive procedures in the first week of life; however, no more than 53% of them received analgesics. The hypoalgesia in

former extremely preterm born 11-year-olds, reported by Walker et al., was most marked in those who had undergone neonatal surgery[26]. In contrast, another study on long-term effects of neonatal surgery (all neonates received opioids postoperatively) showed no differences in the cold and warmth detection thresholds between the neonatal surgery group and the control group at the age of 9 to 12 years[19]. Combining the previous data with the results of present study, we hypothesize that that neonatal injury or surgery is likely to have more pronounced long-term effects on pain processing than neonatal morphine treatment itself.

Chronic pain

In both groups in the present study, prevalence of pain in the three months before the follow-up visit was lower than Dutch reference values (i.e. 58% for boys versus 75% for the girls respectively)[18]. Overall, the prevalence of a pain episode in the last three months did not significantly differ between boys and girls. The prevalence of chronic pain was comparable between the two groups and lower than the reference values (i.e. 11% for boys versus 13% for girls)[18]. Noteworthily, the prevalence of chronic pain in both groups had dropped since the 5-year follow-up visit, i.e. from 14% to 9% in the placebo group and from 15% to 12% in the morphine group[6].

Neurological functioning

The majority of the children had a normal neurological examination. The prevalence of minor neurological dysfunctions (i.e. 39% of the placebo group versus 24% of the morphine group) is comparable to the prevalence in a reference cohort of term born children (i.e. 50% prevalence of minor neurological dysfunctions)[14]. Mild deviations in coordination / balance control – a minor neurological dysfunction that could indicate cerebellar dysfunction – were more common in the morphine group of the present study (P=0.04). Research in rodents suggests that morphine treatment has a negative effect on the development of cerebellar neurons[10]. A study in human preterm born found that they were more at risk for cerebellar injuries during the neonatal period than term born and that at adolescent age their cerebellum volume was smaller than that in term born adolescents[15]. Minor cerebellar dysfunction could therefore be related to the preterm birth, but we cannot rule out an adverse effect from the higher morphine doses on cerebellar functioning.

The pilot follow-up study of the NEOPAIN trial found an overall lower prevalence of neurological soft signs (i.e. 20% in the placebo group (n=5) versus 14% in the morphine group (n=14)); however, the researchers did not detail which neurological soft signs (comparable to minor neurological dysfunctions) were assessed[8]. In that study, the children in the morphine group had 7% smaller head circumference and 4% less

bodyweight than children in the placebo group. Although several children in our study were prematurely born or born small for gestational age, most children in both groups now had normal height, weight and head circumference for their age. The difference in height between the two groups found at the age of five was not longer apparent. The preterm neonates in the NEOPAIN trial received higher doses of morphine, up to 30 mcg/kg/hr of morphine[1,8]. At neonatal age, the children in the morphine group of our study received 10 mcg/kg/hr of morphine; in the case of pain or distress children in both groups received open-label morphine as rescue medication.

Limitations

There are various other quantitative sensory testing modalities, such as mechanical or current detection and pain thresholds[2]. Because 8-to 9-year old children are expected to have a short attention span, we decided to assess only thermal detection and pain thresholds.

Given the range of variation in data on pain thresholds in other studies[4] and the fact that the study site (Zwolle versus Rotterdam) was a significant covariable for the cold pain thresholds in the present study, we suggest that the determination of pain thresholds is sensitive to variations in instructions and methodology. However, this does not influence the comparison between the morphine and the placebo group in the present study. At both study sites the children received the same instructions, however, we did not investigate the inter-tester reliability for giving the instructions.

Reference data were available only for the four tests according to the Method of Limits, and not for the tests according to the Method of Levels. The reference data were collected in a set-up in which the minimum temperature of the thermal sensory analyzer was -10.0 degrees Celsius, compared to 0.0 degrees Celsius in the present study; this hampered the comparison of the cold pain thresholds between the control group and the present study.

Conclusion

We found in the present study that neonatal continuous morphine infusion (10 mcg/kg/hr) had no adverse effects on thermal detection and pain thresholds or overall neurological functioning eight to nine years later. Univariate analysis showed differences in the cold detection thresholds, however, this was not confirmed in the multivariate analysis. Children who had received continuous morphine as neonates experienced more episodes of pain in the three months before the study visit, but the prevalence of chronic pain was comparable between the morphine and the placebo group.

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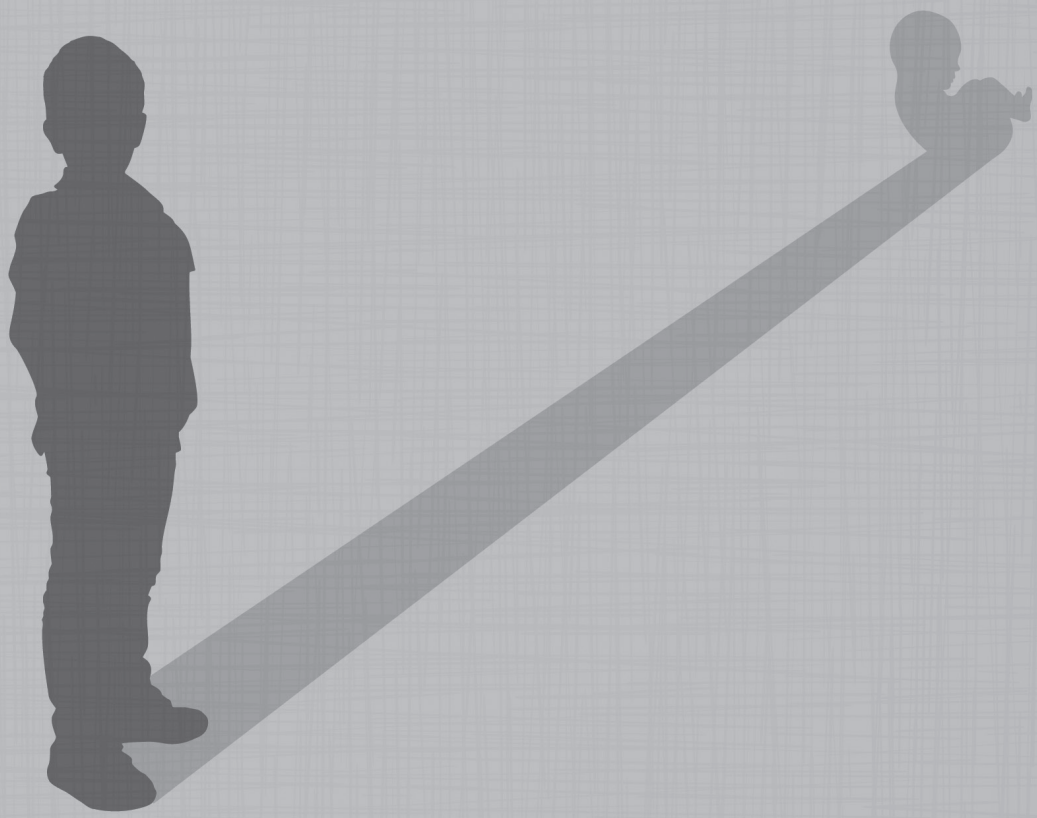
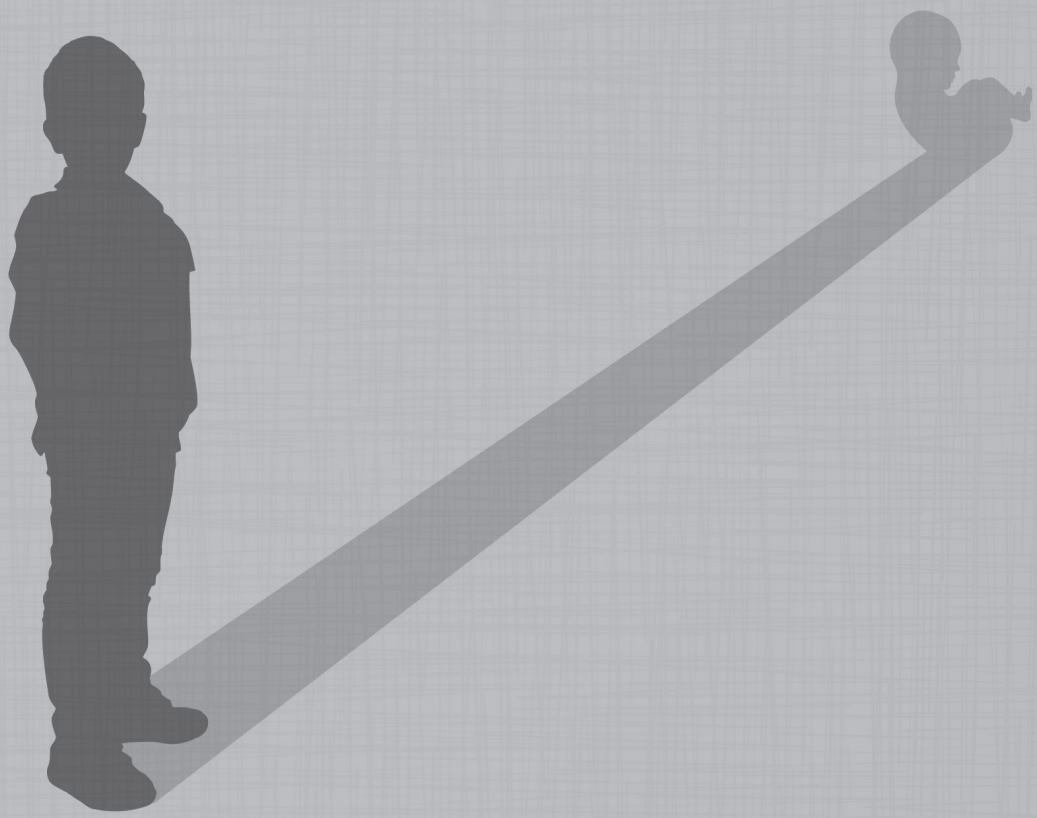
Discussion & Summary



Chapter 7

General Discussion



INTRODUCTION

Most children admitted to a neonatal intensive care unit (NICU) are extremely (<28 weeks gestational age) or very (28 to <32 weeks gestational age) preterm born. Their life expectancy has gone up, and the survival rate in those born at 24 weeks is now even up to 60%, but survivors remain at risk for mental disabilities, cerebral palsy, deafness, blindness and neurodevelopmental disabilities[35,60,75]. Many problems, however, will come to light only at a later age when more brain functions are expected to be matured and environmental demands on for example cognition and behavior will become stronger. A number of follow-up studies show poor academic performance, symptoms of inattention, internalizing behavioral problems and poor executive functions in preterm born children up to young adulthood[2,10]. A major challenge is to find out 'which' factors in the NICU period cause 'what' on the long term. NICU admission means being separated from parents but also implies life-saving, painful interventions such as surgery, endotracheal intubation and mechanical ventilation and venipuncture[65], for which pain treatment, including opioids, is given. A Canadian study documented a decrease in the number of tissue-damaging painful procedures per day from 14 in 1996 to 6 in 2007[42]. Unfortunately, when non-tissue-damaging procedures were included, the daily number of painful events has not significantly dropped over the last decade[17,42,65]. Besides low gestational age, all these above variables may also trigger later adverse outcome. To optimize treatment for our smallest patients the long-term effects of these variables should be studied as well.

Morphine

The studies in this thesis deal with one part of treatment modalities; the long-term effects of morphine given during the neonatal period. Children admitted to a NICU often receive morphine to induce analgesia[17,65]. However, two RCTs have shown that routine morphine administration in mechanical ventilated neonates did not prevent daily and procedural pain[66], incidence of intraventricular hemorrhage (IVH) and periventricular leukomalacia (PVL), or death[5,66]. Cautious use of morphine in ventilated neonates was recommended and its routine use as 'standard of care' was even advised against[5,8,66]. Morphine, however, remains an important analgesic therapy in for example necrotizing enterocolitis and a variety of surgical procedures such as closure of the ductus.

The possible long-term effects of neonatal morphine treatment are still not clear. In rodent models morphine administration was associated with an altered neuroapoptosis[39], an altered neurochemical profile, decreased cell division and neurochemical changes within the hippocampus[59,69]. These alterations are suggested to negatively affect learning capabilities later in life[12,50]. Neonatal pain experiences without morphine as well as

neonatal morphine without pain led to altered pain behavior in rats on the long term[9]. However, when morphine was combined with pain experiences, later pain behaviour was attenuated[9,45]. A recent experimental study in rats provided supplementary evidence for this phenomenon as morphine could only protect the brain from neurological damage when it was combined with mild pain, but not with severe pain[23]. Because of ethical reasons these studies cannot be performed in humans and thus identifying these phenomena in humans will remain very difficult.

Human studies evaluating the long-term effects of morphine are scarce. Three areas have been studied; neuropsychological development, stress response and pain response (Table 1, 2 and 3, respectively). None of these studies found an association between neonatal morphine and adverse cognitive development up to 7 years[24,32,47,58]. Similarly, no effect of neonatal morphine was found on stress response measured by cortisol levels up to 45 months of age[29-31,54]. One study confirmed in a longitudinal analysis the finding from the cross-sectional analyses that basal cortisol levels from 8 to 18 months in children born extremely preterm were higher than those in term born children[31]. Overall, basal cortisol levels had dropped from 8 to 18 months. Another study, in 32 weeks old (post conceptual age) infants born with a very low birth weight (<1500 g), showed that morphine ameliorated the adverse effect of pain on the cardiac autonomic pain response[28]. This could not be confirmed in a later study of the same research group[30]. Both studies showed no indications that morphine ameliorated the effect of neonatal pain on subsequent behavioral reactivity to pain[28,30]. Grunau et al (2009) demonstrated an association between neonatal morphine and poorer motor development at 8 months of age, but this effect was not replicated at 18 months of age[32]. In most of these studies, however, morphine was not the main predictor variable, but 'only' a reported covariable. Severity of illness may be considered a confounder because severely ill children most likely will receive more morphine but at the same time may face a worse long-term outcome due to their disease. It is therefore difficult to unravel the association between these factors. A possible way to examine solely the effect of the administered morphine is a follow-up study of a morphine related RCT. To our knowledge, just two such studies have been published. One included 5 to 6–year-old children[47] who had participated in a RCT[55,56] comparing a morphine cohort with a placebo or pancuronium cohort. The authors found no effect of neonatal morphine use on intelligence, visual motor integration or behavior 5- to 6 years later. A possible long-term effect of pancuronium cannot be ruled out. At the time of the study neuromuscular blockade was used much more then at present. A recent pilot-follow-up study of the NEOPAIN trial in a small cohort of 5 to 7-year-old children (n=15 treated with morphine, n=4 treated with placebo) found no effect of neonatal morphine use on intelligence, but did find indications for adverse effects on head circumference, body weight, reaction time and social behavior[24].

Table 1. Long-term effect of neonatal morphine on brain development in humans

Author, year	Study design	Participants	Age	Dosage Morphine	Type of test				Conclusion
					Cognitive function	Executive functions	Behavior	Other	
McGregor et al, 1998[47]	Follow-up RCT; morphine vs non-morphine (placebo or pancuronium)	NICU population n=57 morphine n=30 non-morphine	5-6 years	(1) 50-100 mcg/kg/h (2) 100 mcg/kg/h (2 hours) + 25 mcg/kg/hour	WPPSI-R	-	CBCL	Movement ABC	No effect of neonatal morphine on intelligence, motor function and behaviour
Rozé et al, 2008[58]	Prospective population-based study; long-term outcome of prolonged sedation and/or analgesia	n=1572 preterms	5 years	Exposure of more than 7 days to prolonged sedative and/or analgesic drugs; yes/no	Kaufman battery	-	-	Disabilities: neuromotor, cognition, hearing and vision	Prolonged sedation and/or analgesia is not associated with poor neurological outcome
Grunau et al, 2009[32]	Cohort study	n=137 preterm, n=74 full-term	8 and 18 months	daily average mg/kg × days Preterms: 2.7 (6.8)	Bayley -II	-	-	Bayley -II	Overall exposure to morphine was associated with poorer motor development at 8 months, but not at 18 months
De Graaf et al, 2011[21] (present thesis)	Follow-up RCT; Morphine vs placebo	NICU population n=49 morphine n=41 placebo	5 years	100 mcg/kg + 10 mcg/kg/h	RAKIT	-	CBCL & TRF	Beery VMI Health-related quality of life Chronic pain	No effect of morphine on main outcome variables; Subtest 'visual analyses' negatively associated with morphine
Ferguson et al, 2012[24]	Follow-up RCT; Morphine vs placebo	NICU population n=14 morphine n=5 placebo	5-7 years	100 mcg/kg + 10-30 mcg/kg/h	Stanford-Binet test (version 5) WRAT4	Progressive Ration task Delayed Matching to sample task	CBCL	Vineland Conners' Physical exam	No effect of morphine on IQ or academic achievement. Morphine associated with decreased head circumference and body weight, more social problems, and increased latencies
De Graaf et al, 2012 (present thesis)	Follow-up RCT; Morphine vs placebo	NICU population n=43 morphine n=46 placebo	8-9 years	100 mcg/kg + 10 mcg/kg/h	WISC-III	CANTAB & BRIEF	CBCL & TRF	Beery VMI	No differences on main outcome variables; Behavioural regulation positively associated with morphine

Kaufman: The Kaufman Assessment Battery for Children, Vineland: Vineland Adaptive Behavior Scales, Conners': Conners' Comprenhensive Behavior Rating Scales, WRAT4: Wide Range Achievement Test

Table 2. Long-term effect of neonatal morphine on cortisol levels in humans

Author, year	Study design	Participants	Age	Morphine dosage		Sampling	Conclusion related to morphine	Other results
Grunau et al, 2004[29]	Cohort study; stress response to novelty in preterm infants	N=54 preterms N=22 controls	8 months	daily average mg/kg × days ELGA: 5.1 (7.6) VLGA: 1.8 (7.3)	Saliva	Stress response to visual novelty; 3 measurements	No relationship between morphine and cortisol response	ELGA preterm infants show a different pattern of cortisol levels than VLGA and term born children
Grunau et al, 2005[30]	Repeated measures crossover design; examine relationships between neonatal pain exposure and subsequent stress and pain reactivity	N=78 preterms	PCA 32 weeks	daily average mg/kg × days ELGA: 1.90 (2.70) VLGA: 0.06 (0.17)	Plasma	Response to stress and pain	No relationship between morphine and cortisol response	Greater cumulative exposure to painful (skin breaking) procedures since birth was associated with lower cortisol response to a stressor
Peters et al, 2003[54]	Cohort study; Relationship between surgery within the first 3 months of life and preemptive analgesia on pain responses	N= 50 surgery first 3 months of life N=50 controls	14 or 45 months		Saliva	Stress response during immunization; 2 measurements	Major surgery in combination with preemptive morphine administration within the first 3 months of life does not alter cortisol levels during immunization	
Grunau et al, 2007[31]	Follow up; Pain, prematurity; morphine co-variable	N=225 preterms infants	3,6,8, 18 months	Daily average morphine dose [mg/kg] ELGA: 6 ± 10 VLGA: 1 ± 5	Saliva	Stress response to novel toy; 2 measurements at 3,6, 8 months; 1 measurement at 18 months	Morphine did not prevent higher cortisol levels at 18 months	ELGA infants; the shift from low basal cortisol levels at 3 months to significantly high levels at 8 and 18 months
De Graaf et al, 2013 (present thesis)	Follow up RCT; relationship between continuous morphine and cortisol secretion	NICU population n=20 morphine N=20 placebo n=39 control	5 years	100 mcg/kg + 10 mcg/kg/h	Saliva	diurnal rhytm; 5 measurements	No effect of neonatal morphine on cortisol levels	NICU children have higher cortisol levels than controls

ELGA, Extremely Low Gestational Age; VLGA, Very Low Gestational Age; FT, Full-term; PCA, postconceptional age

Table 3. Long-term effect of neonatal morphine on pain development in humans

Author, year	Study design	Participants	Age	Morphine dosage	Type of test	Conclusion
De Graaf et al, 2011 (present thesis)	Follow up RCT; morphine vs placebo	NICU population n=49 morphine n=41 placebo	5 years	100 mcg/kg + 10 mcg/kg/h	Chronic pain questionnaire	No effect of neonatal morphine on chronic pain
Grunau et al, 2001[28]	Prospective cohort study; relationship of early neonatal factors and previous medication exposure to subsequent biobehavioral reactivity to acute pain of blood collection	N=136 ELBW	32 weeks PCA	Morphine total exposure (mg/kg) 1.74 (3.44)	biobehavioral reactivity to acute pain of blood collection	Analgesia may ameliorate effects on later pain reactivity
Grunau et al, 2005[30]	Repeated measures crossover design; examine relationships between neonatal pain exposure and subsequent stress and pain reactivity	N=78 preterms	PCA 32 weeks	daily average mg/kg × days ELGA: 1.90 (2.70) VLGA: 0.06 (0.17)	Behavioral and cardiac reactivity to pain of blood collection	Morphine did not appear to ameliorate long-term effects of prior pain on subsequent behavioral reactivity to pain
Valkenburg, 2011 (present thesis)	Follow up RCT; morphine vs placebo	NICU population n=43 morphine n=46 placebo	8/9 years	100 mcg/kg + 10 mcg/kg/h	Chronic pain questionnaire; Thermal detection- and pain thresholds(QST)	No adverse effect of morphine on chronic pain and thermal detection and pain thresholds

NICU, Neonatal Intensive Care Unit; ELBW, Extremely low birth weight (<1500 g); QST, quantitative sensory testing

Unfortunately a good comparison between the human studies is hard to make, because studies differ in design, patients' underlying disease pattern, amount of administered analgesics and outcome variables (Tables 1, 2, 3).

This thesis

We performed a follow-up study in children aged 5 years and 8 or 9 years focusing on the long-term effects of neonatal morphine on brain development (intelligence, behavior and executive functioning), stress response and pain perception. As mechanically ventilated neonates these children had been enrolled in a RCT performed in the Erasmus MC-Sophia Children's Hospital, Rotterdam and the Isala Clinics, Zwolle. They had been randomly allocated to receive continuous morphine or placebo. I will discuss our findings, address the potential importance of the findings, describe pitfalls of follow-up research and give recommendations for future studies.

Brain development

At both 5 and 8 or 9 years of age we found no differences in intelligence, school performance and the cognitive domains of the executive functions between children who received continuous morphine or placebo. The finding on intelligence is in line with previous research in humans[47,58]. Executive functions relate to goal-oriented behavior and adaptive behavior[41], which both are important for school performance. Prematurely born children are known to have problems with certain domains of these executive functions, such as ability to suppress impulses, capacity to manage current and future-oriented task demands, and hold information in mind and their test results were significantly worse than those of term born age matched controls[1,2,4,52]. In the 5-year follow-up study discussed in chapter 2, children in the morphine cohort performed statistically significantly worse than the children in the placebo cohort on 'visual analysis', a subtest of the RAKIT intelligence test. This test is called 'Hidden Figures' and consists of a large complex drawing shown on the top half of a page. The bottom half of the page shows six small drawings. Five of these drawings are partially represented in the complex drawing, and one is completely represented. The child has to identify this 'hidden' drawing. This task requires visual analysis, pattern recognition, matching, and the ability to ignore distracting, irrelevant stimuli. However, we observed that many children could not control their impulses and chose too quickly. Considering that preterm born children were found to have executive function problems at later age, especially inhibition response, it would be worthwhile to examine whether neonatal morphine infusion would influence these functions. In our 8/9 year follow-up program, we used the CANTAB test and the BRIEF questionnaires to this aim. The CANTAB is a computer-based assessment system measuring the neurocognitive component of executive functions. The BRIEF questionnaires are completed by parents and teachers and assess the behavioral component of the executive functions in daily life, i.e. how the child relies on everyday demands in its natural setting[64]. The CANTAB test results showed no evidence for a negative influence of morphine on inhibition response at 8/9 years as suggested in the 5-year follow-up study. Results from the BRIEF questionnaires showed that children in the morphine cohort according to their parents had significantly fewer problems with inhibition and emotion regulation. All in all, neonatal morphine administration seems to have a positive effect on the behavioral, but not on the cognitive component of the executive functions at 8/9 years.

It is reassuring that we did not find major harmful effects of neonatal morphine on learning ability and behavior as suggested in rodent studies[12,50]. Still, the learning and behavioural difficulties found in rodents are based on tests like forced swimming or mazes. These activities are hard to compare with the cognitive and behavioural skills demanded from children, especially when they grow older. Furthermore, these rodents

received significantly higher dosages compared to humans, suggesting a dose-related effect. A first indication for a dose-related morphine effect in humans is seen in the positive effect of neonatal morphine on behavior regulation in our 8/9 year follow-up study and in the negative effect on head circumference, body weight, reaction time and social behavior in 5 to 7-year-olds in the NEOPAIN trial pilot-study[24]. The dosages in this NEOPAIN trial were much higher (10-30 mcg/kg/h depending on gestational age) than those in our original RCT (10 mcg/kg/h in all gestational age groups), which could have influenced later outcome. A major limitation is the lack of a rationale for these high dosages, especially in view of the now available pharmacokinetic data, suggesting that an infusion of 2.5 mcg/kg$^{1.5}$/hr provides adequate analgesia in neonates younger than 10 days[43]. A larger follow-up study of the NEOPAIN trial is needed to examine a possible dose related long-term outcome. As we found that mild deviations in coordination and balance control were more common in the morphine group, we recommend to include a more extensive motor and neurological examination in such a study.

From our results, we cannot draw firm conclusions about possible changes at the neuroanatomical level as found in rodents[39,69]. Only functional magnetic resonance imaging (fMRI) could reveal such changes in combination with specific pain tests, while in experimental settings longitudinal changes in opioids receptor occupancy and distribution would help to "visualize" the central effects of opioids. The latter experiments cannot be conducted in children for ethical reasons, however, seeing that radioactive labelled morphine is needed for PET scanning. Yet, based on the results of our 5 and 8/9 years follow-up studies we conclude that continuous morphine up to 10 mcg/kg/hr can be safely administered to neonates.

Follow-up

These two cases, Sophie and Lucas, are examples of children without severe motor or cognitive disabilities, but who have problems keeping up with the academic demands. These cases also show the advantages and disadvantages of both testing-ages. At 5 years, as for Lucas, the worries about his lack of concentration were noted at an early stage. Lucas and his parents received professional support and eventually at the age of 7 he was prescribed Methylphenidate. This effectively reduced his symptoms of ADHD and improved his school performance. Some problems, however, do not come to light at age 5 years as in the case of Sophie. This phenomenon is called 'growing into deficits', which means that that if "a particular function is not normally well developed at the time of injury, then such a deficit may not necessarily be observed until a later developmental stage[25]. In that case, follow-up at 8 years gains insight in the more complex neuro-cognitive domains like the executive functions, which is valuable for research purposes. In clinical perspective, 8/9 year follow-up is less valuable as many children in

Case 1: Sophie

Sophie is a girl born at gestational age 34.1 weeks with birth weight 2230 grams (appropriate for gestational age). The first six days of her life she spent in a NICU and she received mechanical ventilation for 52 hours. Ultrasounds showed no signs of intracranial bleeding or white matter abnormalities in the brain. Sophie participated in the continuous morphine RCT and was allocated to the placebo cohort. She received no additional morphine. During the six days in the NICU she underwent 73 painful procedures..

Follow-up 5 years

Sophie is doing very well. She is a first grader (kindergarten) of a mainstream primary school. The parents and teachers do not report any problems with motor, cognitive and behavioral development. However, they do report that she is easily distracted or gets overwhelmed in crowded situations. Her IQ is above average (RAKIT: IQ 116) and her visual motor integration score is average (Beery VMI: 106). There were no indications for chronic pain.

Salivary cortisol (nmol/ml) on a regular school day were comparable with norm values; after awakening 18.4 nmol/ml , 30 minutes later 21.8 nmol/ml, 1200h 14.8 nmol/ml, 1530h 10.4 nmol/ml, bedtime 10.7 nmol/ml.

Follow-up 8 years

Sophie is in 4th grade of mainstream primary school and experiences problems on several developmental domains. Although she works hard, her skills for reading, writing and mathematics are lagging behind. Her IQ is average (WISC-III NL: 94) and her visual motor integration score also average (Beery VMI: 101). Sophie scores below average on the CANTAB test focusing on planning skills. Parents and teacher recognize this, as both BRIEF questionnaires reveal problems with planning and inhibition. The teacher describes that Sophie has troubles with finishing a task which requires more than 1 step, she has concentration difficulties and does not evaluate her work. The CBCL score shows a clinical score for attention problems. TRF shows no behavioral problems. Sophie had a normal neurological examination**.** There were no indications for chronic pain. Her detection thresholds for cold were 30.5˚C (MLI) and 30.8˚C (MLE), for warmth 37.3˚C (MLI) and 35.8˚C (MLE). Her pain threshold for cold was 11.4˚C and that for heat 50.0˚C. Her thresholds for cold (MLI) are within 1 SD of the mean of our reference population. Her warm detection threshold (MLI) and heat pain thresholds (MLI) are 1 SD above the mean indicating less sensitive thresholds than the reference values.

MLI=Method of Limits, MLE=Method of Levels

need of additional support or special education already received this. The results of the neuropsychological assessment served more as supporting evidence.

The disadvantage of our cohort is the small sample size. Additionally, only 12 out of the 46 placebo children (26%) in our 8/9 year follow-up did not receive any morphine

Case 2: Lucas

Lucas is a boy born at gestational age 32.2 weeks with birth weight 1740 grams (appropriate for gestational age). He was admitted to the NICU for 26 days and received mechanical ventilation for 279 hours. Ultrasounds showed no signs of intracranial bleeding or white matter abnormalities. Lucas participated in the continuous morphine RCT and was allocated to the morphine condition. He also received additional morphine on top of the study medication. He underwent 221 painful procedures during his NICU admission.

Follow-up 5 years

Lucas is going to first grade (kindergarten) of a mainstream primary school. Parents and teachers alike are concerned about his behavioral development. He finds it difficult to sit still, to concentrate on a task and he gets easily distracted. The teacher questions his cognitive abilities and suggests he may be better off at a school for special education. His IQ is below average (RAKIT: IQ 87) and his visual motor integration score is below average (Beery VMI: 83). There are no indications for chronic pain. Based on the results of the test and concerns of parents and teachers we referred him to the department of child psychiatry for an extensive psychological assessment and extra support. No cortisol data available.

Follow-up 8 years

Lucas was diagnosed with ADHD and takes Methylphenidate. He now goes to a school for special education. He receives extra support for spelling. He has problems with mathematics; works slow and disorganized. His IQ is average (WISC-III NL: 91) and his visual motor integration score is low (Beery VMI: 79). The CANTAB test shows no signs of problems with executive functions. His teacher, however, describes severe executive function problems on the BRIEF questionnaire, especially with metacognitive skills, for example taking initiative, working memory, planning and evaluation of behavior. Both CBCL and TRF show clinical scores on attentional problems and social problems. Both parents and teacher describe that Lucas' behavior is much better manageable and his school performances are better since he is on medication. Lucas showed mild deviations in coordination. There were no signs of chronic pain. His detection thresholds for cold were 30.0˚C (MLI) and 30.2˚C (MLE), for warmth 34.8˚C (MLI) and 32.9˚C (MLE). His pain threshold for cold was 8.0˚C (MLI) and that for heat 37.9˚C (MLI). His warm detection (MLI) and cold detection and pain thresholds (MLI) are within 1 SD of the mean of our reference values. His heat pain threshold (MLI) is 1 SD below the mean, indicating a more sensitive threshold than the reference values.

MLI=Method of Limits, MLE=Method of Levels

at all as a neonate. Therefore the effect of neonatal morphine on later development should be confirmed in larger studies. We are awaiting a follow-up study of the NEOPAIN study, in which 449 neonates received placebo and of whom 179 did not receive any morphine at all. On the other hand the morphine dosages were very high especially seen in the light of the newly suggested dosages for neonates[18,43]. The relevance of the

findings of the NEOPAIN study for clinical practice is therefore questionable. Another important issue is the fact that both our original RCT as well as the NEOPAIN study used an intention-to-treat design in which patients remained in the placebo cohort independent whether they received additional morphine or not. Furthermore, patients were included irrespective of "proven" pain at the start of opioid use. So a number of patients might have received opioids in the absence of pain.

Many different tests and questionnaires measuring a wide range of developmental areas can be selected for neuropsychological assessment battery. The selection should at least be based on the following considerations. First, the psychometric properties of the measurement instruments should have been tested 'good' and reported in international peer-reviewed journals. Second, considering that preterm born children are at risk for behavioral problems and learning deficits[1,2,35,37], parents often cooperate in follow up research because they have questions about the cognitive or behavioral development of their child. To evaluate whether their child endures problems in one of these areas the used instruments should have reliable, preferable Dutch, norm values to enable comparison with 'healthy' peers. Third, the duration of the total assessment is important. A 5-year-old will not be able to hold attention for more than 2 to 3 hours. For example, a sustained attention task seems suitable to get an objective picture of attention skills. In practice, children with an attention problem will fail to finish this task – leading to missing data. Administering questionnaires like the CBCL and BRIEF-parents, in combination with the TRF and BRIEF-teacher, would possibly give sufficient information about behavior and executive functions.

In our 5-year follow-up program we used the RAKIT intelligence test. We suggest, however, using intelligence tests of the Wechsler family (WPPSI-III and WISC-III) as they are internationally known. Unfortunately, the WPPSI-III (for 2-7 year olds) was not validated for the Dutch population when we started our follow-up program, but nowadays it is. In the Dutch school system, children will enter third grade at the age of 6, and then start writing and reading. The teacher of Sophie (case 1, page 6) was very interested in the results of the intelligence test as it helped her decide whether Sophie was ready to enter third grade or not. Therefore, and also considering the noted problems with reading and writing at 8/9 years in our total study cohort, a more comprehensive assessment of language skills at the age of 5 would be valuable, for example using the Schlichting Test for Language Production-II and/or Schlichting Test for Language Comprehension.

In general, teachers provided valuable information. In the Netherlands up to 95% of primary schools use the Dutch National Pupil Monitoring System[3,20], in which the child's academic achievements are detailed; spelling, reading and mathematics. Although we

did not find a morphine effect on academic achievements, around 20% of the 8/9–year-old children of our cohort received special education. This is in line with the 12% found in a follow-up study by Aarnoudse-Moens et al (2011) of preterm born children[3], but much higher than the corresponding 4.9% in the general Dutch population[51]. Another 20% received extra support in mainstream primary schools and approximately 50% of the children experienced problems with one or more academic skills. This monitoring system is often also used in the first and second grade (both kindergarten) and would provide useful information for the 5-year follow-up as well[1].

In addition, executive functioning assessed with the CANTAB test and the BRIEF questionnaires was an important outcome variable in the 8/9 years follow-up study. However, the results of the two instruments were not highly associated. Such discrepancies between cognitive measures of executive functions and the BRIEF questionnaires are described in previous studies as well[6,48]. Probably, behavior regulation is less needed during CANTAB testing, or during intelligence testing, as these tests are performed in a well-structured laboratory setting, resulting in optimal performance. But behavior regulation is imperative in daily functioning, especially at school, when a child is in a less structured environment. The CANTAB information is valuable as it reveals whether a child is capable to perform in a most optimal situation. For example, Lucas (Case 2, page 7) is described in the BRIEF questionnaires as having problems with his working memory. Taking all information of the BRIEF and CANTAB results together, these problems in all likelihood were not caused by deficits in his memory functions, but rather by his high distractibility. A disadvantage of the CANTAB is the small, none-Dutch norm population, which precludes comparison with peers. Meanwhile, the international developmental neuropsychological test battery NEPSY-II-NL has been validated in the Netherlands including a Dutch norm population for children in the age of 5-12 years[44]. This may be a better choice for future follow-up research.

Nowadays the neonatal follow up program of the Sophia Children's hospital invites children born < 32 weeks, children with abnormal MRI scans of the brain, abnormal neurological development in the neonatal period or abnormal development of hearing or vision. Thus, both Sophie and Lucas are not invited for this program, despite their academic and behavioral problems. In chapters 2 and 3 of this thesis we showed that gestational age was not a predictor of adverse outcome. Although this may be explained by the relatively low number of term born children in our study, we cannot exclude that these mechanically ventilated children born at a gestational age of >32 weeks – and even term born children requiring NICU treatment –also may develop problems. This would imply that the national guidelines for inclusion criteria for the neonatal follow-up program should be broadened.

Stress system

In chapter 4 we evaluated – by measuring cortisol secretion – any influence of NICU admission on stress levels at 5 years of age. Production of cortisol follows a circadian rhythm, with highest concentrations in the morning and lowest in the evening (Sherman et al 1985). We therefore collected five samples over the day (diurnal rhythm), which may be a more reliable measure of the HPA-axis physiology than just one sample[57]. We included children of the RCT of Simons et al[65,67]. Both the morphine and placebo cohort had significantly higher secretion of cortisol during the day than children of the control cohort. This result is in contrast with a study by Buske-Kirschbaum and colleagues in which cortisol levels did not differ between preterm born children and healthy controls[16]. This study, however, included no more than 18 children in a wide age-range from 8 to 14 years. The wide age-range may have influenced their results, as cortisol levels decrease with increasing age[36]. Other groups have shown higher basal cortisol levels up to 18 months[31] and higher cortisol levels in response to a visual novelty at 8 months of age[29]. We are, however, the first to show that the diurnal rhythm of cortisol levels in a sample of NICU children is higher than that in a sample of children without a history of hospitalization. In our study gestational age did not significantly predict higher cortisol levels, perhaps because the numbers of children born >32 weeks and >37 weeks were too small. We suggest that a study in a larger cohort with children born at different gestational ages might have found an effect of gestational age. On the other hand, these higher cortisol levels may not be due to premature birth itself, but rather to painful interventions or medical treatment during NICU stay resulting in a wind-up of the HPA axis with long term effects. The results may also be a confirmation of the concept of 'fetal programming' in which exposure to adverse intrauterine and early extrauterine influences may 'program' the developing brain leading to altered physiological systems[14,16,49,63].

Nevertheless, there is some reason to worry: high cortisol levels are associated with cardiovascular, endocrine and metabolic diseases[19,61] as well as with psychiatric disorders such as social fear, anxiety disorders and depression in children[13,14,27,33,46,74]. Our original RCT in ventilated neonates showed significantly lower noradrenaline levels in the morphine condition, however, suggesting a protective effect of morphine on the stress system[67]. With this is mind we determined in chapter 4 the diurnal rhythm of cortisol in the 5–year-old children who had participated in our neonatal RCT comparing continuous morphine administration with placebo. The cortisol levels were not significantly different between children in the morphine and placebo cohort. This could suggest that morphine given during the neonatal period does not affect the stress system at the age of 5 years. An important limitation, however, was that only 6 out of 20 placebo children received no additional morphine in the first 28 days of their lives. Although we adjusted for this additional morphine in our analyses, this limitation may have created bias.

Considering the results of this study and previous studies[16,29,31] it seems that former NICU children have higher cortisol levels, implicating a state of chronic stress. Determining chronic stress is now possible with a new method of determining cortisol level in scalp hair. Based on the assumption that hair grows approximately 1 cm per month, analysis of a 6 cm long hair would reflect a period of 6 months[70,72]. Sampling is by taking a lock of hair with a diameter of approximately 3-5 mm scalp-near from the back of the head[68,71]. This was found to be a feasible and a low-burden technique in 5 to 12-year-old children [70,72]. Therefore, hair cortisol could be an interesting option for future research in this population, especially as it is easier than collecting 5 saliva samples over the day as was done in the present study. However, first normative data of cortisol in hair need to be collected in the general population.

Another strategy is measuring cortisol during a stressful situation, for example with the Trier Social Stress Test for Children (TSST-C)[15]. This test includes a public speaking task and a mental arithmetic task in front of an audience. Stress levels of preterm children during this stress test were not different from those of term borns[16]. However, in chapter 3 we showed that children in the morphine cohort had better behavior regulation skill than children in the placebo cohort. It could be hypothesized that better ability to regulate behavior in general holds true for a stressful situation as well.

Pain sensitivity

One of the primary aims of the original RCT was to evaluate the effect of morphine on pain responses[66]. Considering that repeated pain exposure may cause hypersensitivity and lower pain threshold in preterm neonates, it was hypothesised that morphine administration might protect preterm neonates from short- and long-term harmful effects of pain. However, pain scores during endotracheal suctioning were not significantly different between the morphine and placebo cohort[66]. In chapters 2 and 6 of this thesis we investigated longer-term effects of continuous morphine administration; i.e. in terms of chronic pain and thermal sensitivity. Both at the ages of 5 and 8/9 years the incidence of chronic pain did not differ between the morphine and placebo cohort and was comparable to a Dutch norm population of children of the same age[53]. Furthermore, thermal detection- and pain thresholds at the age of 8/9 years were not different between the morphine and placebo cohort. Thus, we can conclude that morphine 10 mcg/kg/h neither has a protective effect nor an adverse effect on later chronic pain and pain sensitivity.

Also, the warm detection threshold and both cold and heat pain thresholds of children of both morphine and placebo cohort were comparable with reference values of healthy children (n=139). In a previous study by Hermann et al, however, the NICU population had significant higher pain thresholds for heat than a control cohort and therefore

were considered as less sensitive for pain[34]. Note worthily, the pain thresholds found in this study were comparable with ours. The discrepancy in findings is hard to explain but may be due to lower thresholds than our reference values in the control cohort of Hermann and colleagues.. Our control cohort included 139 children whereas Hermann and colleagues assessed 20 control children[34]. Our control cohort was recruited from two mainstream primary schools and these children had no medical history and no cognitive problems. There were no indications that instructions were misunderstood. Our reference values are also higher than the reference values of Blankenburg et al[11]. This may be due to different instructions to the children but also to a different method of obtaining thresholds in the study of Blankenburg et al.[11], namely when the subject felt 'aching', 'stinging, or 'burning'. In our study, children were explicitly asked to press the button when they perceived the stimulus as painful. The differences in thresholds between our study and the studies of Hermann et al. [34] and Blankenburg et al. [11] point at the need to reach consensus about which quantitative sensory testing (QST) methodology should be used internationally. Furthermore, clear guidelines and testing protocols must become available.

Despite our strict protocol and careful training of researchers, we were not able to prevent that study site (Zwolle versus Rotterdam) was a significant covariable for the cold pain thresholds. This suggests that the determination of pain thresholds is sensitive to only small variations in instructions and methodology. IQ proved to be another significant confounder during testing. In chapter 4, in which we studied the feasibility of QST with the Thermal Sensory Analyzer-II (TSA) in 5-year-olds, IQ seemed to have a significant impact on the results of one particular TSA method, the method of limits. In this method children push a button when a stimuli is perceived. This makes the test dependent of reaction time. The method of limits has been used most extensively in previous research [26,34,62,73]. In addition, IQ also significantly contributed to the detection threshold, but not to pain thresholds at 8/9 years of age measured with this method of limits. Considering that all children seemed to fully understand the instruction, this effect of cognitive functioning might be associated with reaction time. Note that we also found a significant IQ effect on the warm detection thresholds measured with the other method, the method of levels, which is independent of reaction time. This IQ effect could therefore also be related to reduced concentration or problems with information processing.

The IQ effect, but also center differences and differences between norm populations call into question the reliability and/or validity of QST. Hirschfeld et al. examined the reliability based on a short-term retest analysis showing a fair to moderate agreement between measurements of both left and right hand[36]. But what about the validity? QST seems to be a valid assessment as it helps clinicians to identify sensory loss and nerve lesion, in

which the contralateral side of the measured area often serves as reference value[7]. In clinical populations like ours, QST is used to detect differences in somatosensory perception corresponding to both large non-nociceptive (A-β) and nociceptive small (A-delta, C) fibers. However, Hirshfeld et al. describes QST as a 'psychophysical' method[36]. The 'physical' part consists of the presented thermal or mechanical stimulus triggering the nerve fibers. The psychological part begins when the stimulus is perceived and action must be taken by pressing a button or responding orally. This fleeting moment between perceiving the stimulus and reaction allows bias of IQ as shown in chapter 4 and for example reaction time in people with a mental disability. Sophie, the case described in page 6, did not press the button at all 5 heat stimuli at 50.0˚C. It could be questioned if she really did not perceive this as painful. Would she not perceive holding a hand in water in the same temperature as painful? We observed, however, that she did the utmost to complete the test successfully, and she may have been thinking: "the higher, the better". This goes to say that QST may be more of a measure of pain behaviour than a measure of nociception. In further studies examining the long-term impact of neonatal pain and/or analgesic treatment on nociception, QST can still be used for triggering a pain sensation. It should however, be combined with fMRI to detect differences in brain activity during pain. Structural MRI could be used in addition to detect differences in volumes of specific brain areas related to pain (i.e. insula, thalamus, anterior cingulate cortex).Thus, influences of IQ, reaction time or behavior can be excluded. In an exploratory fMRI study, preterm children showed exaggerated brain responses to a heat painful stimulus compared with healthy controls, whereas term NICU children exhibited a pattern of brain activation very similar to the controls[38]. The sample sizes of this study were however relatively small and a larger cohort should be included to provide more definitive answers.

Future research

In this thesis we demonstrated that continuous morphine infusion in preterm infants on mechanical ventilation did neither have a positive nor a negative effect on intelligence, school performance, salivary cortisol secretion and pain perception at ages 5 and 8/9 years of age. Children in the morphine cohort showed even better behavior regulation skill than children in the placebo cohort. However, the adverse outcome of morphine on head circumference, body weight, reaction time and social behaviour demonstrated in the pilot-study of the NEOPAIN trial should not be ignored [24]. The positive effect of morphine on behaviour in our study compared to the negative effect on reaction time and social behaviour in the pilot-follow-up study of Ferguson et al (2011) could be an indication of dose-dependent effects, in view of the 2 to 3-fold higher morphine dosages of opioids dependent of gestational age used in the NEOPAIN trial[5]. Considering this pilot-study and the previous RCTs[5,66], it would not be an ethical decision to set up a new

RCT with higher dosages ofmorphine. A new study should take into account pharmacokinetic data and apply a population pharmacokinetic /pharmacodynamic approach to determine dosage and only include patients with pain confirmed by validated, age-appropriate pain assessment instruments. Long-term effects of higher dosages should be evaluated in a follow-up study of the NEOPAIN trial[5].

The question is whether it would be worthwhile to set up a follow up study of our morphine RCT at older ages, for example 14 and/or 21 years? Based on our outcomes at 5 and 8/9 years, differences between the morphine and placebo cohort are not to be expected. However, neuro-cognitive development will not be fully matured up to young adulthood[40]. In addition, the better behavioral regulation of the children in the morphine cohort may have its impact on academic achievements later in life.

Still, to study the long-term effects of neonatal pain and morphine treatment QST should be combined with functional MRI in order to measure brain activity during pain. Additionally, structural MRI such as T1 and DTI can be used to measure volume of pain related brain areas and the white matter tracks. A major drawback of the original trial is that the newborns received morphine irrespective of 'proven' pain. It would be interesting to compare brain activity and brain volumes of the present cohort of children with children who unquestionably endured painful procedures and received morphine. Ideally "function" of the CNS assessed by MRI and diffusion tensor imaging (DTI)[22] should be combined with "function" as described in this thesis. Since 2011 a large follow-up study is ongoing in Erasmus MC-Sophia, studying the long-term effects of neonatal pain and morphine treatment at the age of 8-18 years supported by the Dutch research council. This study compares detection- and pain thresholds, structural MRI, brain activity during pain and neuropsychological development between children with a medical history and healthy controls. Children with a medical history are divided into 5 groups: (1) children of the cohort described in this thesis, (2) children who received neonatal ECMO treatment, (3) children with major abdominal or thoracic non-cardiac surgery in the first 3 months, (4) children who have been operated on within the first months of life for a giant congenital Tierfell naevus, and (5) children who required methadone treatment during weaning of opioids.

Although the administration of 10 mcg/kg/h continuous morphine did not prove effective for procedural pain, neonates in our hospital still receive morphine after surgery and in cases of severe pain. Recent pharmacokinetic research showed that for neonates younger than 10 days who require opioid analgesia after non-cardiac surgery, a loading dose of morphine followed by an infusion at 2.5 mcg/kg$^{1.5}$/hr, will give adequate morphine and metabolite serum concentrations[43]. A recent randomized controlled trial in our

hospital showed an opioid-sparing potential of paracetamol in infants undergoing major surgery[18]. Optimal dosing of paracetamol is now being evaluated in our hospital as well as the use of 2.5 mcg/kg morphine in ELBW infants in collaboration with National Institutes of Health. In addition, these studies provide the opportunity to examine the long-term effects of a lower dose of morphine as well as the long-term effects of paracetamol.

However, studies examining the impact of morphine on the short- and long-term may be outdated, now paracetamol i.v., has become available[18]. Still, the debate on possible long-term effects of the other pain treatments will go on. Therefore, studies evaluating the impact of a pain treatment should record detailed information of the neonatal period itself; i.e. surgeries, illnesses, treatments, interventions, medication, pain scores, growth, nutrition etc. In this way, future follow-up studies can adjust for influential co-variables. Furthermore, not short-term primary outcome variables, but also long-term primary outcome variables should be considered, such as neuropsychological outcome variables. This will result in a more sophisticated study design and enough power to evaluate the long-term effects of neonatal pain treatment.

Shadow of the past?

Will neonatal morphine administration hang over these children as a shadow of the past? The answer to this question would be 'no' considering our finding that the morphine treated children had better behavior regulation skills at age 8 or 9 years than children treated with placebo. Still, our main outcome variables such as intelligence, school performance, stress levels and pain perception did not reveal such effect and therefore we maintain the conclusion of our initial RCT that continuous morphine should not be 'standard of care' in ventilated neonates. We can conclude that morphine given in a continuous dose of 10 mcg/kg/h does not harm brain development, does not affect the stress system, and does not alter detection- and pain thresholds.

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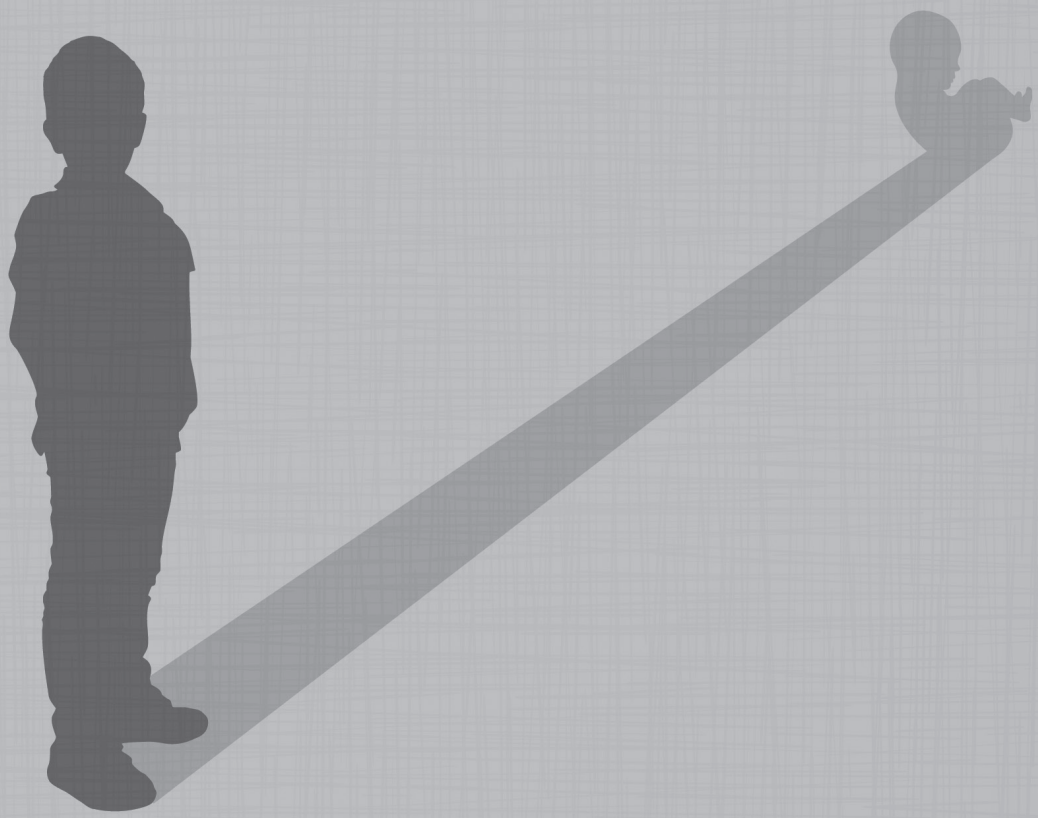
Chapter 8

Summary/ Samenvatting



SUMMARY

Neonates receiving intensive care regularly undergo painful life-saving interventions and treatment procedures. A randomized controlled trial (RCT) performed in the Sophia Children's hospital, Rotterdam and Isala Clinics, Zwolle, in 2000-2002 did not suggest a positive effect of morphine treatment on pain intensity in ventilated newborns. Nor did it prevent severe neurological outcome i.e. incidence of intraventricular hemorrhage (IVH) and periventricular leukomalacia (PVL), or death, and therefore continuous morphine was not recommended as 'standard of care'[1]. In the years after, the effectiveness of morphine in procedural pain was questioned and attention shifted to a more suitable analgesic therapy, e.g. paracetamol i.v.. Still, morphine is widely given to neonates after surgery. We know that morphine may have short-term side-effects, such as respiratory depression, but little is known about possible long-term effects. Furthermore, the available evidence is contradictory. Building on the findings from the above-mentioned original RCT we set out to study solely the longer term effect of continuous morphine treatment at neonatal age on the child's (1) neuropsychological development; (2) stress system; and (3) pain perception – i.e. at 5 and 8 or 9 years.

The **first part** of this thesis addresses the possible influence of neonatal morphine administration on the child's neuropsychological development.

In **chapter 2** we studied the general functioning of these children in terms of intelligence, visual motor integration, behavior, chronic pain and health related quality of life at the age of 5 years. Ninety of the 150 children in the original RCT participated; 49 children who had received continuous morphine and 41 children who had received placebo. Univariate analyses showed significantly lower intelligence quotient (IQ) scores for children who had received morphine: i.e. a mean IQ score of 94 (SD 14.5) versus a mean IQ score of 100 (SD 12.0) in children who had received placebo. No other group differences were found. After adjustment for additional open-label morphine and a propensity score for clinically relevant co-variables, the difference between groups in IQ score disappeared. However, continuous morphine and the additional open-label morphine were negatively associated with scores on visual analysis, a subtest of the intelligence test. This test also requires good response inhibition - a domain of executive functioning - which is a well known problem in preterm born children. Follow-up at older age is recommended to monitor these higher-neurocognitive functions.

In **chapter 3** we indeed explored neurocognitive development at later age, 8 or 9 years. Eighty-nine children of the original RCT participated, but only 80 children were actually tested. Participation of the other nine children consisted of parents completing

the questionnaires that all parents received. Seventy-four of the tested children were also seen at the age of 5 years. Our results show that continuous morphine infusion in the neonatal period has no significant negative consequences on the child's general functioning in terms of intelligence, visual motor integration, and behavior. Children in the morphine group showed significantly fewer externalizing problems according to the parents but more internalizing behavior according to the teachers. These children also showed significantly fewer problems with executive functions in daily life as rated by parents for the subscales inhibition and organization of materials and for planning/ organizing as rated by the teachers. After adjustment for IQ and the propensity score, executive functioning as rated by the parents remained statistically significantly better in the morphine-treated group. Additional morphine given did not significantly influence any of the outcome variables.

We concluded that continuous morphine infusion of 10µg/kg/h during the neonatal period overall does not harm general functioning and may even have a positive influence on executive functions at 8 to 9 years.

The **second part** explores the long-term effects of continuous morphine on the stress system.

In **chapter 4** we describe that intensive care treatment is thought to be not only a painful, but also a stressful experience. The original RCT showed that continuous morphine had a positive effect on the stress system by lowering the noradrenalin levels[2]. As part of our follow-up program at age 5 years, parents collected saliva samples of their child. Saliva contains the so-called 'stress-hormone' cortisol, one of the end products of the stress system; the hypothalamic-pituitary-adrenal (HPA) axis. Saliva was collected five times over a normal school day to determine a diurnal rhythm. We also collected saliva of a control group of children of comparable age. The first question was if the entire group of children who participated in the original RCT had an altered diurnal rhythm of cortisol secretion compared to children without a neonatal hospital admission. Second, we compared this diurnal rhythm between the groups of children who had received continuous morphine infusion or placebo. After adjustment for relevant co-variables, the results showed that 5-year-old children who had received intensive care had higher cortisol levels than the healthy controls, suggesting early programming of the HPA-axis. Gestational age did not contribute significantly to later cortisol levels. Importantly, morphine infusion in the neonatal period did not ameliorate or exacerbate the association with upregulation of cortisol secretion at school entry age.

The **third part** of this thesis addresses the effects of neonatal morphine on one's detection thresholds and pain perception later in life.

Thermal detection thresholds have rarely been assessed in 5-year-olds. **Chapter 5** describes a pilot-study including children who participated in the original RCT. Children's perception for cold and warm stimuli was determined with the Thermal Sensory Analyzer (TSA) device by two different methods; the method of limits, dependent of reaction time, and the method of levels, independent of reaction time. First we evaluated whether the different methods would yield different cold and warm detection thresholds. Results showed that the method of levels yielded more sensitive thresholds than did the method of limits. Next we established possible associations between intellectual ability and the detection thresholds obtained with either method. IQ was statistically significantly associated only with the detection thresholds obtained with the method of limits and not with the method of levels. Therefore, the method of levels is to be preferred over the method of limits in 5-year-old preterm born children, as it establishes more sensitive detection thresholds and is independent of IQ.

In **chapter 6** we focused on the thermal detection thresholds and pain sensitivity, incidence of chronic pain and neurological functioning at the age of 8 or 9 years. Univariate analysis revealed that children in the morphine cohort were more sensitive for the detection of cold (according to the method of levels). However, this was not confirmed in the multivariate analysis. In addition, the detection thresholds of cold (the method of limits), detection thresholds of warm (method of limits and method of levels) and pain thresholds of cold and heat (method of limits) were comparable between the morphine and placebo cohort. Overall, the entire cohort was more sensitive for the detection of cold (method of limit) compared to the reference data. This might be due to methodological variations. Analysis of the detection threshold of warm and pain thresholds of cold and heat (according to the method of limits) revealed no differences. Although children in the morphine cohort had experienced more episodes of pain in the three months before the study visit than children in the placebo cohort, the prevalence of chronic pain was comparable. The overall prevalence of minor neurological dysfunctions was not significantly different between the morphine and placebo cohorts. With regard to minor neurological dysfunctions, mild deviations in coordination and muscle tone were more common in the morphine cohort than in the placebo cohort. All in all, we conclude that neonatal continuous morphine infusion (10 mcg/kg/hr) had no adverse effects on thermal detection and pain thresholds or overall neurological functioning at 8 or 9 years.

In **chapter 7**, the results of our studies are discussed. Our overall conclusion is that morphine given in a continuous dose of 10 mcg/kg/h does not harm the development of the brain, nor the stress system nor alters chronic pain and detection- and pain thresholds.

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SAMENVATTING

Pasgeborenen die opgenomen zijn op een intensive care afdeling ondergaan regelmatig levensredende, maar pijnlijke behandelingen. Uit gerandomiseerd onderzoek met een controlegroep (Engels: RCT voor Randomized Controlled Trial) in ons eigen ziekenhuis en de Isala Klinieken Zwolle bleek dat toediening van morfine de pijnintensiteit niet verminderde van beademende pasgeborenen. Ook had het geen positief effect op het voorkomen van complicaties zoals intraventriculaire bloedingen en periventriculaire leukomalacie , en op de overlijdenskans. Daarom werd continue morfinetoediening niet geadviseerd als standaardzorg[1]. Later ging men ook twijfelen aan de effectiviteit van morfine voor procedurele pijn en verschoof de aandacht naar mogelijk beter geschikte pijnstillers zoals paracetamol. Toch krijgen veel pasgeborenen nog steeds morfine na een operatie. We weten dat morfine nadelige effecten heeft op de korte termijn, zoals een ademhalingsdepressie, maar weinig is bekend over de lange termijn. Bovendien is de wetenschappelijke literatuur tegenstrijdig. Dit was voor ons aanleiding om een follow-up studie van de hierboven genoemde RCT uit te voeren. Het doel was om na te gaan hoe het met deze kinderen ging op de leeftijd van 5 jaar en vervolgens op de leeftijd van 8 of 9 jaar, met nadruk op op (1) neuropsychologische ontwikkeling; (2) stresssysteem; en (3) pijnperceptie .

Het **eerste deel** van dit proefschrift beschrijft de mogelijke invloed van morfinetoediening op de neuropsychologische ontwikkeling.

In **hoofdstuk 2** hebben we het algemeen functioneren van deze kinderen onderzocht, zoals intelligentie, visueel motorische integratie, gedrag, chronische pijn en gezondheidsgerelateerde kwaliteit van leven op de leeftijd van 5 jaar. Negentig van de 150 kinderen uit de originele RCT namen deel aan de follow-up studie; 49 daarvan hadden vroeger continue morfine gekregen; 41 een placebo. Univariate analyses lieten een significant lager intelligentie quotient (IQ) score zien bij de kinderen die morfine hadden gekregen; namelijk gemiddeld 95 (SD14.5) versus 100 (SD 12.0) bij de kinderen die een placebo hadden gekregen. Er werden verder geen andere verschillen gevonden tussen de twee groepen. Echter, na statistische correctie voor extra toegediende morfine en bepaalde klinische co-variabelen was er geen verschil meer in IQ score tussen beide groepen. De continue morfine, en ook de extra gegeven morfine, was echter wel negatief geassocieerd met de prestatie op de visuele analysetaak, een subtest van de intelligentietest. Deze test vereist ook een goede responsinhibitie – een domein van de executieve functies – wat een bekend probleem is bij prematuur geboren kinderen. Follow-up op latere leeftijd wordt geadviseerd om deze hogere neurocognitieve functies verder te onderzoeken.

In **hoofdstuk 3** hebben we inderdaad deze neurocognitive ontwikkeling onderzocht op 8 of 9-jarige leeftijd. Negenentachtig kinderen van de originele studie hebben deelgenomen, maar slechts 80 kinderen zijn ook daadwerkelijk getest. De deelname van de overige negen kinderen bestond uit het invullen van de vragenlijsten door de ouders. Dit waren dezelfde vragenlijsten die door alle ouders van het onderzoek werden ingevuld. Vierenzeventig van de geteste kinderen waren ook op de leeftijd van 5 jaar getest. Bij de kinderen die vroeger morfine hadden gekregen bleken geen significant negatieve gevolgen op het algemeen functioneren in termen van intelligentie, visueel motorische integratie en gedrag. Deze kinderen lieten, afgaande op wat de ouders rapporteerden, significant minder externaliserend gedrag zien dan kinderen uit de placebogroep, maar meer internaliserend gedrag volgens leerkrachten. Ook hadden deze kinderen significant minder problemen met functioneren in het dagelijks leven, als gerapporteerd door zowel de ouders als de leerkrachten. Zelfs na statistische correctie voor IQ en bepaalde variabelen bleef het functioneren van deze kinderen zoals beoordeeld door de ouders significant beter dan van de kinderen uit de placebogroep. De extra gegeven morfine had geen significante invloed op de uitkomstmaten. Wij concluderen dat toediening van continue morfine in de dosering van 10µg/kg/h bij pasgeborenen over het algemeen geen schadelijk effect heeft op het algemeen functioneren – en mogelijk zelfs een positief effect op het executief functioneren – op de leeftijd van 8 of 9 jaar.

Het **tweede deel** van dit proefschrift verkent de mogelijke invloed van toediening van continue morfine op het stresssysteem op latere leeftijd.

In **hoofdstuk 4** beschrijven we dat een intensive care opname niet alleen pijn met zich meebrengt, maar ook stress veroorzaakt. In de originele RCT bleek dat continue morfine een positief effect had op het stresssysteem, gezien de daling van noradrenalinespiegels[2]. Als onderdeel van ons follow-up programma voor 5-jarige kinderen namen ouders speekselmonsters van hun kind. Speeksel bevat het zogenaamde 'stress-hormoon' cortisol, één van de eindprodukten van het stresssysteem dat de hypothalamus-hypofyse-bijnieras wordt genoemd (Engels: HPA voor hypothalamic-pituitary-adrenal). Omdat we naar het dagritme van cortisol wilden kijken vroegen we de ouders vijf monsters af te nemen verdeeld over een normale schooldag. Hetzelfde hebben we gevraagd aan ouders van kinderen in een controlegroep van dezelfde leeftijd. Deze controlekinderen waren als pasgeborenen niet in een ziekenhuis opgenomen geweest. Als eerste vroegen wij ons af of het dagritme van cortisol zou verschillen tussen alle kinderen van de RCT (dus morfine en placebogroep samen) en de controlekinderen. Ten tweede trokken we de vergelijking tussen de kinderen die in de originele RCT continue morfine hadden gekregen en de placebogroep. Na statistische correctie voor bepaalde co-variabelen

bleek dat de kinderen uit de RCT, dus zowel kinderen uit de morfine als de placebogroep, hogere cortisolspiegels hadden dan de controlekinderen. Dit lijkt te suggereren dat er sprake is van vroege programmering van de HPA-as. Zwangerschapsduur had geen significante invloed op de latere cortisolspiegels. Een belangrijke bevinding van deze studie is dat de morfine niet gezorgd heeft voor een verlaging of een verhoging van de cortisol levels in de RCT groep.

Het **derde deel** van dit proefschrift gaat over de effecten van morfinegebruik op de pasgeborenenleeftijd op de waarnemingsdrempels voor koude en warmte en pijnperceptie op latere leeftijd.

Het bepalen van waarnemingsdrempels voor temperatuur is nauwelijks onderzocht in 5 jaar oude kinderen. **Hoofdstuk 5** beschrijft een pilot-studie waaraan een aantal kinderen deelnamen van de originele RCT. Hun waarnemingsdrempels voor koude en warmte werden getest met een speciaal apparaat, de Thermal Sensory Analyzer (TSA), volgens twee methoden: de 'method of limits', waarbij reactiesnelheid een rol speelt, en de 'method of levels', waarbij dit niet het geval is. Allereerst hebben we onderzocht of deze twee verschillende methoden ook verschillende uitkomsten opleveren. Dat bleek zo te zijn, want de 'method of levels' leverde sensitievere drempels op dan de 'method of limits'. Ook bleek IQ significant geassocieerd te zijn met de waarnemingsdrempels bepaald met de 'method of limits', maar niet met de 'method of levels'. We concludeerden dat bij deze kinderen de 'method of levels' te verkiezen is boven de 'method of limits', omdat deze methode sensitievere waarnemingsdrempels opleverde en onafhankelijk is van het IQ.

Op 8 of 9-jarige leeftijd, beschreven in **hoofdstuk 6,** hebben we ons niet alleen gericht op de waarnemingsdrempels, maar ook op de pijngevoeligheid, de incidentie van chronische pijn en het neurologisch functioneren. Univariate analyse liet zien dat de kinderen die vroeger morfine hadden gekregen eerder koude waarnamen dan de placebogroep (volgens de 'method of levels'). Dit kon echter niet bevestigd worden in de multivariate analyse. Waarnemingsdrempels voor koude (method of limits), waarnemingsdrempels voor warmte (method of limits en method of levels) en pijndrempels voor zowel koude als warmte (method of limits) waren vergelijkbaar tussen de morfine- en placebogroep. Beide groepen waren sensitiever voor koude (method of limits), dus voelde de koude eerder, vergeleken met de referentiewaarden. Dit zou kunnen komen door een afwijkende procedure bij het vaststellen van de referentiewaarden. Maar wat betreft de waarnemingsdrempels voor warmte en pijndrempels voor koude en warmte (method of limits) waren er geen verschillen met de referentiewaarden. Ondanks dat kinderen in de morfinegroep in de drie maanden voorafgaand aan het bezoek aan de poli vaker

pijnlijke momenten hadden meegemaakt dan de kinderen in de placebogroep, was de incidentie van chronische pijn in beide groepen gelijk. Dit gold ook voor de incidentie van kleine neurologische dyfuncties, alhoewel milde afwijkingen in de coördinatie van spiertonus meer voorkwamen in de morfinegroep dan in de placebogroep. Al met al concluderen we dat continue toediening van morfine in de dosering van 10µg/kg/h bij pasgeborenen geen negatieve gevolgen heeft op de waarnemingsdrempels en pijngevoeligheid of op de neurologische ontwikkeling op de leeftijd van 8 of 9 jaar.

In **hoofdstuk 7** worden de resultaten van onze studies bediscussieerd. De algemene conclusie luidt dat continue toediening van morfine in de dosering van 10µg/kg/h bij pasgeborenen geen schadelijke gevolgen heeft voor de neuropsychologische ontwikkelingen het stresssysteem, niet leidt tot minder of meer chronische pijn, en de waarnemingsdrempels voor koude en warmte en de pijnperceptie niet beïnvloedt.

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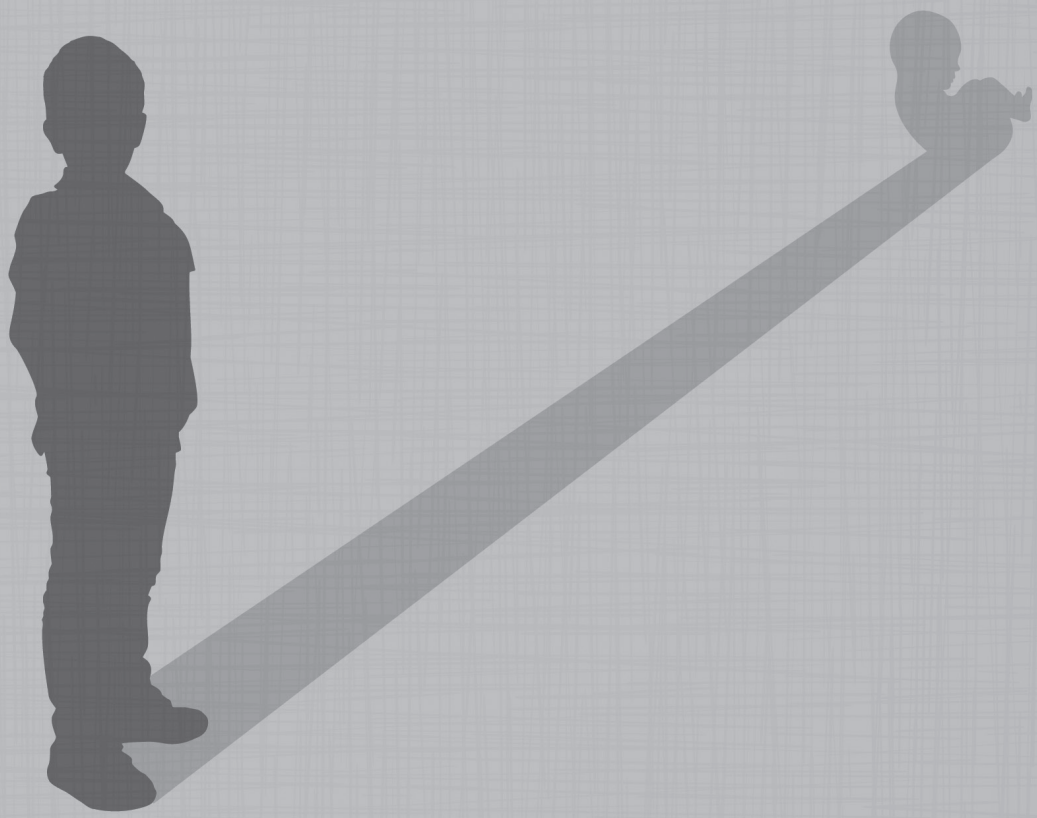
APPENDICES



CURRICULUM VITAE

Johanna (Joke) de Graaf was born in Leiderdorp, the Netherlands, on September 29th 1980. After graduating on Atheneum level at Visser 't Hooft Lyceum in Leiden in 1998, she enrolled in the Child Care and Education program at the Free University Amsterdam, the Netherlands. In 2001 she continued this study at Leiden University, combined with a position as research-assistant in a project focusing on early home visitation in families at risk for child maltreatment. In 2004 she completed her master-thesis on this project and was awarded the Master's degree in Child Care and Education. Not long thereafter she started working at the Department of Pediatric Surgery of the Erasmus MC-Sophia Children's Hospital in Rotterdam, and there conducted the research presented in this thesis.

She lives in Leiderdorp and shares her life with Arjen Rietkerk; they have two beautiful daughters Evi (2010) and Hannah (2012).

PHD PORTFOLIO

Name PhD student	Joke de Graaf
Erasmus MC Department:	Pediatric Surgery
PhD period:	January 2006 - June 2013
Promotor(s):	Prof.dr.D.Tibboel
Supervisor:	Dr. M. van Dijk

	Year	Workload (Hours)
General courses		
Biomedical English Writing and Communication, Rotterdam	2007	80
Nihes Course Classical Methods for Data-analysis, Rotterdam	2007	160
Engels presenteren en discussiëren, Rotterdam	2008	80
Cursus Vineland; vragenlijst adaptief functioneren, Leiden	2008	8
Cursus ANT; Amsterdamse Neuropsychologische Taken	2008	8
Nihes Course Regression Analysis	2009	80
Basiscursus Regelgeving en organisatie voor Klinisch onderzoekers (BROK)	2011	40
Presentations		
ISPP, Vancouver (poster)	2007	30
LNF, Amsterdam (oral)	2011	40
Conferences		
Jackson Reese Symposium, Rotterdam	2006	8
Circle of Life, Rotterdam	2006	8
Is dit normaal?	2007	8
PHD-day	2007	8
POPS symposium	2007	8
Pediatrisch Psychologie symposium	2009	8
PHD-day	2011	8
Teaching activities		
Several presentations during working groups for pain experts and psychologists about the long-term effects of morphine activities	2006-2012	60

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