Giuseppe Buonocore
Carlo Valerio Bellieni
Editors

Neonatal Pain

Suffering, Pain, and Risk of Brain Damage in the Fetus and Newborn

Second Edition

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Introduction: Pain and Suffering from the Womb Onwards?

What is pain? To paraphrase Augustine of Hippo: "If nobody asks me, I know; if I try to explain it, I don't know" (Confessions 11.14.17).

Pain is the only sensation that we cannot remember. We can remember the stimulus that provoked pain or its organic consequences, but we cannot recall pain as we recall flavours, noises, and images. It is difficult to describe pain, but we can describe its features, which are of three types:

- The stimulus. We recognize a stimulus as potentially painful even if we cannot see the sufferer's reaction, because we appreciate its intensity and nature (e.g. a lancet on the skin).
- Bodily consequences. Examples are lesions, hormone production (cortisol, endorphins, epinephrine), and changes in physiological parameters (heart rate, blood pressure, sweating).
- Behavioural changes.

All three of these features are evident in the case of newborns. Newborns are "psychobiologically social beings" [1] and can feel anxiety and fear. This book will show that they can feel pain even before birth.

But what is "pain"—a term often confused with "suffering"? Cassell wrote: "A search in the medical and social-science literature did not help me in understanding what suffering is: the word 'suffering' was often coupled with the word 'pain', as in 'pain and suffering'" [2]. Although pain and suffering are closely identified in the medical literature, they are phenomenologically distinct. "Pain has a felt quality, a felt intensity. Suffering, on the other hand, is not located in the body" [3], or "Pain refers to extreme physical distress and comes in many varieties: throbbing, piercing, burning. Suffering, by contrast, refers to a state of psychological burden or oppression, typically marked by fear, dread or anxiety" [4].

"Suffering can be defined as the state of severe distress associated with events that threaten the intactness of the person" [2]. Schopenhauer usefully defined suffering as "the gap between what we demand or expect from life and what actually comes to us" [5]—an idea recently echoed by van Hoof: "Suffering is to be understood as frustration of the tendency towards fulfilment of the various aspects of our being" [6]. Do newborns have desires? Clinical observation of newborns is enough to suggest a nature marked by deep desires: growing, feeding, seeking milk, and

crying to obtain it are signs of a desire for health [7, 8]. But desires are a person's main feature are newborns and fetuses persons? Boethius, in his *Liber de persona et duabus naturis*, defined personhood as "an individual substance of rational nature" (*naturae rationalis individua substantia*; Chap. 111, PL 64, 1343), and newborns/fetuses are individuals and with a rational nature, though they do not yet exercise it. Thus, it is reasonable to say that newborns and even fetuses are persons, with all their unexpressed desires and, consequently, suffering.

This book can help us to define what pain and suffering are. Pain is a fundamentally "physical" phenomenon, *the clash arising from an attack on one's physical integrity*, whereas suffering is something broader, with pain as one of its sources and desire as its condition. We can define it as *the clash arising from an attack on the integrity of one's self as a person*.

In conclusion, we can say that newborns and fetuses can feel pain and suffer [9]. This book will show that their personhood becomes more and more evident with the acquisition of progressive skills beginning in prenatal life. Recognizing human dignity and human suffering from life in the womb onwards is a clinical duty in the service of better treatment. This book has been written to overcome anything that would come between an awareness of this fact and the shared effort to provide effective treatment of pain and stress in the preverbal patient.

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Note to the Second and Updated Edition

Ten years after, we need to double the success of the first edition of *Neonatal Pain*. Several progresses appeared in the field of neonatal pain treatment and assessment, and they required a new and fresh update. This edition faces the problem of long-term consequences of pain on the infantile brain, of pain treatment during prenatal surgery, and much more.

We thank all the authors of the first edition for their patient work and all the new authors for their stimulating approach to this discussion.

We sincerely look forwards to knowing the readers' reactions towards this new book.

Carlo Valerio Bellieni, Giuseppe Buonocore

Part I

Delivery and Pain

Gender Differences in Pain Since Birth

1

Anna Maria Aloisi, Irina Butkevich, and Stefano Pieretti

In a number of animal and human studies, males and females have been shown to differ in their responsiveness to noxious stimuli. Indeed, sex and gender are important factors in the modulation of pain. Chronic pain is more common in women than in men, with some painful diseases commonly reported only among women. It is becoming very evident that gender differences in pain arise from an interaction of genetic, anatomical, physiological, neuronal, hormonal, psychological and social factors which modulate pain differently in the sexes. Experimental data indicate that both a different modulation of the endogenous opioid system and sex hormones are factors influencing pain sensitivity in males and females.

Several reviews on the topic of gender differences in pain mechanisms, control and treatments have been published in the last two decades [1]. The increasing literature refers to a broad range of topics, including preclinical studies on mechanisms underlying male and female differences in nociception and its control, clinical research on gender differences in pain perception and modulation, epidemiological investigations of sex differences in pain prevalence and a growing number of studies examining sex differences in responses to pain therapies [1].

In this brief review, we summarize important findings regarding gender and pain, and we will discuss findings regarding sex differences in animal models of pain and in clinical pain prevalence and severity. We will conclude with a brief commentary on future directions in this interesting field of knowledge.

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Since the pioneering work of Berkley [2], large-scale epidemiological studies have consistently revealed a higher female prevalence of several painful diseases. Women report more severe levels of pain, more frequent pain in more areas of the body and pain of longer duration than men. Such painful conditions particularly involve the head and neck, e.g. migraine, chronic tension-type headache and temporomandibular disorders, but also include fibromyalgia, irritable bowel syndrome and interstitial cystitis [3]. However, while women are more affected by these chronic painful syndromes, the condition is not exclusive to this sex, and there are also conditions more common in men than in women such as cluster headache, a typical male pathology. Moreover, despite these findings, the relationship between gender and pain is not simple since other studies have found no sex differences or inconsistent results when examining pain severity in clinical populations [3].

Some of the observed differences can arise from specific recurrent problems occurring over a long period of a woman's life such as gynaecological syndromes, as well as from the greater female longevity, or are related to diseases with a higher male than female prevalence. Furthermore, the prevalence of different kinds of pain in both sexes can change across the lifespan, as occurs for migraine, fibromyalgia, temporomandibular disorders and gastrointestinal, abdominal, joint and back pain. As recently reported, menopause can play an important role in changing pain sensitivity. Interestingly, although the loss of oestrogen can lead to a decrease in lifelong painful conditions such as headache, menopause can also be accompanied by 'new' painful conditions such as osteoporosis and joint inflammation [4]. No sex differences have been reported for some pathological conditions such as cancer [5], although sex differences in the type of cancer and its stage and in the effectiveness of pain treatments in these clinical conditions could also influence the presence, magnitude and direction of sex differences in cancer pain [3]. Other confounding factors that can influence sex difference estimates in pain are the reported gender differences in pain symptoms and signs of some syndromes such as appendicitis, migraine, IBS, rheumatoid arthritis and coronary artery diseases [6]. It is recognized that psychological and sociocultural mechanisms can influence pain perception, expression and tolerance in both sexes, thus confounding gender-related pain analysis. Nevertheless, the overall findings from epidemiological and clinical studies demonstrate that women are at higher risk for many common pain conditions than men. Data on pain intensity are less consistent and influenced by several methodological factors, including mode of patient selection in clinical studies and the sex differences in the effects of pain treatments.

1.1 Sex and Experimentally Induced Pain

Differences in responses to experimental pain in both sexes have been investigated mainly in healthy people using a wide variety of stimuli (mechanical, electrical, thermal, ischemic, chemical). Pain responses have been evaluated by different measures including time and intensity to the first sensation of pain, pain tolerance and self-report measures of pain intensity. The results varied between studies regardless

of the type of stimulus used, indicating that sex differences in nociception depend on multiple factors such as the type of stimulus, testing or end point paradigm, body location, temporal rhythms, reproductive status and age and the presence of disease or illness [6]. In contrast, a recent systematic review concluded that the last 10 years of experimental research did not provide clear and consistent results concerning sex differences in human pain sensitivity [7]. However, from the data used for this review and the analysis of the studies, it appears that females feel pain with greater sensitivity than males, although the statistical significance of the sex difference varies across measures, as previously reported [6]. On the other hand, it is interesting to note that despite similar behavioural responses by men and women to a painful stimulus (pain intensity, threshold), some neurophysiological measurements (fNMR, PET) often show a different or opposite response to the same stimulus [8]. These data strongly indicate a different functional involvement of the central nervous system with possibly 'different' plastic changes in those areas probably involved in pain chronicization.

In this regard, it is important to underlie that sex differences in experimental pain were observed also in neonates. Indeed in a recent study [9], using the DAN scale, a validated neonatal pain scale, we observed that, in pain scores assessed in neonatal male and female babies, females' pain scores resulted significantly higher than males'. These evidences clearly show that sex differences in pain perception are present since birth and can work in the subject's brain to affect future pain chronicization events. This possibility was repeatedly confirmed in experimental animals in which a painful/stressful stimulus received while pups induces long-term changes in pain sensitivity until adulthood [10].

1.2 Mechanisms of Sex Differences in Pain

The specific mechanisms underlying the observed gender differences in pain are not yet clear, and it has been suggested that an interaction of biological, psychological and sociocultural factors probably contributes to these differences.

Androgens and oestrogens are essential for the development and maintenance of the reproductive system, and many studies suggest that they also play an important role in the observed differences between males and females in the response to pain. Changes in oestrogen plasma levels were found to be correlated with recurrent pain in women [11], and postmenopausal women undergoing oestrogen replacement therapy showed an increased incidence of temporomandibular (TM) joint pain [12]. However, TM joint pain and fibromyalgia are also related to the menstrual cycle phases, and rapid oestrogen changes may also be associated with increased pain [13]. Fibromyalgia symptoms are associated with the luteal phase, when both oestrogen and progesterone levels are high [14], but not with the follicular phase, when only oestrogens are high.

Pain perception was found to vary according to the menstrual cycle phases in women with chronic pain perception [15]. In experimental models of pain, oestrogens appear to be pronociceptive in males since rats injected intracerebroventricularly

with oestradiol for 2 days showed higher levels of formalin-induced licking than rats injected with saline [16]. However, oestrogens also seem to play an important role in inducing antinociception. Simulation of pregnancy in ovariectomized rats, with high plasma levels of oestrogens and progesterone, increased the pain threshold [17], and this effect was also present in males [18]. Recently the antinociceptive effect of oestrogen was confirmed in a model of neuropathic pain in mice [19]. The authors demonstrated that male and female mice react differently to structural and functional changes induced by sciatic nerve ligature, used as a model of neuropathic pain. Male mice showed a gradual decrease of allodynia and a complete recovery, while in females the allodynia and gliosis were still present 4 months after neuropathy induction. Administration of 17β-oestradiol was able to significantly attenuate this difference, reducing the allodynia and inducing a complete recovery also in female mice. Furthermore, 17β-oestradiol-treated mice showed a functional improvement of the injured limb, a faster regenerative process of the peripheral nerves and decreased neuropathy-induced gliosis [19]. With regard to the effect of androgens on pain, an inverse relationship was found between plasma testosterone and work-related neck and shoulder disorders in female workers [20]. Another evidence for an analgesic effect of androgens is the clinical finding that the levels of gonadal and adrenal androgens such as testosterone and DHT are lower in both female and male rheumatoid arthritis patients than in controls. Interestingly, androgen administration induces a significant improvement of clinical symptoms, probably through inhibition of the immune system [21, 22]. In male rats, when supraphysiological levels of testosterone were administered to both male and female rats, the licking duration, which was longer in female than male controls, decreased only in females, whereas no decrease in flexing or jerking behaviour was observed [23]. These results indicate that a high level of testosterone did not affect the nociceptive input, since jerking and flexing were unchanged, but did induce a 'male-like' response in females with regard to licking, the most complex supraspinal formalin-induced response. This suggests that the already lower licking levels in males are kept low by testosterone and that females are sensitive to changes in testosterone plasma levels. Interestingly, these experimental data were recently confirmed in women [24].

The combined oral contraceptive pill (COCP) has been implicated in the development of a number of chronic pain conditions. Modern COCP formulations produce a low endogenous estradiol, low progesterone environment similar to the early follicular phase of the natural menstrual cycle, with a variable effect on serum androgen levels. Vincent and co-authors [24] used behavioural measures and functional magnetic resonance imaging to investigate the response to experimental thermal stimuli in healthy women, in both a natural and COCP-induced low endogenous oestradiol state, to investigate whether alterations in central pain processing underlie these observations in COCP users. The findings suggested that, in a low endogenous oestradiol state, testosterone may be a key factor in modulating pain sensitivity via descending pathways.

Other experiments aimed at evaluating the long-term effect of a painful stimulus in rats confirmed that male gonadal hormones have an inhibitory, adaptive effect on

the behavioural and neuronal responses to repeated nociceptive stimulation [25]. These data are not surprising considering the distribution of sex hormones and their receptors in areas of the peripheral and central nervous systems associated with nociceptive transmission [26, 27]. Furthermore, sex hormones appear to modulate cortical processing of pain-related stimuli [28–30]. A regional increase in baseline μ-opioid receptor availability and greater activation of endogenous opioid neurotransmission during pain in women in the high-oestrogen state was also reported. During the low oestrogen condition, however, significant reductions in endogenous opioid tone were observed at the level of the thalamus, nucleus accumbens and amygdala, which were associated with hyperalgesic responses [31]. The important effect on gonadal hormones by painkillers should be underlined. Data are clear concerning the hypogonadism induced by opioids and other commonly used analgesics [32]. Thus, the endocrinopathies occurring in these patients can strongly affect their quality of life and the possibility to completely recover from the original pathology. These findings suggest that the interaction of the opioidergic system with gonadal hormones plays a role in the observed sex-based differences in pain sensitivity.

Several studies have indicated that genotype may contribute to sex differences in pain. Since the early experimental data of Liebeskind and collaborators on mice strain differences in swimming-induced analgesia, preclinical research has shown that genotype influences nociception, and these findings have been extended to humans in recent years [33]. For example, hereditary sensory and autonomic neuropathies (HSANs) are monogenic pain disorders in which pain sensibility is substantially absent. Rare inherited disorders may provide models to explain genetic variability in more common pain states, and these syndromes appear to be linked to genes encoding proteins of different functional classes, e.g. ion channels, enzymes, transcription factors and trophic factors.

Many studies have suggested that interactions between the immune system and the nervous system modulate nociception via the crucial role of microglia [34]. Sex hormones also regulate the expression of peroxisome proliferator-activated receptors (PPARs), which in turn can modify the expression of cytokines associated with nociception. Examining the sexually dimorphic expression of PPARs, the authors found that a PPARα agonist reversed allodynia in males but not in females or castrated males, whereas a PPARγ agonist reversed allodynia in females but not in males or testosterone-treated females. These findings appear to be important for future research on pain since they indicate the need for sex-separated experimental studies and further suggest that different clinical strategies could be adopted to optimize pain management in men and women.

Conclusions

Differences between men and women in pain prevalence, the seeking of medical treatment of pain syndromes, pain behaviour and responses to analgesic drugs have long been reported. The role of social, cultural and biological factors in the sex difference in pain perception has been discussed. During the last two decades, a large amount of data has been collected on differences between the sexes in responses to pain, including pain thresholds, tolerance and response to

pain treatments. Sex differences in nociception have been well documented in the literature. It has been shown that women perceive more pain than men, and this has been demonstrated for clinical pain and for experimental pain in humans and animals. Sex differences in pain perception are frequently substantial, with moderate to large effect sizes. Multiple factors are considered responsible for sex differences in pain perception and for the great prevalence of chronic pain conditions in women. Biological factors such as sex hormones are thought to be one of the main mechanisms explaining sex differences in pain perception, probably from birth. Further research to elucidate the mechanisms underlying sex differences in pain responses is needed to reduce these disparities in pain.

Conflict of Interest There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias the conduct and findings of this study.

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Stress and Pregnancy: CRH as Biochemical Marker

2

Silvia Vannuccini, Caterina Bocchi, Filiberto Maria Severi, and Felice Petraglia

2.1 Introduction

The initiation, maintenance, and termination of pregnancy are regulated by a complex interaction between the fetus and the mother, mediated by placenta, through the action of several growth factors, neurohormones, and cytokines [1–5]. Among these hormones, human placenta, decidua, chorion, and amnion produce CRH (corticotropin-releasing factor (CRF)) [6, 7], the well-known hypothalamic peptide involved in the endocrine adaptations of the hypothalamic-pituitary-adrenal (HPA) axis in response to stress stimuli [8–10]. CRH and neuropeptides play a role in both physiological (parturition, life, and work stress events) and pathological stress conditions (preterm labor, intrauterine growth restriction, pregnancy-induced hypertensive disorders) occurring during gestation.

2.2 CRH and CRH-Related Peptides

CRH is a 41-amino acid peptide released from the median eminence of the hypothalamus, acting in the anterior pituitary to stimulate the release of adrenocorticotropic hormone (ACTH) and related peptides in response to stress events, and modulating behavioral, vascular, and immune response to stress [8, 10]. In mammals, the CRH family consists of at least four ligands: CRH, urocortin (Ucn) [11], Ucn2, and Ucn3 [12]. All these peptides have been found in the human placenta and fetal membranes [13–15] and are suggested to be involved in the mechanisms of pregnancy maintenance and parturition [16]. Urocortin has a sequence similar to fish urotensin (63%)

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and human CRH (45%) [11]. As CRH, its addition to cultured pituitary cells stimulates the release of ACTH in a dose-dependent manner, indicating that a common signaling pathway exists for both CRH and urocortin [17].

CRH and Ucns interact with two distinct receptors: R1 (classified into R1α, R1β, R1γ, and R1δ subtypes) and R2 (R2α, R2β, and R2γ subtypes) [18, 19]. Urocortin binds to type 1 and type 2 CRH receptors, with a particularly high affinity for the type 2 receptor [20]. Although Ucn activates both CRH receptors, the lack of a pervasive Ucn projection to CRH-R2-expressing cells [21] and the absence of CRH/Ucn projections to brain anxiety centers [22] pointed to the existence of additional CRH-related peptides. This was confirmed when Ucn2 (also named stresscopin-related peptide) and Ucn3 (also named stresscopin) were isolated [12].

CRH-binding protein (CRH-BP), a 37-kDa protein of 322 amino acids, mainly produced by the human brain and the liver [23], is another of the CRH-related peptides. It has been demonstrated that CRH-BP is able to bind circulating CRH and urocortin, thus modulating their actions on pituitary gland [24, 25] by preventing their binding to their own receptors.

2.2.1 Location in Gestational Tissues

CRH, Ucns, CRH-BP, and receptors are expressed in human placenta, decidua, and fetal membranes. Placental CRH mRNA is located in the cytotrophoblast, syncytiotrophoblast, and intermediate trophoblast at term [6, 7, 26]. Moreover, CRH mRNA is also expressed by the subepithelial layer of the amnion, the reticular layer of the chorion, the decidual stromal cells, and human umbilical vein endothelial cells [6, 27–31].

Placental and decidual cells collected at 8–11 weeks or 38–40 weeks of gestation express urocortin mRNA, and immunohistochemical investigations localized urocortin staining in syncytial cells of trophoblast as well as in amnion, chorion, and decidua of fetal membranes [14], but the urocortin mRNA expression in human placenta does not change throughout gestation [32]. Ucn mRNA and peptide are also expressed by fetal membranes, collected in the first and third trimesters, amnion epithelial cells, the subepithelial layer of the amnion, and the reticular layer of the chorion [33–35]. Human trophoblasts, fetal membranes, and maternal decidua express mRNA and immunoreactive Ucn2 and Ucn3 throughout gestation [13]. Their localization shows some differences with Ucn and CRH [36, 37]. Ucn2 and Ucn3 are localized in syncytiotrophoblast and extravillous trophoblast cells, while Ucn2 is localized to blood vessel endothelial cells, leading to the suggestion of a role of Ucn2 in regulating the placental vascular endothelial behavior. With respect to the fetal membranes, Ucn2 is distributed only in amnion, while Ucn3 is found in both amnion and chorionic cells [13].

Fluorescent in situ hybridization and immunofluorescence studies demonstrated that syncytiotrophoblast cells and amniotic epithelium are the cell types expressing CRH-R1α, CRH-Rc [38], and CRH-R2β mRNA [39]. CRH receptors (mRNA and protein) have been also described in human myometrium [40, 41] and endometrium [42].

The syncytial layer of placental villi at term intensely expressed CRH-BP mRNA and immunoreactivity, whereas rare positively hybridized cells were observed within the cytotrophoblasts and mesenchymal cells. Large decidual cells, amniotic epithelial cells, and chorionic cytotrophoblast stained positively for CRH-BP mRNA and protein. Thus, production of CRH-BP occurs in human trophoblast and intrauterine tissues and may represent one of the major mechanisms used by target tissues to control CRH activity during pregnancy [43].

2.2.2 In Vitro Effects

In nonpregnant women, the close relationship between catecholamines, HPA axis, and stress events represents a classic finding of neuroendocrinology [44], given that increased production of catecholamines and CRH characterizes the adaptive responses to stressful events [45]. Placental ACTH is a product of the proopiomelanocortin (POMC) gene and has the same structure of pituitary ACTH, retaining its immunogenic and biologic activity [46, 47]. Placental ACTH is localized to the cytotrophoblast in the first trimester and to the syncytiotrophoblast in the second and third trimesters [48].

2.2.2.1 Placental Hormonogenesis

The addition of CRH and urocortin stimulates placental ACTH release [6]. The effect is mediated by CRH receptors, as the co-incubation of cultured placenta cells with specific CRH receptor antagonists inhibits the release of ACTH induced by CRH and urocortin. Furthermore, the addition of CRH-BP reverses the effects of CRH on ACTH in human placenta. Indeed, CRH-BP binds CRH in vitro with great affinity: on a perfused pituitary cell column system, the bioactivity of CRH is reduced by co-incubation with CRH-BP [49], whereas in vivo the presence of the binding protein shortens the half-life of immunoreactive CRH [50].

CRH, urocortin, and ACTH stimulate the release of prostaglandin F2α (PGF2α) and prostaglandin E2 (PGE2) from cultured amnion, chorion, decidual, and placental tissues [51, 52], and these effects are inhibited in the presence of specific antisera to CRH and to ACTH. In placenta, but not in amnion or decidua, the stimulatory effect of CRH on PGF2α and PGE2 output is attenuated in the presence of an antibody to ACTH, thus supporting the possibility of paracrine stimulation by CRH and ACTH of prostaglandin production in intrauterine tissues [53]. Urocortin has CRH-like effects on placental cells and tissue explants, because it stimulates ACTH and prostaglandin secretion [54]. CRH markedly stimulates the release of immunoreactive oxytocin from cultured placental cells in a dose-dependent fashion [55].

2.2.2.2 Blood Flow Regulation

Several in vitro experiments have demonstrated that CRH has vasodilatory effects in the human fetal-placental circulation. The effects are mediated by nitric oxide (NO) and by cyclic GMP, as blocking the synthesis of these molecules causes marked attenuation of CRH-stimulated vasodilatation [56]. The addition of CRH to preconstricted placental vessels is able to attenuate all constrictor mechanisms

without variation in CRH ability as a vasodilator agent. CRH-induced vasodilatation appears to be mediated by a CRH receptor, as the vasodilatory response to CRH is antagonized in the presence of CRH receptor antagonists [57, 58]. CRH-induced vasodilatation occurred at concentrations comparable to plasma CRH levels found in the maternal and fetal circulations [35], and CRH is approximately 50 times more potent than prostacyclin as a vasodilator agent [57, 58].

Urocortin has the same effects as CRH: administered intravenously in rats, it is more potent than CRH in causing hypotension, and, with respect to placental circulation, it causes vasodilatation, reducing fetal-placental vascular resistance via CRH type 2 receptors, and being more potent than CRH [59]. As the fetal vessels of the human placenta are not innervated, control of blood flow in this vascular bed is partly dependent on locally produced and circulating vasoactive factors [60]. As syncytiotrophoblast is the main source of CRH during pregnancy [36], placental CRH may access the fetal-placental circulation to cause dilatation through paracrine or endocrine mechanisms. It may be released locally to affect the vascular smooth muscle and endothelium, or it may be secreted into the fetal-placental circulation and travel to its site of action through the placental vascular system.

2.2.2.3 Myometrial Contractility

The family of CRH-related peptides is suggested to play important roles in the control of myometrial contractility during pregnancy and labor [3, 61, 62]. CRH also regulates myometrial contractility, exerting diverse roles at different stages of gestation. In fact, CRH is involved in both relaxation and contraction of myometrium, and this has been demonstrated to be likely dependent on different patterns of expression and biologic effects of CRH receptors (CRH-Rs) [3, 63–66]. CRH-R1 contributes to the maintenance of myometrial relaxation during pregnancy through activation of the adenylyl cyclase/cAMP pathway [67, 68]. In contrast, at term CRH binding induces phosphorylation of CRH-R2 variants, with subsequent stimulation of the phospholipase C/inositol triphosphate, ERK1/2, and RhoA pathways and increase of myosin light chain (MLC20) phosphorylation, promoting myometrial contractility [62, 69]. However, the hypothesis of clearly distinct roles for CRH-R1 (prorelaxation) and CRH-R2 (procontractile) has been challenged by the finding of a region-specific change in CRH receptor subtypes in the uterus identifying CRHR2 as one of the fundal genes significantly increased during labor [70, 71], while CRH-R1 has been shown to be expressed in the lower segment of the uterus and upregulated, rather than downregulated, with the onset of labor [70]. Putative dynamic and changing alternative splicing of CRH-Rs within the myometrium during pregnancy and labor may in part explain this phenomenon.

Placental CRH may also stimulate fetal pituitary ACTH, which triggers secretion of fetal adrenal dehydroepiandrosterone sulfate (DHEAS) [72], which in turn is used by the placenta for conversion to estrogen by the process of aromatization [73]. This increase in estrogen then could serve as a trigger for the cascade of events leading to labor and parturition. In fact, estrogens increase uterine contractility by increasing myometrial excitability and myometrial responsiveness to oxytocin and other uterotonic agents, as well as stimulating the synthesis and release of prostaglandins

by fetal membranes [73]. Furthermore, estrogens stimulate proteolytic enzymes in the cervix, such as collagenase, which break down the extracellular matrix, permitting the cervix to dilate.

Human myometrium expresses Ucn [33, 74], and a twofold increase of contractility is observed when Ucn is added after PGF2 administration [54]. Ucn activates diverse intracellular signaling pathways that contribute to the activation of myometrial contractility [75], such as p42/p44 MAPK [76, 77]. Moreover, Ucn stimulates matrix metalloproteinase 9 (MMP-9) protein level in the culture medium of chorionic trophoblast, syncytiotrophoblast, and amniotic epithelial cells [78], suggesting a local role in tissue remodeling and cervical ripening at the time of labor. A role for Ucn2 in the control of myometrial contractility during human pregnancy has been demonstrated, involving binding to CRH-R2 and sequential activation of PKC, leading to 20 kDa myosin light chain (MLC20) phosphorylation. In fact, Ucn2 gene expression is significantly greater in human laboring gestational tissues (placenta, fetal membranes, and myometrium) compared with nonlaboring, both at term gestation. A similar profile of increasing of Ucn2 mRNA and protein expression with advancing gestation in the mouse was observed. In the myometrium, although an independent effect on contraction was not shown, Ucn2 accelerated the procontractile effect of PGF2 likely via upregulating the expression of the PGF2 receptor and by increasing Prostaglandin-endoperoxide synthase 2 (PTGS2) expression. In addition to stimulatory effects on prostaglandin production, Ucn2 also upregulated myometrial expression of proinflammatory cytokines via CRH-R2. A positive feedback loop between Ucn2 and inflammatory cytokines likely therefore exists, because Ucn2 expression was increased by the inflammatory stimulus TNF-α, probably through NF-κB signaling [79].

2.2.3 Secretion in Biological Fluids

The human placenta expresses large amounts of CRH (>1000 times higher than in myometrium and choriodecidua) resulting in high CRH levels in maternal serum during pregnancy [26]; indeed CRH levels increase exponentially to approximately 800 pg/ml during “late” third trimester and peak (2000–3000 pg/ml) during labor. The peptide becomes undetectable within 24 h after delivery [80, 81]. From intrauterine tissues, CRH is released into the maternal and umbilical cord plasma as well as the amniotic fluid. Plasma CRH levels are low in nonpregnant women (less than 10 pg/ml) and become higher during the first trimester of pregnancy, rising steadily until term [23, 82, 83]. CRH is also measurable in fetal circulation, and a linear correlation exists between maternal and fetal plasma CRH levels, despite umbilical cord plasma CRH levels being 20–30 times lower than in maternal circulation [80].

CRH-BP is measurable in maternal plasma, and levels remain stable in nonpregnant women and during gestation until the third trimester of pregnancy [84]. The existence of a binding protein for CRH explains why there is not a dramatic increase of ACTH despite high levels of CRH during the third trimester of pregnancy [85, 86]. In fact, it was confirmed that most of the endogenous CRH in both maternal plasma and amniotic fluid is carrier bound and therefore has reduced bioactivity.

Maternal plasma CRH-BP concentrations decrease markedly in the last 4–6 weeks before labor [87], returning to approximately nonpregnant levels during the first 24 h postpartum. Thus, opposite changes in concentrations of CRH (higher) and CRH-BP (lower) in maternal plasma occur at term, so that the availability of bioactive CRH increases during the activation of labor.

2.3 Labor and Delivery

During pregnancy, CRH derived from the placenta is thought to play a crucial role in the regulation of fetal maturation and the timing of delivery, and CRH has also been implicated in the control of fetal-placental blood flow. Elevated CRH concentrations, as compared with gestational age-matched controls, occur in patients in preterm labor [88, 89]. The exponential curve depicting the CRH increase is shifted to the left in women who will subsequently deliver preterm and to the right in women who will deliver postdates (Fig. 2.1). This has led to the suggestion that CRH production is linked to a placental clock which determines the length of gestation [90–92]. Several pieces of evidence support the link between placental CRH and the stress of parturition in humans. During spontaneous labor, maternal plasma CRH levels rise progressively, reaching the maximum values at the most advanced stages of cervical dilatation [1, 30, 81, 85, 93] In addition, individuals who undergo

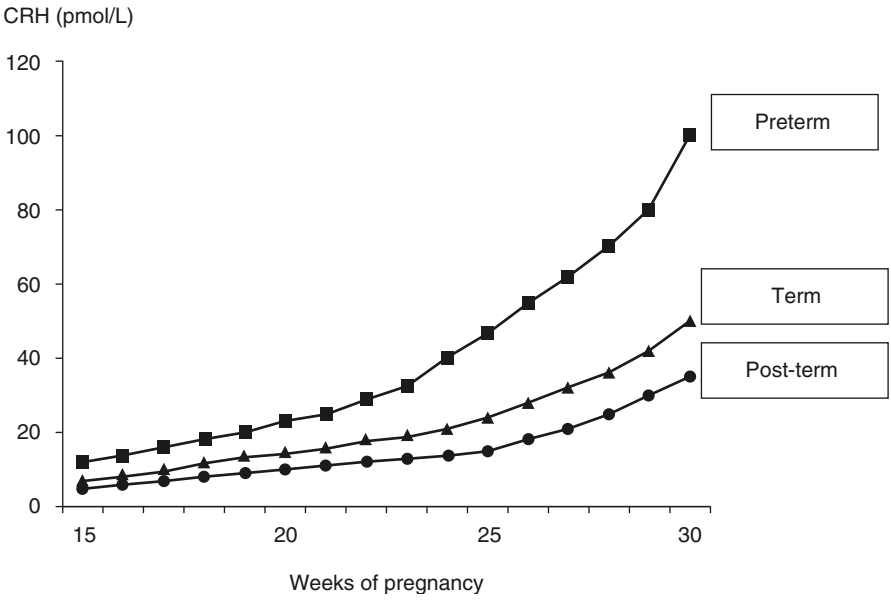

Fig. 2.1 Changes of maternal plasma CRH levels during pregnancies that ended in term, preterm, and post-term deliveries

elective cesarean delivery have placental, plasma, and amniotic fluid CRH levels significantly lower than those who have had spontaneous vaginal delivery [90]. By contrast, during spontaneous physiological labor, a significant decrease has been observed in CRH-BP levels in maternal plasma [85, 90], cord blood [90], and amniotic fluid [94]. The very rapid rise of CRH in late pregnancy is associated with an E3 surge and critically altered P/E3 and E3/E2 ratios that create an estrogenic environment at the onset of labor [95].

With respect to urocortin, maternal levels at labor were higher than those previously reported during pregnancy, but they did not change significantly at the different stages of labor when evaluated longitudinally [96].

As regards post-term pregnancy, maternal blood Ucn does not change in post-term compared to term laboring women. However, higher Ucn levels have been found in women undergoing induction of labor for post-term pregnancy and responding within 12 h, compared to induced and nonresponding women, reinforcing the hypothesis of a major fetal contribution for this neurohormone in the mechanisms of physiologic and pathologic labor [97].

2.4 Preterm Labor

Preterm birth (PTB) is a major complication of pregnancy and remains a leading cause of neonatal morbidity and mortality worldwide. Women with preterm labor have maternal plasma CRH levels significantly higher than those measured in the course of normal pregnancy [98, 99] (Fig. 2.1). This finding suggests that the increase in CRH levels in women with preterm labor is not due to the process of labor itself but may indeed be part of the mechanism controlling the onset of labor. The continued elevation of CRH preceding clinical evidence of uterine contraction suggests that CRH secretion is not sufficient to induce initiation of labor, and other factors are required in this event [90]. Maternal plasma and cord blood Ucn levels are higher in women delivering preterm compared to those delivering at term, while Ucn mRNA expression does not change between term and preterm placenta. These data, together with the finding that Ucn levels in arterial cord blood are higher than in venous cord blood and in maternal plasma, suggest a fetal rather than an exclusively placental source of the peptide at preterm parturition [100].

Maternal plasma CRH is higher in women with threatened preterm labor who give birth within 24 h from admission compared with those delivered after 24 h or with normal pregnant women at the same stage of gestation. This difference was observed at 28–32 weeks' and at 32–36 weeks' gestation, but not before 28 weeks [3]. Maternal and fetal plasma CRH-BP levels are low in preterm labor [101, 102], resembling the physiological pattern observed at term. Because CRH-BP modulates the CRH-induced ACTH and prostaglandin release from decidual cells as well as the myometrial contractility activated by CRH, the precocious fall in CRH-BP levels may be involved in the pathophysiology of preterm labor.

Moreover, recent data showed that chorioamnionitis associated with PTB activates placental Ucn pathways in vivo [103].

2.4.1 Chorionamnionitis

Intrauterine infection is a stress situation associated with a significant CRH elevation in placental extracts, maternal plasma, and amniotic fluid [3]. In addition, women with intrauterine infection (i.e., microbial invasion of the amniotic cavity) had significantly higher amniotic fluid and umbilical cord plasma CRH and CRH-BP levels but unchanged levels of ACTH and cortisol concentrations [104]. However, it is well known that CRH triggers ACTH and cortisol secretion [105]. The increased levels of CRH-BP in cases of maternal infection may prevent the CRH-induced stimulation of ACTH and cortisol, even in the presence of high CRH concentrations in amniotic fluid and umbilical cord plasma.

A possible explanation for these increases may be found in the genomic characterization of CRH-BP, which has revealed acute phase response elements. One of them is known to bind the transcription factor NF-κB, which regulates immunoglobulin and interleukin transcription, and is thought to play a role in the response to inflammation [106]. On the other hand, cytokines stimulate CRH expression and secretion [107, 108], so the increased levels of CRH-BP in the presence of intrauterine infection may play a role in regulating inflammatory responses evoked by CRH.

Placental expression of stress-related pathways is activated in infective process, with a significant impact of preterm prelabor rupture of membranes (pPROM) with chorioamnionitis on placental CRH peptides and receptors. In fact, CRH, Ucn2, and CRH-R1 mRNA expression were higher, while Ucn and CRHR-2 were lower in pPROM with chorioamnionitis than in preterm birth and pPROM. Ucn3 mRNA expression was lower in pPROM with and without chorioamnionitis than in preterm birth. The addition of lipopolysaccharide in trophoblast explants decreased Ucn, Ucn3, and CRH-R2 and increased CRH, Ucn2, and CRH-R1 mRNA expression in a dose-dependent manner, suggesting CRH's potential importance in infection-mediated PTB [103]. In fact, trophoblast samples collected from preterm birth associated with chorioamnionitis show upregulation of Ucn2 and downregulation of Ucn and Ucn3 in comparison with preterm deliveries not associated with chorioamnionitis. These changes have been confirmed in vitro by treating placental trophoblast with lypopolysaccharide, suggesting their potential importance in infection-mediated PTB [103]. Indeed, urocortin stimulates IL-4 and IL-10 secretion and reverses LPS-induced TNF-alpha release from trophoblast cells through action on CRH-R2 receptors, suggesting that this peptide may play a possible role as an anti-inflammatory agent [109].

2.5 Preeclampsia and Fetal Growth Restriction

Preeclampsia (PE) and pregnancy-induced hypertension (PIH) represent other obstetric complications in which a deregulation of CRH has been found. Preeclampsia affects 10–12% of pregnancies, requires intense monitoring and clinical supervision, and is potentially threatening to mother and fetus [110–113]. Preeclampsia is associated with abnormal placentation, due to altered cytotrophoblast proliferation

and invasion, causing reduced placental perfusion and impairment of placental angiogenesis, with insufficiency and failure of remodeling of the spiral arteries [114, 115]. Trophoblastic abnormalities play a role in the development of preeclampsia, and alterations in the secretion of placental hormones and/or factors may be considered manifestations of the earlier stages of the disease. During pregnancy, CRH and CRH-related peptides appear to regulate the fetoplacental circulation via activation of the nitric oxide (NO)/cGMP pathway. Pregnancies with abnormal placental function such as preeclampsia (PE) are characterized by increased maternal plasma CRH concentrations and reduced placental CRH receptor 1alpha (CRH-R1alpha) expression. CRH and CRH-related peptides can positively regulate the placental NO/cGMP system. This pathway appears to be impaired in PE and may contribute toward dysregulation of the balance controlling vascular resistance [116, 117].

CRH may play a crucial role in the implantation and the anti-rejection process that protects the fetus from the maternal immune system, primarily by killing activated T cells through the Fas-FasL interaction. In experimental animals, type 1 CRH receptor (CRH-R1) blockade by antalarmin, a specific type 1 CRH receptor antagonist, decreased implantation sites by approximately 70%. CRH is also involved in controlled trophoblast invasion, by downregulating the synthesis of the carcinoembryonic antigen-related cell adhesion molecule 1 by extravillous trophoblast cells. CRH-R1 blockade by antalarmin increased trophoblast invasion by approximately 60%. Defective uterine CRH/CRH-R1 system during early pregnancy may be implicated in the pathophysiology of recurrent miscarriage, placenta accreta, and preeclampsia [118].

In patients with pregnancy-induced hypertension (PIH), maternal CRH levels at all stages of pregnancy are significantly higher than in healthy controls [119–121]. Moreover, maternal concentrations of CRH are greatly increased in preeclampsia [50, 122] in the presence of plasma CRH-BP levels significantly lower than those in healthy controls [123]. In addition, cord venous plasma CRH concentrations are significantly higher in patients with preeclampsia and higher than those in cord arterial plasma, indicating secretion of CRH from the placenta into the fetal circulation [122, 124, 125]. Moreover, both placental CRH release into fetal plasma and CRH peptide content are higher in PE than in uncomplicated pregnancy [126]. In addition to CRH, other hormones with vasodilatory actions and involved in the stress response such as ACTH and cortisol are increased in fetuses from normotensive pregnancies [126] as well as in those with fetal growth restriction [127, 128].

Intrauterine growth retardation (IUGR) is often accompanied by upregulation of the HPA axis. In the fetal baboon, HPA axis is upregulated in late gestation because both ACTH and cortisol are increased in peripheral plasma of IUGR fetuses. In fact, a lack of cortisol negative feedback was observed: glucocorticoid receptor peptide expression is not downregulated and leptin receptor expression is decreased in the fetal hypothalamic paraventricular nucleus effectively reducing leptin inhibitory effects on fetal HPA axis [129]. Concentrations of CRH in fetal circulation are significantly increased in pregnancies complicated by abnormal umbilical artery flow velocity waveforms, thus representing a stress-responsive compensatory mechanism in the human placenta [130]. It is unknown whether this deranged secretion is

part of the primary pathophysiology of these conditions or occurs as a secondary response to the increased vascular resistance in abnormal pregnancies. Additional data confirm that pregnancies complicated by PE and IUGR are associated with abnormal placental vascular resistance and abnormally high umbilical vein CRH levels [128], reinforcing the concept of the importance of CRH in the control of human fetoplacental circulation.

Ucn levels are higher in women affected by PIH, PE, or PE associated with IUGR when compared with healthy women, while Ucn mRNA expression does not change in pathologic or normal placenta; these data, together with the finding that Ucn levels in arterial cord blood are higher than in venous cord blood and in maternal plasma, again suggest a fetal major source of the peptide in pregnancy-related hypertensive disorders [131]. Recently, placental mRNA expression of Ucn2 and Ucn3 has been evaluated in relation to PE [132]: all PE placentas appeared to express significantly higher Ucn2 and Ucn3 mRNA compared to controls. Interestingly, early PE samples show stronger immunoreactivity for Ucn2 than for Ucn3, while Ucn3 immunostaining was stronger in late PE samples. Moreover, Ucn2 transcript levels have been shown to increase in placental explants exposed to in vitro hypoxia reoxygenation, suggesting that increased placental expression of the peptides may reflect a response to the oxidative stress as well as involvement in the pathogenesis of PE.

Interestingly, Ucn has been found to contribute to the pathogenesis of IUGR possibly through negative regulation of placental system A activity, which represents a placental amino acid transporter whose normal activity is fundamental for maintaining fetal growth [133]. CRH induced the expression of the FasL protein in human macrophages and potentiated their ability to induce the apoptosis of a Fas-expressing extravillous trophoblast (EVT)-based hybridoma cell line in co-coltures. The aberrant expression of CRH in preeclampsia may activate the FasL-positive decidual macrophages, impair the physiological turnover of EVT, and eventually disturb placentation [134].

2.6 Fetal Programming

Prenatal exposure to maternal HPA axis and placental hormones represents primary mechanisms underlying the effects of maternal psychological distress on subsequent infant and child development. During human pregnancy, there is a complex relation between psychosocial and biological markers of prenatal stress. Both sources of stress have programming consequences for the human fetal nervous system, birth outcome, and risk for subsequent health and disease [135–137].

Conclusions

In the event of stressful conditions, such as reduced placental blood flow, chronic hypoxia, or infection, the human placenta responds with increased CRH and urocortin secretion, with the aim of dilating the placental circulation, promoting oxygen and substrate availability, promoting term and preterm labor, and also

preparing the fetus for birth, increasing cortisol secretion for lung maturation. Thus, the placental release of CRH as a stress factor is the final pathway triggered by the human placenta to help the mother and the fetus to escape a hostile environment.

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Pain Control During Labour

3

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Labour pain refers to a complex, subjective and multidimensional experience characterised by severe pain.

During the first stage of labour (from the beginning of regular uterine contractions to cervical dilatation is completed), the pain is predominantly visceral and mediated by the T10–L1 segments of the spine, while during the second stage (from the end of the first stage to the delivery of the foetus), an additional somatic component is present and mediated by the S1–S4 segments.

Pain during labour affects both the mother and child. It raises maternal levels of catecholamines, which may have detrimental effects on a fetal well-being (particularly when placental function is poor). Catecholamines, on the one hand, increase maternal heart rate, stroke volume and heart contractility, causing an increase in the myocardial workload and oxygen consumption; on the other hand, they also increase peripheral vasoconstriction, causing a decrease in placental perfusion. Moreover, labour pain is associated with hyperventilation, which leads to respiratory alkalosis

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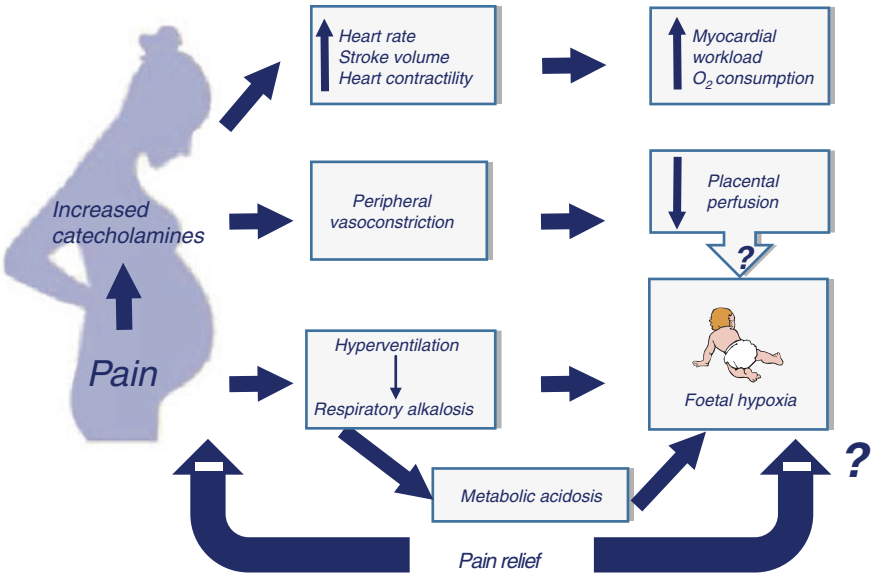

Fig. 3.1 Hypothetical effect of pain relief on foetal well-being. Labour pain increases maternal catecholamine levels and causes hyperventilation, inducing cardiovascular, respiratory and metabolic changes with potentially detrimental effects on a less well foetus (e.g., hypoxia and metabolic acidosis). Pain relief provided during labour may improve foetal well-being

and ultimately causes metabolic acidosis and foetal hypoxia. Adequate pain treatment provides relief to the mother and, in theory, may improve foetal well-being (Fig. 3.1).

Optimal labour analgesia should (1) ensure good pain relief; (2) be safe for both the mother and foetus/neonate; (3) be easy to manage and adaptable to the mother's needs; (4) have known, predictable effects; and (5) not interfere with the dynamics of labour and motor blockade.

The management of labour pain is an essential component of obstetric care and a major goal of intrapartum care.

Many factors influence the perception of labour pain: the woman's physical and psychosocial characteristics and cultural beliefs, as well as the birth environment and the care provided by birth assistants. Since standardised approaches to the management of labour pain may not meet the needs of all women, it is important that pain-related care be individualized and offered as a choice from a variety of non-pharmacological and pharmacological methods [1].

3.1 Non-Pharmacological Analgesia

Non-pharmacological analgesia comprises a variety of methods, such as *continuous support*, *transcutaneous electrical nerve stimulation (TENS)*, *acupuncture*, *hydrotherapy*, *hypnotherapy*, *massage*, *movement and positioning*, *breathing exercises and psychological support*.

Continuous one-to-one intrapartum support is the basis of labour care. A recent meta-analysis of 22 large-scale randomised clinical trials (RCT) reported that, as compared with women who received conventional support, women allocated to continuous one-to-one intrapartum support care were less likely to receive intrapartum analgesia (relative risk [RR] 0.90, 95% confidence interval [CI] 0.84–0.96), report dissatisfaction with care (RR 0.69, 95% CI 0.59–0.79) and undergo caesarean section or instrumental vaginal delivery [2].

In another recent meta-analysis of three RCTs involving about 200 women, *birth ball exercises* were noted to provide statistically significant improvement in labour pain. Furthermore, *electro-acupuncture* at EX-B2 and SP6 acupoints seemed to reduce labour pain and shorten the duration of the active phase of labour.

However, because large evidence-based RCTs are lacking, well-designed randomised controlled studies are needed to evaluate the use of non-pharmacological analgesia for the relief of labour pain.

3.2 Pharmacological Analgesia

Pharmacological methods for labour analgesia include the administration of *nitrous oxide*, *parenteral opioids and neuroaxial analgesia*.

3.2.1 Nitrous Oxide

Nitrous oxide is an inhaled anaesthetic and analgesic gas commonly used in general anaesthesia and dental care. Among its many benefits are a significant reduction in pain perception in comparison with women who received no analgesic treatment, without increasing the incidence of caesarean section, vaginal operative delivery or Apgar score of <7 at 5 min [3].

While nitrous oxide was found to provide less effective pain relief than epidural analgesia [4], the lag time between requested analgesia and pain relief is short, and the woman's sense of personal control is increased, probably because the gas is self-administered. It does not interfere with labour progress or the labouring woman's ability to push, and it does not appear to have adverse effects on the neonate; however, it limits patient mobility and may impair memory of labour.

The administration of nitrous oxide may be indicated when neuroaxial analgesia is contraindicated or unavailable.

3.2.2 Parenteral Opioids

Parenteral opioids like pethidine (meperidine), fentanyl, butorphanol, nalbuphine, tramadol and morphine, all provide good pain relief when given in high doses; however, they can cause respiratory depression and hypotension and are associated with a decrease in myometrial contractility in the mother and severe respiratory depression in the newborn. Remifentanil is a potent opioid with pharmacological

properties that make it a potentially ideal parenteral analgesic for labour pain relief. Several studies reported, however, that remifentanil may induce significant respiratory depressant effects, with episodes of desaturation, hypoventilation and apnoea in labouring women. As compared with other opioids, it reduces pain more significantly with a similar side-effect profile, but it provides a modest short-lasting labour analgesia that is consistently less than that obtained with neuroaxial analgesia.

The administration of remifentanil may be appropriate when neuroaxial analgesia is refused or contraindicated or pain control with neuroaxial analgesia is not guaranteed. In such cases, careful monitoring of maternal and foetal parameters is recommended [5].

3.3 Neuroaxial Analgesia

Neuroaxial analgesia is the mainstay analgesic for intrapartum pain relief and the most effective and safest technique available today. It is most commonly administered by continuous lumbar epidural or bolus request and combined spinal-epidural analgesia. The effect of epidural analgesia on the progression of labour has been extensively studied. According to a recent Cochrane meta-analysis, in comparison with no analgesia, epidural analgesia does not influence the first stage of labour, while the duration of the second stage is about 15 min longer [6]. It is associated with an increased risk of instrumental vaginal delivery but not of caesarean delivery [7].

Epidural labour analgesia can be administered either as a continuous infusion by bolus or by patient-controlled epidural analgesia.

Patient-controlled epidural analgesia (PCEA) allows self-administration of a bolus by the labouring woman and reduces the time interval between pain onset and administration of analgesia. It is associated with less motor block and lower local anaesthetic dose versus continuous infusion. Two systematic reviews found that PCEA combined with continuous infusion is associated with a higher incidence of instrumental vaginal delivery than PCEA alone and that the evidence for the benefits or risks of adding a background infusion to PCEA versus PCEA only is inconclusive [8, 9].

There are many epidural regimens that can alleviate labour pain, yet none is considered as a gold standard because of the complexity of standardising drug dosage schemes and their combinations. Consistent with modern concepts of individualized pain management, epidural labour analgesia relies on a combination of opioids and local anaesthetics to improve the efficacy of analgesic treatment for visceral pain without affecting motor function.

Combined spinal-epidural analgesia is delivered with a single intrathecal injection of opioids with or without local anaesthetics plus epidural analgesia. It gives

rapid pain relief, minimal motor blockade and faster cervical dilatation. It is associated with more frequent spontaneous deliveries and fewer instrumental deliveries than epidural analgesia, but depending on the dose of fentanyl administered, it may carry an increased risk of caesarean section due to abnormal foetal heart rate.

In our hospital, epidural analgesia during labour is used in 32% of vaginal deliveries. Like other authors, we have observed no significant differences in the rate of caesarean sections or a significant increase in oxytocin levels between patients receiving epidural analgesia and controls. In contrast with the published data, we have noted no increase in instrumental vaginal deliveries probably because we administer epidural analgesia only during the first stage of labour. Consistent with published data, we have noted no association between low Apgar score and epidural analgesia during labour.

Conclusions

The choice of labour analgesia should be made by the woman together with her obstetrician and anaesthesiologist and should be neither forced nor refused (Fig. 3.2).

When continuous one-to-one intrapartum support is not completely effective in obtaining pain relief, the woman can choose from a variety of non-pharmacological and pharmacological analgesia strategies. Her choice will depend on several factors, including her own cultural beliefs and psychological characteristics, as well as the environment and care support provider.

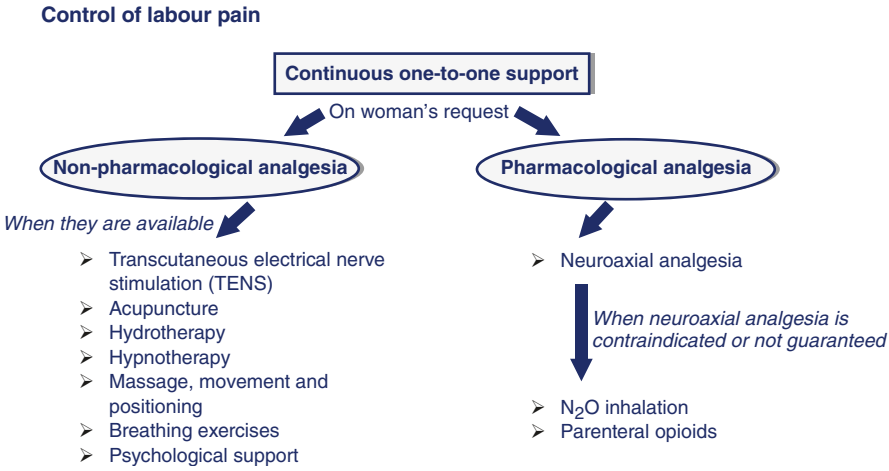

Fig. 3.2 Flowchart of pharmacological and non-pharmacological strategies for the control of labour pain

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Part II

Fetal Pain

Fetal Stress: Ultrasound Study of Fetal Behavior

4

Caterina Bocchi, Silvia Vannuccini, Filiberto Maria Severi, Carlo Valerio Bellieni, and Felice Petraglia

4.1 Fetal Behavior and Ultrasound

The development of ultrasound techniques opened a window on the prenatal world. Since the early 1990s, bidimensional ultrasonography has played an important role in the study of certain fetal behaviors in attempts to understand fetal well-being. Certain fetal attitudes can be likened to those subsequently seen in newborns [1]. Study of fetal circulation by Doppler ultrasonography in parts of the fetal body such as the middle cerebral arteries has also clearly shown that certain pathophysiological and pathological conditions cause a redistribution of normal fetal circulation, indicating a change in fetal status [2, 3].

Fetal behavior can be defined as fetal activities observed or recorded with ultrasonic equipment. The introduction of three-dimensional and four-dimensional (i.e., three-dimensional in real time) ultrasonography has enabled a more detailed morphological evaluation of the fetus, improving not only the definition but also the perception of certain fetal attitudes, such as facial expressions, in response to specific stimuli [4]. Analysis of the dynamics of fetal behavior has led to the conclusion that fetal behavioral patterns directly reflect developmental and maturational processes of the fetal central nervous system [5].

4.1.1 Fetal Motor Development

The uterus is an optimal, stimulating, and interactive environment for fetal growth. Touch, the first sense that develops in the fetus, is fundamental for communication and human experience. The uterus is a protected but not isolated environment,

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where the embryo moves from an early age. Myocardial movements can be detected at week 4. These are followed by head rotation and movements of the arms and legs. By week 10, the embryo can be observed bringing hands to head, opening and closing the mouth, and swallowing. By week 14, the repertoire of movements is complete. In fact, after the development of skin sensitivity, around 10 weeks, repeated stimulations result in hyperexcitability and a generalized movement of all limbs; after 26 weeks, this generalized movement gradually gives way to more coordinated behavioral responses [6]. Infants delivered at 26–31 weeks, for example, show coordinated facial expressions in response to heel prick, although these are immature compared to older infants [7].

4.1.2 Habituation

Fetal movements may be spontaneous, reflecting individual needs of the fetus, or may be evoked, reflecting fetal sensitivity to its environment [8]. The word *habituation* is used to indicate decrease or cessation of an evoked response after repeated presentation of the same stimulus. It is based on the brain's capacity to process short-term and long-term information. Habituation to visual, acoustic, olfactory, and taste stimuli has been extensively studied in newborns [9]. Habituation depends on the capacity of the central nervous system to learn and to recognize a stimulus: once habituation has occurred, the stimulus is ignored. Normal habituation to stimuli is therefore considered by researchers to express neurological well-being as it is based on *learning* [10–13].

4.1.3 Fetal Memory

With regard to learning capacity, fetal memory was first recognized in 1925. Recent experiments show that newborns have functional memory, development of which evidently began in the prenatal period. Prenatal memory is presumably rudimentary, developing quantitatively and qualitatively as the baby matures [14]. Hypotheses about fetal memory functions currently being studied include the recognition and bonding with the mother, breastfeeding, and acquisition of language. It's well known that newborns remember tastes and odors perceived in the uterus and that these prenatal perceptions may influence future preferences of the baby. Recent studies have shown that the uterine environment has an acoustic background consisting of sounds between 50 and 60 dB, among which the mother's voice, which is also transmitted by the bones, stands out. The sounds heard during prenatal life are recognized by fetuses and may have a relaxing effect on the baby. The structural and functional development of hearing is complete around week 23, and this is the first sense to become completely mature. The fetus responds first to low frequencies and then to higher frequencies as gestation proceeds. Newborns have been found to recognize music that the mother listened to during pregnancy and to recognize sounds that became familiar after week 30 [15].

Habituation to acoustic and vibrational stimuli has been tested by applying a sound at 80–110 dB every 5 s (range 4–7 s). At an intensity of 109 dB, fetal heart rate (FHR) increases, whereas at 103 dB, fetal movements increase. Habituation is inversely proportional to gestational age and does not depend on whether the fetus was quiet or active before application of the stimulus [12].

4.1.4 Fetal Behavioral Responses to Maternal Stimuli

During pregnancy, fetuses are responsive to the external environment, specifically to maternal stimulation. During this period, brain circuits develop to prepare neonates to respond appropriately. The detailed behavioral analysis of fetus' mouth movements in response to mothers' speech may reveal important aspects of their sensorimotor and affective skills. Fetuses are sensitive only to specific maternal vocalizations, and fetal-matched responses are rudimentary signs of early mirroring behaviors that become functional in the postnatal period. Thus, fetuses seem to be predisposed to respond selectively to specific maternal stimuli and such responses may play a role in the development of behavioral and emotional attunement with their mothers long before birth [16].

Maternal touch of the abdomen is a powerful stimulus, producing a range of fetal behavioral responses. Fetuses displayed more arm, head, and mouth movements when the mother touched her abdomen as compared to maternal voice in situ. The increase in their activity was also indicated indirectly by the decrease of arm-crossing movements in older fetuses. The difference in the responses by older and younger fetuses to maternal touch may lend support to the early observation of that older fetuses respond preferentially to touch compared to younger fetuses. They decreased their arm and head movements to maternal voice. Fetuses in the third trimester showed increased regulatory (yawning), resting (arms crossed), and self-touch (hands touching the body) responses to the stimuli when compared to fetuses in the second trimester. The decrease in arm and head movements as a response to maternal voice supports the results of using direct maternal voice to stimulate the fetus. A decrease in FHR to maternal voice in situ and an increase in FHR to recorded voice were also demonstrated [17].

4.1.5 Fetal Blink-Startle Reflex

The fetal blink-startle reflex is a complex reflex that appears around weeks 24–25, becoming very evident at week 28 [18]. Its presence indicates integrity of the seventh and eighth cranial nerves, and it is evoked by external stimuli. Long-term habituation of this reflex involves the brainstem. Electromyography (EMG) studies in adults have shown that it is altered in schizophrenia and psychoses and by caffeine intake. A study was conducted on 22 pregnant women undergoing the third trimester routine ultrasound. Inclusion criteria of the study population were normal singleton pregnancy at 30–34 weeks with mothers fasting for at least 2 h, newborn

with Apgar Score at 5 min greater than 8, and negative neurological examination. A vibrational-acoustic stimulus, produced by an Amplicord model 95S laryngophone, was applied repeatedly to the maternal abdomen before the beginning of the routine ultrasound examination scheduled at 30–34 weeks' gestation. The blink-startle reflex was evaluated in a quiet environment, doing a coronal scan of the fetal face at a suitable magnification. The stimulus was applied every 10 s until there was no fetal response to two consecutive stimuli. The fetuses required four stimuli to habituate (mean 4.54, range 1–9) [19]. The data of the present study, in which the blink-startle reflex was tested in a population of normal fetuses, provides a useful reference for our population. It will make it possible to detect future changes in this reflex, which could be a useful sign of neurological damage in high-risk pregnancies.

4.1.6 Fetal Behavior and Neurologic Development

Fetal facial expressions are thought to be indicative of normal fetal neurologic development [20]. Fetuses display a broad spectrum of facial expressions—as seen during emotional expression by adults. Mouthing is significantly more common than all of the other facial expressions, and scowling and sucking are the rarest expressions. Facial expressions can be used as an indicator of normal fetal neurologic development from the second to the third trimester [21]. 4D ultrasound may be a valuable tool for assessing fetal neurobehavioral development during gestation. Indeed, the follow-up of fetal activity by 4D ultrasound could allow distinction between normal and abnormal fetal behavior, which would help in early identification of fetal brain impairment [22]. The Kurjak's antenatal neurodevelopmental test (KANET) is a new prenatal screening test for assessment of fetal behavior [23]. The following parameters are included in the KANET test: isolated head anteflexion, overlapping of cranial sutures and head circumference, isolated eye blinking, facial alteration, mouth opening (yawning or mouthing), isolated hand and leg movements, hand-to-face movements, finger movements and thumb position, and gestalt perception of general movements (overall perception of the body and limb movements with their qualitative assessment). KANET has the potential to detect and discriminate normal from borderline and abnormal fetal behavior in normal and in high-risk pregnancies, which means that it could become a valuable diagnostic tool for fetal neurological assessment [1, 2].

4.1.7 Fetal Behavior and Pain Expression

Facial "distress" movements are essential components of the development of mature pain expression [24–26]. The Neonatal Facial Coding System was used to provide detailed descriptions of facial activity in preterm infants during painful procedures [27]. Measures containing facial movements include deepening of the nasolabial furrow, open lips, and horizontally and vertically stretched mouth,

which are incorporated in the coding scheme of fetal facial expressions from 24 to 36 weeks of gestation [28]. Refined methods of coding fetal facial movement allow to identify the progression of increasingly complex facial movements in utero as well as the formation of the fetal facial "pain/distress" gestalt. Results indicate that as fetuses mature, they show increasingly complex facial movements using up to 7 of the 19 facial movements occurring at the same time. The number of co-occurring movements making up the pain facial gestalt increased with fetal age [26]. 4-D images of the fetus have also been reported to show fetuses "crying" after external stimuli [29]. Even anencephalic fetuses withdraw from noxious stimulations, demonstrating that this response is mediated at a subcortical level [30]. Anyhow, even infants with significant neonatal neurological injury due to a parenchymal brain injury respond to noxious stimulation with a pattern of behavioral reactions similar to infants without brain injury [31]. There is much incertitude about whether these reactions can constitute pain or just a reflex without negative implications such as stress or suffering [32]. Anyhow, fetal reactivity is demonstrated by the observation that fetuses spend 9% of their daytime in wake state, and states F3 (calm wake) and F4 (active wake) up to 21% of daytime in term fetuses were described [33]; even the state F5 (crying) was observed in a fetus [29].

Therefore, ultrasound equipment opened an interesting window on intrauterine life, allowing the study of early fetal behavioral development as a source of information for fetal neurodevelopment and fetal reaction to stress and pain.

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Prenatal Affective Exchanges and Their Subsequent Effects in Postnatal Life

5

Catherine Dolto

This paper is the result of 23 years of haptotherapeutic clinical work with men, women, and children in difficulties and of pre- and postnatal haptonomic accompaniment of pregnancies, normal as well as pathological ones. After training as a pediatrician and several years of psychoanalysis and extensive work with my mother, Françoise Dolto (including attending her seminars), I discovered haptonomy in 1979. I committed myself to this practice, which seemed to promise many new possibilities for therapeutic fields, especially in relation to the prevention of problems in parent–child relationships. What this practice has actually brought has far surpassed my hopes.

It was Frans Veldman, a Dutch doctor, who discovered and developed haptonomy. He defines it as the science of affectivity and psychological–tactile (haptic) contact. It takes its name from the Greek verb *hapto*, which means "I touch in order to cure, to put together." It is a phenomenological, empirical (from the Greek *emperia*, experience), and human science; it enables one to approach the human being in the reality of the meeting, in the "here and now," and in his or her entirety, avoiding any dissociation or hierarchy between body, psyche, and affectivity. Haptonomy allows us to step out of the psyche/soma dichotomy in which we have been enclosed for centuries.

Haptonomy proposes a sophisticated theory of the human person and human development very different from that of psychoanalysis, but cognitive discovery of this theoretical corpus will never enable real understanding of its essence. In haptonomy, understanding passes through feeling and experience before moving on to naming and analyzing. Hard cognitive work is, however, absolutely necessary to acquire a complete grasp of this complex thought.

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Haptonomy is very much inspired by phenomenology. It builds a "phenomenality," which means a corpus of observable, identifiable, and reproducible phenomena which characterize the human affective life.

Frans Veldman understood that within the psyche, could be identified and grouped everything concerning perceptions, sensations, emotions, feelings, the grouping of which he named "the affective." The way the affective operates is linked to subcortical channels with related changes of tone and hormonal secretions. In this way, one can understand how the affective approach enables to address the human being as a whole, since the affective binds the spirit and the body.

Haptonomy's theoretical, multidisciplinary, neurophysiological, anatomoclinical, and psychological background enables it to embrace the perpetual relationship which binds the body and mind. Haptonomic phenomenality opens the door to a multilayered world where each level is related to the others through the effects of affective-confirming contact and of personal experience of tenderness. The feeling of being complete brought about by this contact is essential for human development. This approach demonstrates that there is not one single memory but several memories, more or less archaic, more or less consciously active, which take place in our whole corporeality and the entries to which are sensorial. Emotions, being experiences, play a critical role in a person's overall construction as well as in the construction of memories. The hapto-psychotherapeutic method leads us to think that the question of infantile amnesia should be reconsidered in the light of new discoveries. Very often children tell the story of their birth, or describe it, without even knowing what it is. But this has nothing to do with the phenomena of recovered memories.

5.1 Contact

The skin is tremendously important. It is the first sensorial organ the child may use in utero (the skin receptors are functional as of the 7th week of gestation) and the first organ of communication and exchange. The sense of touch is the one sense that is always reciprocal. In order to avoid this reciprocity (which they regard as a pitfall), medicine and paramedical disciplines have advocated an objective touch, which unfortunately results in a distance between the person approaching and the one being approached. At the other end of the spectrum, think how comforting an instinctive bear hug can sometimes be. Haptonomic phenomenality shows that the objective touch, being stressful, distorts the exchange. It induces reactions which hinder the clinical exam and warp the diagnosis. We use a particular contact, which needs to be learned. We call it psychotactile affective-confirming contact. Furthermore, perceptions and sensations coming from the interior of its body mean a great deal to the child.

5.2 Affective Confirmation

According to haptonomy, the subject, being an autonomous source of desire, searches for security as early as conception. For this, the subject discriminates, both very early and in a fine way, between what is good and what is bad for him.

The psychotactile affective-confirming contact provides affective confirmation. It is indispensable for the proper development of the subject, who can then unfold all the possibilities gathered in his significant constellation, of which phylogenetic and ontological data are basic elements. In order to express itself, this genetic data needs representation, experiences, and meetings, in which the subject feels the experience of his being good for the other, in a mutually experienced affective environment of security, plays a decisive part. These exchanges, made of emotions, belong to the field of epigenetic evolution right from prenatal life. We know how important the epigenetic influence can be: it explains cerebral plasticity, which lasts until the end of life and enables one to escape the tyranny of one's genes, which is comforting.

The German notion of *lust*, poorly translated as "pleasure" in English, is key to understanding the development of a healthy being. The experience of pleasure, in an environment of affective security and reciprocity, enables the individual to mature and his intellect to blossom and promotes psychic well-being. Because of the structural importance of pleasure (*lust*), we speak about sensuality and not sensoriality, from the beginning of fetal life. "Sensuality" means here sensorial perceptions effected by a feeling of pleasure or displeasure and of security or insecurity. This already plays a big part in prenatal life. To explain all this, haptonomy elucidated concepts and a theory which cannot be given in detail here. However, I am going to cite some critical milestones to help you understand the following clinical elements.

5.3 Tone

"Representation tone" is a vital, integral, physical concept which embraces muscular tone; tension in tendons, ligaments, and capsules; interstitial tissue turgor; arterial tension; lymph circulation tension; as well as "psychotone"—the tension and strength of psychic expression. Representation tone is usually perceived as a quality of presence other people can feel and to which they react (body language, in lay speech). In daily life, we speak about a nice presence, an anxious presence, an insecure presence, or, by contrast, a peaceful, open, and reassuring presence. Everybody knows how babies are receptive to the quality of presence of the people around them. They perceive the representation tone of nearby adults in a nonconscious way, on an affective, prerational, and prelogical level. Reciprocally, they answer with an adapted representation tone. Perception of this tone is even more important during prenatal life. When the child is in the womb, it feels all variations affecting its environment, whether comfortable or uncomfortable, or secure or insecure, enabling freedom of movement or forced immobility.

5.4 Prenatal Accompaniment

We receive the couples in individual sessions, if possible starting right at the beginning of pregnancy, but often until the 4th or 5th month of pregnancy. At a later stage of the pregnancy, if a situation of distress arises for the child, the mother, or

the father, an experienced haptotherapist can intervene to propose a different kind of work adapted to the urgent situation. I often receive women at risk of a premature delivery.

This kind of approach relies on the desire and involvement of both parents. If the father does not wish to experience the pregnancy in this way, we do not do it. Otherwise, we would risk hindering the relationships within the parent–child triad in a potentially negative way, not knowing when and how their affective bonds will be affected. If the father has disappeared for good, the mother will be asked to choose another person to accompany her. This person—usually a woman—will not be a substitute for the father, who is present in the child's life whatever the situation, but will enable the child to avoid a dual relationship with the mother, which would be oppressive for both of them. The third person, whether family member or not, is the one who opens up the relationship and thus prevents mother–child fusion.

We meet the couples at least eight times before the birth, more if necessary. The prenatal work must absolutely be followed by postnatal sessions, which are carried out in a very specific way. The last session, a very important one, takes place when the child stands up and has started walking for at least 2 months. This accompaniment to parenthood cannot be compared with birth preparation, even though it certainly contributes positively to this major event, which is totally modified, particularly with regard to pain, by the development of the affective mother–father–child relationship.

5.5 The Intimate and the Extimate in the Secrecy of the Mother's Womb

5.5.1 From the Mother's Point of View

The psychotactile affective-confirming contact activates the subcortical channels as well as the whole limbic system and has direct effects on hormonal secretions (endorphins, cortisol) and on the muscular tone of the striated muscles, through the regulation of the neuromuscular spindles. The tone in the smooth muscles is modified in relation with striated muscles but through ways which are still unknown. As soon as a woman has established affective contact with the child she is bearing in her womb, her muscle tone modifies, creating an important change in her perceptions of her own body. She feels relaxed and at ease, and the muscle tone of her uterus becomes much more supple, even though she may have a hard time on an emotional or physical level. Her breath changes, without her even knowing it; her joints become more flexible. The child perceives these changes and immediately reacts to them by a slight movement of the spine.

Thanks to the possibilities offered by the affective contact, a woman can rock her child from within, inviting it to move toward her heart or her pelvis, to one side or the other, by the modification of tone that she thus induces in her womb. (The uterine muscles and abdominal muscles are experienced collectively as a tender,

welcoming place for the child.) The child is moved in the direction in which she invites it. If it is awake, it goes along with the movement. In this way, the mother can invite one twin toward the top and the other one toward the bottom, enabling a specific playtime with each of the children. The discovery of these possibilities is always a very joyful surprise for the mother. It is even more important for a depressive mother, or for an ambivalent mother who hesitated to keep her child, or for a mother who feels powerless to help a child who is in danger.

5.5.2 From the Father's Point of View

The father first alerts the child to his presence through his manner of accompanying the mother. We teach fathers different ways to bring comfort to the mother, through rocking and easing the arch of the back. This simple contact, if it is affective confirming, modifies the mother's muscular tone. It brings to her, and thus to the child, relaxation and ease. If a tense and tired woman lays down, the child starts moving intensely, in a jerky and abrupt way, enjoying the relative ease in the uterus provided by this position. However, if the father applies a light and tender touch, the child stops moving and remains quiet under his hand, which reassures both mother and child. After 5–10 min of quiet rest under this soothing hand, it will start its games again with a calmer, softer motion.

Fathers also play a very important part for their children through their voice. Unlike the mother's voice, which always vibrates the womb—the child's home—in the same way, the sporadic voice of the father envelops the child from different points and enables it to consider space. As of the 3rd month of pregnancy, children react to a voice which is directed at them. If they like it, they go nearer (unless the mother prevents them from doing so). As is well known, until the beginning of the third trimester of the pregnancy, hearing is not functional, but the skin, which is very sensitive, catches vibrations. I have been told that ancient obstetricians saw the skin of the fetus as a big ear. We experience this every day.

5.5.3 From the Child's Point of View

Let me say first that children give motor answers. They come nearer, go away, or sway by moving their pelvis, by leaning or pushing on the uterine wall, in an infinite number of variations. Twins play together. But motivity is a very subtle language; moving is not answering; stamping one's feet is not swaying. It is important to understand rhythm, amplitude, and the direction of each movement within the complexity of interactions between anatomy and physiology. It is clinically so obvious that parents very quickly feel these subtleties. When everything is fine, the mother accompanies her child from within in each of its movements, without even noticing it, thanks to what we call prelogical, prerational affective awareness. But she can also immobilize her child if she is not feeling well, if she is afraid, or

if she is having a conflict, consciously or not, with the person who is trying to approach her child.

We find out that, long before birth, children are paying attention to everything that surrounds them: messages coming from their mother through the highly subtle interactions which bond them but also exterior sounds and atmospheres. They receive them through their mother's feelings but also directly. They seem to be alert to any kind of sign. If a gentle and light hand calls them and then withdraws, they look for it: a real "hide and seek" game starts as long as the child is available. If the hand comes quietly, children will come and nestle under it and stay there. All children imperceptibly sway to the mother's breath. If we invite them to magnify their movement and they are awake and available, they take control of the swaying: its amplitude, rhythm, length, and direction. They can choose a lateral or up and down swaying or a whirling around their axis, this movement being always very slow. These are real dances we perceive very clearly under our hands, to the point that the parents and the accompanying person feel the child is now rocking them. As of the 4th month of pregnancy, children are able to choose one kind of swaying, to memorize it and, moreover, to propose it to their parents, provided that the mother is available. Some children like only one kind of swaying; others change and go from one to the other every 5–10 s. We communicate with them through slight changes of hand weight and pressure. They make themselves known, each in their own way, surprising their parents, who thus get to know them long before the birth meeting. Mothers say "when I go towards my child, it is as if he lights up." One can easily imagine what this means in terms of epigenetics and cerebral plasticity.

These dances are not only joyful manifestations in reaction to our invitation or on their own initiative; they are also valuable signs of their state. A child who is not well will neither sway nor invite his parents.

Thanks to the early contact, these children are mobile, playful, attentive to the world, and sensitive to tenderness. When they have to go in neonatal services and when the prematurity drama abruptly rushes them into an incubator, they desperately suffer while discovering their new, constricted condition. They are pinned to their little bed by gravity and deprived of their usual reassuring gestures (sucking their thumb, playing with their umbilical cord, placenta, or feet, masturbating, or swallowing amniotic liquid) because of their lack of motor coordination. They have lost their freedom and any kind of potential initiative; they are now submissive and become passive. Many of them escape into their own world and anesthetize their perceptions; they dissociate themselves, which later on will influence their way of living.

This can also be true for other children, without haptonomic preparation, but who experience the same treatment. I often brought newborns, just out of neonatal services, suffering from what I call the "Sleeping Beauty syndrome." They are passive, waiting to be animated and to be brought back to life. You really have to call upon them strongly, physically by psychotactile affective-confirming contact but also by speech on the emotional and psychological levels, to help them get out of this survival state, which is not really life.

5.6 Interactions Within the Triad

We are obviously in an extremely subtle universe. It is a tiny theater where each one plays a part. But we should not be fooled: even when the child is very small, even when its big answers seem small to us, in the palm of the hand, in the interior feelings of the mother, all of this is extremely precise. The accompanying person needs meticulous training and a long maturation period in order to be totally confident with his or her action. Such a subtle approach in such a sensitive period requires constant ethical reflection and responsibility.

5.7 The Exchange Dynamics

Through the tender contacts provided by its parents, the child receives affective confirmation, thanks to which it develops a feeling of a basic security. Reciprocally, the child answering to its parents gives them affective confirmation and they become mutually established. This ternary dynamic, this circulation of affective confirmations between the members (this is similar in multiple pregnancies), is essential.

When a mother cannot be with her child, the child does not express itself much, and even if it moves, it never really enters a dialogue or proposes anything. When the mother is in conflict with the father, she does not accompany the child in its answers, and we can feel that the child has difficulty going closer to its father. A certain kind of "viscosity" shows the child's difficulty in clearing its own way, almost in spite of its mother. Sometimes the child is totally immobilized without the mother knowing what she is doing. Our work is to help her become aware of this and understand why things are happening this way so that she can overcome this difficulty. Most of the time, it is quite easy to find the path back toward maternal feelings. What make things difficult is anxiety, fear, and uncertainty. Modern medicine often creates these feelings to the point that they can become pathogenic. As of the in utero life, the child looks for contact with its mother, and its quality of perception is stunning. If the child is in a moment of "dance," as I like to call these swaying sequences, it stops as soon as the mother thinks of something else and is no longer connected with it. These maternal withdrawals may be for insignificant or dramatic reasons. We adapt our approach depending on their origin. Sometimes they end up in laughter, when the mother got lost in a funny thought about the child, which led her to lose contact with the here and now of the relationship. Sometimes they end up in tears.

When the pregnancy is developing fine, all this brings pleasure to the parents and their child; they mature and change together. Newborns who have been well accompanied are born with excellent axial tone; they can immediately support their head. They are at the same time very awake, peaceful, and smiling. They are usually rather precocious. With them, you have to review pediatric semiology. Actually, this suggests that the haptonomic child is not precocious, but rather that all other children are slowed down by their lack of security and of affective confirmation. When one feels secure, one can go quicker and further.

5.8 Tragedies

Such a vast and complex subject deserves a lengthy treatment. What I feel I can strongly state today is that, as of the life in utero, children can help their parents going through difficult moments. Are they already driven by a therapeutic urge? This is difficult to prove, but they concretely assert themselves in crucial moments in a singular manner. When women are ambivalent, self-derogatory, feeling guilty for not being the ideal mother they had imagined, and hurt by receiving news of a developmental or inherited disorder in the child or by mourning, very often the child is the one who comes to help them in a most effective way. It goes up to her heart, where it rocks quietly, while she is crying or saying very painful or violent things. Sometimes it goes up to her heart in the deepest moments of despair. That is the child's way of telling her that she is a good enough mother. Even if she considered having an abortion or if she does not feel capable of being a mother, the child shows her that here and now she is its mother and that they are doing fine. It is obviously a great help for the mother, who is thus surprisingly able to get over very painful hurdles.

I must admit that I am not always able to say what is really happening during this period which I like to call the "totally intertwined mother and child." Who signals whom? It is not always possible to know, but a good accompaniment should only help to enable the bond to be permeable between the two, and then these astonishing and undoubtedly effective–affective exchanges will happen under our eyes. When a woman is not well, the presence of the father (or the third person) is essential, because the child reacts to his approach, and this helps the mother to come back to her child. A bipolar situation would lead them to a dead end, a total incapacity to change, symptomatic of a bond frozen in pain and reinforced by the feeling of guilt. But the third person, contacting the child, enables it to assert itself and find its path toward its mother alone.

The suspicion of a handicap, for which you might have to wait weeks before getting a confirmation or an invalidation, leaves long-lasting traces. Some parents come to me because they want to contact their child who is going to suffer a medically required abortion because of a severe malformation. These sessions are always moving and, strangely enough, both sad and happy. Parents have the opportunity to "hold the hand" of their condemned child until the end. During its short in utero life, it will have received the goodness of their affection. That is exactly how you accompany a sick child to the end of its life, too. We work then for the future of the parents and brothers and sisters already here or to be born. Experience has taught us that mourning is easier thus, whereas a mourning not properly done would weigh on several generations.

When the karyotype is normal, the child still needs to be helped to face life. Traces of these moments of doubt may poison the life of a family for years. Parents then have to retie a bond of confidence with it. The affective exchanges give the child the courage to live. This is pretty obvious when the child has to go through a very medicalized birth and a period in neonatal services. Children with a good accompaniment fight for life but remain quiet. They have developed a strong tolerance for frustration. This happens very quickly. Sometimes, very late in the gestation,

we contact for the first time children who have endured a lot of ultrasound scans or even some fetal surgery. Usually when I apply my hands, even though the mother knows and trusts me, the child goes through a moment of panic and of abrupt agitation before slowly regaining peace. These children are intensely open to contact, as if their ordeals had given them, more than others, a taste for communication. Experience shows that just one meeting with the parents can be enough; if they were really perceptive, the qualitative change is so radical that the child comes into the world quiet and peaceful, like one who has been accompanied for a long time.

Inhibition and depression are inseparable and it is interesting to discover that they are already tied in prenatal life. Depression inhibits maternal feeling or a mother's capacity to express it. She tightens her womb around the child and thus inhibits the relational dynamics of the child. This has definite effects on the constantly, quickly evolving child. Anxiety and anguish have the same inhibiting effects. The child withdraws; loses its impulses, just as if it were trying to fade away; and then suddenly moves very abruptly or stamps his feet. Even if the child's development is not, most of the time, modified to the point of being medically serious, postnatal work with children shows that there are some traces left on the personality. Direct and indirect traces coming from the experience of the mother before and during the birth strongly influence her behavior with the baby.

There is no doubt that early prenatal traumas or problems in the weeks following the birth leave much deeper influences than is usually thought even until now. During the first 9 months of life, what Françoise Dolto suggested to us has been confirmed: children (and their parents) experience anniversary syndromes related to difficult periods of the pregnancy. The anguish of the 8th or 9th month can be considered as an anniversary syndrome of the anguish experienced by the mother (and/or father) and hence by the child around its birth. If a child or its parents go through an ordeal at 4 or 5 months into the pregnancy, we have to recommend that the parents be very attentive to their baby when it reaches 4 or 5 months after birth. Very often they experience some sort of dysfunction. Merely speaking with them about what happened for them and their parents at 4 or 5 months in the womb makes everything return to normal. This is extremely important for premature babies.

This knowledge should not upset parents and practitioners facing traumatic events and their inevitable effects. On the contrary, knowledge of these powerful affective interactions around pregnancy enables us to help a great deal by giving the chance to speak about old or current difficulties in an affective proximity with the child. Allowing each other—especially the child—their place along with the dignity of their story creates an immediate therapeutic effect, and they become free of a burden that would have been too heavy. Some children start psychotherapy work around 6 or 7 years old with a pathology that makes me immediately think about a birth story. If this happens to be the case, we just need to tell the story, making the child the hero of the story—his story, from which he came out victorious—which proves him he has powers which he can rely on. We then see the child straightening up and entering his epic, being proud of it, and no longer crushed by it. This should always be tried before giving up when a child or a teenager who had early prenatal or postnatal problems is in trouble.

Memories of prenatal pain or difficulties during the first separation, the birth and the delivery, arise throughout life each time there is an abrupt transition, a separation, each time it is necessary to get mobilized to play a part in the world. In these moments, the effects of early maternal depression, prenatal tragedies, or traumatic birth reappear, particularly during adolescence (but also at the menopause or when retiring). We notice that the child or the teenager experiences heavy inhibitions to act and to dare, which might be in conflict with their strong desire to express themselves. The child who is born in fear, in anxiety, who has spent months in an incubator, and who tried to be born, but in vain, or, on the contrary, has been born asleep when he was not ready bears traces of this trauma, such as forbidden dynamics; lack of confidence in himself, in life, and in others; and lack of courage and strength. We can work on all this in a much simpler way than we think. It is surprising to see the power and duration of the positive or negative impacts of these first deep affective imprints. People suffer into old age because of the nature of the bond they had with their mother as small children or in good cases are supported and carried through the hardest ordeals by the mutual trust and the affective security they experienced in the most archaic part of their life. The first psychoaffective layer has an incredible value.

There would be fewer pathological cases if we accepted that the accompaniment of children and parents during this very sensitive period should be sustained by much ethical and political consideration, always trying to respect the necessity of mutual affective security. The first months of life, from conception, echo all the way along the path they initiate and unquestionably orient and affect society as a whole. It is always possible to help later, but prevention avoids much suffering. These statements call upon our individual and collective responsibility.

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Foetal Pain

6

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6.1 Introduction

We have decided to publish the available evidence on foetal pain, even because several authors have argued that although a preterm baby can feel pain, a foetus of similar gestation age is anaesthetized while in the womb by some endogenous sedatives [1]. This contrasts with the evidence that prenatal life is not entirely spent in sleep and that the foetus is aroused by noxious stimuli; but the main concern with this theory is that foetal endogenous sedative's (adenosine, pregnenolone and prostaglandins) mean level overlap those in common mothers' blood, without provoking anaesthesia in mothers, and, consequently, with no chance of provoking but a middle sedation in the foetus but absolutely no analgesia [2].

The relationship between the mother and the foetus starts immediately through biochemical dialogue, which is fundamental to good implantation: β-HCG induces the production of progesterone by the mother, while many molecules, such as early pregnancy factor (EPF), cause immunological tolerance in the mother [3]. During the foetal period also, the conceptus is a "protagonist", promoting cellular traffic with the mother and to initiating labour. For these reasons, in the field of prenatal medicine, we need to consider the foetus as a patient, and the old concept of foetal well-being must be transformed in the new science of foetal medicine, which includes both treatment and diagnosis.

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In our centre the main approaches to foetal therapy are transplacental, invasive ultrasound-guided and (at present only in experimental animals) open foetal surgery. Many of these procedures require transgression of the foetal body (e.g. thoracentesis, paracentesis, cystocentesis, pyelocentesis, shunt placement and foetal tissue biopsy), so, in our opinion, the important question is "does the foetus feel pain"?

6.2 Foetal Pain Indicators

The definition of pain proposed by the International Association for the Study of Pain ("an unpleasant sensory and emotional experience associated with actual or potential tissue damage, that is described in term of such damage") is not adapted to dealings with the newborn or the foetus, because it assumes recognition and verbal expression of experience. We therefore need to use "indicators" of foetal pain. From the literature we can distinguish different types of indicators:

- Anatomical
- Cytochemical
- Neurophysiological
- Hormonal/haemodynamic
- Behavioural

6.2.1 Anatomical Indicators

Peripheral cutaneous sensory receptors develop early in the foetus. They appear in the perioral cutaneous area at around 7–8 weeks of gestational age and later in the palmar regions (10–10.5 weeks), abdominal wall (15 weeks) and then all over the body (16 weeks). The peripheral sensory neuron synapses on a dorsal horn interneuron, which stimulates the ventral horn motor neuron. These synapses are responsible for motor reflexes, which allow withdrawal of the limb from noxious stimuli (8 weeks) [4].

Many studies have focused on the presence of thalamic fibre synapses to cortical plate to assess whether foetuses feel pain: there are no studies of thalamocortical fibres in relation to foetal pain, so we will analyse studies on other thalamocortical circuits. Kostovic and Rakic have shown that the density of cortical plate synapses increases around 26 weeks of gestational age, but histological analysis of eight foetuses showed that thalamic projection reached the visual cortex at 23–27 weeks of gestational age [5]. Krmpotic-Nemanic et al. showed that the auditory cortex was reached at between 26 and 28 weeks of gestation, and in one case cortical plate penetration was shown at 24 weeks [6]. These data are confirmed by a study of eight foetuses that demonstrated mediodorsal afferents to frontal cortex at 24 weeks [7].

Several studies have shown that before thalamic fibres reach the cortex, they synapse on subplate neurons, that is, a waiting "compartment" before the cortical

plate. Thalamic projections reach the somatosensory subplate at 18 weeks [8]. There is evidence that neurons in the subplate zone initiate excitatory neurotransmission in the cortex, influencing the development of foetal cortical circuits [9]. These neurons may play a role in nociception transmission to the cortex.

6.2.2 Cytochemical Indicators

In the human foetus, substance P appears in the dorsal horn at 8–10 weeks' gestation [10] and enkephalin at 12–14 weeks [11].

6.2.3 Neurophysiological Indicators

EEG is a measure of the electrical activity of cortical neurons. A primitive EEG is present from 19 weeks; from 22 weeks it is possible to recognize a continuous EEG pattern that is typical of the awake state and of REM sleep in the neonate. Somatosensory evoked potentials, which test the activity of the spinal cord, transmitting visceral pain sensation, are measurable from 24 weeks [12, 13].

Positron emission tomography has showed that glucose utilization is maximal in the sensory areas of the foetal cortex, implying high levels of activity [14].

In neurobehavioural studies in drug-addicted patients, the recording of foetal cardiotocographic patterns showed a cerebral involvement (between 27 and 35 weeks of gestation) of these patterns, demonstrating the presence of opiate receptors in these foetuses [15, 16].

6.2.4 Hormonal/Haemodynamic Indicators

Stress hormones, normally released by adults experiencing pain, are released in massive amounts by the foetuses subjected to needle puncture to draw a blood sample [17, 18]. Redistribution of blood flow occurs after acute painful stimuli [19].

In a fundamental study for research into foetal pain, Fisk has shown that after intrahepatic vein (IHV) procedures, foetal plasma cortisol and endorphin increased two to six times, whereas the foetal middle cerebral artery (MCA) pulsatility index (PI) decreased by two standard deviations, consistent with the centralization or "brain-sparing" response. These hormonal and haemodynamic responses can be prevented by the administration of opioid analgesic (fentanyl) in the foetus [20, 21]. In another study, Fisk et al. demonstrated that noradrenaline (norepinephrine) and corticotropin-releasing hormone (CRH) concentrations increase after acute stress and that these events are independent from maternal responses [17, 22, 23].

The presence of opiate receptors in the foetal bladder was demonstrated by Noia et al., who measured the term of foetal micturition in drug-addicted patients by serial ultrasound evaluation until 18th week [16, 24].

6.2.5 Behavioural Indicators

Behavioural indicators of pain include withdrawing from painful stimuli and changes in vital signs and facial movements. Preterm neonates of 26 weeks' gestation exhibit cutaneous withdrawal reflex after acute stress [25], and scalp sampling increases heart rate in some foetuses [26]. Some studies have identified a special set of facial expressions, similar to those of adult pain perception, that is present during invasive procedures in the premature neonate of 30 weeks' gestation [27–30].

6.3 Long-Term *Sequelae*

There is increasing evidence that early painful or stressful events can sensitize an individual to later pain or stress. Many invasive procedures resulting in acute pain, chronic pain and prolonged stress—and also antenatal maternal psychological problems such as complications of past pregnancies or the current one, anxiety before and after invasive karyotyping or mental disorders of the mother—can be dangerous if they happen during a critical window associated with epochal brain development.

Characteristics of the immature pain system in foetuses (such as a low pain threshold, prolonged periods of windup, overlapping receptive fields, immature descending inhibition) predispose them to greater clinical and behavioural sequelae from inadequately treated pain than older age groups [31]. Repetitive pain in neonatal rat pups can lead to an altered development of the pain system associated with decreased pain thresholds during development [32]. Evidence for developmental plasticity in the neonatal brain suggests that repetitive painful experiences during this period may alter neuronal and synaptic organization [19]. It is evident from animal study that prenatal stress can modify adaptive capacities throughout the entire life of animals: the hypothalamic–pituitary–adrenal (HPA) axis mediates the animal's responses to perinatal stressful events and thus serves as a neurobiological substrate of the behavioural consequences of these early events.

In an elegant study, Vallee et al. [33] showed that stress potentiates the age-related increase in circulating glucocorticoid levels in rats. They also demonstrated a reduction of hippocampal glucocorticoid receptors, which could be responsible, at least in part, for this prolonged corticosterone secretion observed after stress in prenatally stressed rats. In addition, they showed that prenatal stress enhances age-related memory impairments; thus, they hypothesized that elevated glucocorticoid levels could cause neuronal loss in the hippocampus, with subsequent impairment of cognition and memory [33].

In the primate model, exposure to a 2-week period of exogenous ACTH is associated with impaired motor coordination and muscle tonicity, reduced attention span and greater irritability [34]. Exposure to stress in utero is associated with higher levels of ACTH and cortisol in stressed newborns and with lower scores for attention and neuromotor maturity after birth [35, 36].

As we know, the HPA and immune systems are mutually regulatory, and their interactions partially determine the effects of stress on immune function. Premature

alteration of the HPA axis can cause alteration of the immune function, and thus prenatal stress could also have long-term implications in respect of infectious and autoimmune diseases.

In primates prenatal stress effects also appear to vary with the stage of pregnancy at which disruption occurs. Disruption during early pregnancy increases cellular immune responses, whereas prenatal stress exposure during mid- to late pregnancy can be immunosuppressive in adult offspring [37].

In rats, Gorczynski observed that stress-related immunosuppression (as measured by antibody responses and skin graft rejection) was most pronounced in offspring born from prenatally stressed dams [38]; prenatal stress accelerates the onset and increases the prevalence of diabetes in the nonobese diabetic (NOD) mouse model of insulin-dependent diabetes [39].

In humans, several studies demonstrated that early pain or stress (both in utero and in the neonatal period) impairs the physiological development of the nervous system, the HPA axis and the immune system, producing long-term altered susceptibility to pain, to inflammatory diseases and to psychiatric disorders in later life.

It is important to understand the concept of "development plasticity", which means that one genotype can become many phenotypes depending on different environmental conditions during development [40]. Preterm neonates who had experienced 4 weeks of neonatal intensive care unit therapy manifested decreased behavioural responses and increased cardiovascular responses to the pain of a heel prick compared with neonates born at 32 weeks [41]. Taddio et al. demonstrated that children undergoing ritual circumcision immediately after birth without any kind of anaesthesia react more vigorously to vaccination at 2 months of age than those who received anaesthesia before surgery [42]. Other studies focused on hypersensitivity and hyperalgesia in the wound area after surgery [43].

The best evidence for an effect of maternal stress on the physical development of the baby comes from a study which has examined the links between prenatal stressors and foetal brain development. Information about stress was obtained from 3021 pregnant women by questionnaire. The 70 most stressed patients were compared with 50 controls. The authors found that antenatal stress is associated with a lower gestational age, lower birth weight, smaller head circumference and worse score on the neonatal neurological examination [44].

Zappitelli et al. showed that the mother's emotional state has a role in abnormal development of the neural dopaminergic system and can lead to attention deficit hyperactivity disorder (ADHD) in early childhood [45].

To test the role of maternal stress during pregnancy in psychiatric and behavioural disorders, a retrospective epidemiological study was conducted. One hundred and sixty-seven persons were identified whose fathers had died before they were born; a control group comprised 168 persons whose fathers had died during their first year of life. The number of diagnosed schizophrenics treated in psychiatric hospitals and the number of persons committing crimes were significantly higher in the index than in the control group. The results suggest that, especially during months 3–5 and 8–9 of pregnancy, maternal stress may increase the risk of psychiatric disorders in the children, perhaps mediated through the inborn temperament of the child [46].

The studies by Bracha et al. of schizophrenia in twins are very interesting. Concordances in subtypes of monozygotic twins can be used to investigate the influence of prenatal development in the aetiology of mental illness. The results of Bracha et al. indicate that simple monozygotic concordance rates may overestimate the heritability of schizophrenia and that prenatal development may also be important in its aetiology. These authors thought that the second prenatal trimester is the critical period of the massive neural cell migration to the cortex and of the migration of fingertip dermal cells to form ridges. By determining differences in the fingertip ridge count of monozygotic twins discordant for schizophrenia, they demonstrated that prenatal stressors may contribute to the aetiology of schizophrenia. They analysed 30 pairs of monozygotic twins, 23 pairs in which the twins were discordant for schizophrenia and 7 pairs in which both twins were normal. In the discordant for schizophrenia pairs, no pair had the same number of digital ridges, despite their homozygosity. The authors concluded that twins, even homozygous ones, can react differently to the same maternal stress during the second trimester of pregnancy [47, 48].

It is our daily experience that invasive procedures can induce the pregnant mother to stop up "the channels" with her baby during the time when she is waiting for the answer on a prenatal diagnosis. Women who have an amniocentesis put their feelings on hold: what happens is a kind of separation of body and mind. The woman alienates herself from her own body and, by implication, from the child growing within her [49]. In our opinion this can be a severe insult to the developing foetus, and psychological support for these women could be an act of future social prevention.

6.4 Discussion

Some authors distinguish nociception from pain: the former is just an activation of anatomical pathway, while the latter requires the presence of consciousness [50, 51]. Recently, Lee and colleagues published a systematic review that caused an interesting debate among scientists [52]. This is the first review about foetal pain. Many states in the USA are considering legislation requiring informed consent regarding analgesia for the foetus during abortion procedures after 20 weeks of gestation. Georgia and Arkansas have already approved such statutes [53, 54], so the authors ask: "Does the fetus have the functional capacity to feel pain?" They undertook a systematic review via PubMed of English language articles on foetal pain. A multidisciplinary team revisited all the articles. They conclude that evidence regarding the foetus's feeling of pain is limited, and anyway it is unlikely that there is pain perception before the third trimester. The authors state that pain is an emotional and psychological experience that requires conscious recognition of a noxious stimulus. The presence of an anatomical pathway and other indicators does not mean that pain perception exists [52]. Derbyshire affirms that pain perception requires the development of representational memory that is acquired only after birth [55]. Other authors

state that studies on pain in preterm infants are not applicable to foetal pain perception, because "the fetus is actively maintained asleep (and unconscious) throughout gestation and cannot be woken by nociceptive stimuli". According to the authors, if the foetus is never awake, it cannot be conscious and thus cannot feel pain. They try to demonstrate that the foetus is always asleep because in utero there are some chemical suppressors of foetal behaviour and cortical activity such as adenosine, allopregnanolone, pregnanolone and prostaglandin D_2. They also point to the warm temperature in the uterus and the presence of amniotic fluid, which induces sleep and protects the foetus from tactile stimulation. All these factors produce inhibition of cortical activity that is responsible, in their opinion, for the differences between foetus and neonate in their responses to stimuli, such as the response to hypoxia, which causes an arousal reaction in the neonate and depression in the foetus. In our opinion, it has been widely shown in the scientific literature that foetuses have access to a great deal of sensation in the uterus: they can perceive sound, the changing taste of the amniotic fluid, touch and pressure stimuli to the mother's abdomen, changes in light and dark and changes in balance [56–58].

The review by Lee and colleagues has many limitations. The first issue to discuss is the question of whether consciousness is a condition necessary for the feeling of pain. According to the Oxford English Dictionary, pain is "a strongly unpleasant bodily sensation such as is produced by illness, injury or other harmful physical contact" [59]. Probably when a pregnant woman asks if her foetus can feel pain, she does not mean a conscious rationalization of pain. This is important for well-informed counselling of the women. Moreover, as Austin suggests [60], some of statements from which the author draws his conclusions must be questioned. The first is a semantic matter: according to the International Association for the Study of Pain, "each individual learns the application of the word [pain] through experiences related to injury in early life". This definition has limited biological and clinical application: in order to experience pain, the individual must first learn what pain is, and in order to learn what pain is, he must first experience it. This is a never-ending circular argument.

The second point is "Derbyshire claims that the perception of pain requires the development of representational memory; but, although memory may be necessary for the interpretation of pain, it is not for its perception. Even if the foetus does not recognize pain, the experience still remains unpleasant [60].

Despite their limited scientific significance, foetal pain indicators must be borne in mind especially because they are clinical signs of pain during surgery in unconscious and anaesthetized adults and paediatric patients [61].

The evidence that early exposure to noxious stimuli has adverse effects on future neural development is increasing [12, 62]. It follows that noxious stimulation may not need the presence of consciousness to alter the course of sensory development.

In our opinion this debate is strongly motivated by the question around voluntary termination. Claiming that abortion can cause foetal pain has important consequences for the personal ethics of both the parents and the clinicians—but this fear must not impede a scientific and honest search for the truth.

Conclusions

The American Academy of Pediatrics, Fetus and Newborn Committee wrote a statement on the prevention and management of pain and stress in the neonate. The objectives of the statement are to:

- Increase awareness that neonates experience pain. Provide a physiological basis for neonatal pain and stress assessment and management by healthcare professionals.
- Make recommendations for reduced exposure of the neonate to noxious stimuli and to minimize associated adverse outcome.
- Recommend effective and safe interventions that relieve pain and stress [63].

Opioid analgesia for minor and major procedures has been shown in randomized trials to reduce metabolic and biophysical stress responses, postoperative morbidity and mortality and abnormal imprinting of subsequent pain responses in infancy [64].

Premature neonates are foetuses out of the intrauterine environment. Prevention or treatment of pain is a basic human right regardless of age, and the humane care given to premature babies needs to be extended to the foetus.

We hope that all foetal medicine units will really take care of the well-being of the foetus, which is to take care of the well-being of the person for his entire life.

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Analgesia During Fetal Surgery

7

Gloria Pelizzo

7.1 Introduction

Thanks to advances in high-resolution ultrasound and MRI, an increasing number of conditions are diagnosed early during gestation, and new insights into their pathophysiology have been reported. Some of these conditions are life-threatening in utero or due to possible irreversible organ damage may benefit from prenatal surgical intervention [1].

Malformations that qualify for fetal surgery should satisfy the following prerequisites [1, 2]:

1. Prenatal diagnostic techniques should identify the malformation and exclude other lethal malformations with a high degree of certainty.
2. The defect should have a defined natural history and be known to cause progressive irreversible injury to the fetus after delivery.
3. Repair of the defect should be feasible and should reverse or prevent the pathological process.
4. Surgical repair must not entail excessive risk for the mother or her future fertility status.

As reported in Table 7.1, there are several diseases that can be treated with *intra utero* procedures either directly on the fetus or through the placenta and cord [2–5].

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Table 7.1 Congenital malformations and fetal treatment

Congenital malformation	Fetal treatment
Pulmonary cystic adenomatoid malformation	Lobectomy or thoraco-amniotic shunt positioning
Myelomeningocele (MMC)	MMC repair
Sacrococcygeal teratoma (SCT)	Fetal resection
Urinary tract obstruction	Decompression with shunt positioning
Twin-to-twin transfusion syndrome	Laser fetoscopy
Congenital diaphragmatic hernia (CDH)	Tracheal occlusion—FETO procedures
Congenital heart disease (CHD)	Aortic and/or pulmonary valvuloplasty, intracardiac stent positioning

7.2 Contents in Fetal Pain

All invasive procedures on the fetus focus on the questions: when does a fetus have the functional capacity to feel pain? If that capacity exists, what forms of anesthesia or analgesia are safe and effective for treating fetal pain [6]?

In the early 1980s, the Subcommittee on Taxonomy of the International Association for the Study of Pain defined pain as an "unpleasant sensory and emotional experience, associated with actual or potential tissue damage, that is observable through some form of detectable behavior" [7].

Even though debate exists over whether the fetus perceives pain, a number of articles confirm that the fetus can experience and respond to painful events with sudden fetal movements. It is also known that the fetus reacts to painful stimuli by various motor, autonomic, hormonal, and metabolic changes at relatively early stages of gestation [8, 9].

At the end of the second trimester of gestation, the fetus has the appropriate neural structures to perceive pain. From the eighth week of intrauterine life, the first nociceptive receptors develop, and by the 20th week of gestation, they are found in all parts of the body. By the 23rd gestational week, the central nervous system is anatomically and functionally receptive to nociception. The complete myelination of nociceptive pathways begins around the 30th week and is completed after birth [9–16]. The formation and myelination of central nociceptive areas (thalamus, sensory cortex, limbic system, hypothalamus, and cerebral cortical association areas) are realized in the postnatal period up to 1 year. The completion of the central and peripheral areas for pain control allows the multimodal perception of stimuli, with memory [9–16].

Theoretically, there are no age limits for the perception of pain [8, 17]. Many US states recognize this principle and, in defense of fetal rights, have enacted statutes/legislation requiring physicians to inform women seeking abortions 20 or more weeks after fertilization (i.e., 22 weeks' gestational age) that the fetus has the "physical structures necessary to experience pain," as evidenced by "drawing away from surgical instruments." The physician must also offer anesthesia or analgesia "administered directly" to the fetus. Physicians who do not comply may be subject

to substantial fines, license revocation, and civil suits for punitive damages [18, 19]. Although this legislation does not affect most US abortions, because only 1.4% are performed at or after 21 weeks' gestational age, this legislation raises important scientific, clinical, ethical, and policy issues [6, 19].

To date, there is no method for recording fetal pain during pregnancy. In recent years, potential behavioral patterns in preterm infants who have experienced pain have been reported as a model to study fetal pain perception. Exposure to repeated pain in very preterm infants (VPT, ≤32 weeks gestational age [GA]) in the neonatal intensive care unit (NICU) has been associated with altered cognitive and motor development [20, 21] after birth. Preterm patients, who have been exposed to varying long-term levels of pain-related stress (e.g., skin-breaking procedures) in the NICU, present with major health-care issues associated with a heightened risk of impaired neurobehavioral development [22] even in the absence of major postnatal morbidities or brain injuries. Blunted reactivity to physical stressors (i.e., clustered nursing procedures) in VPT infants at an age of 32 weeks post-conception is a function of the number of skin-breaking procedures experienced during the NICU stay [21]; these stressful events constitute a relevant early adverse experience capable of altering the development of the hypothalamic-pituitary-adrenal (HPA) axis, as measured by salivary cortisol reactivity [23, 24]. Dampened reactivity is a well-known effect of early chronic exposure to stress in humans and might reflect the way the HPA axis maintains stability by increasing negative feedback regulation. Neonatal pain quantified as the number of neonatal skin-breaking procedures adjusted for clinical confounders might at least partially explain reactivity to socio-emotional stress at 3 months and the higher risk of later adverse, internalized behavioral outcomes. Internalized behaviors, anxiety/depression, withdrawal, and somatic problems, prevalent in children born very preterm as compared with full-term neonates, are evident by 2 years corrected age (CA) [25–27] and persist to school-age late adolescence and young adulthood [28–34].

The most plausible hypothesis to explain this scenario is that the preterm infants' physiological learning begins with stressful experiences in the NICU as well as programming stress susceptibility perceived later in life [35].

Animal studies have established that early-life adversity, including maternal separation, pain exposure, and fetal surgery, may induce long-term behavioral changes [15, 36–40].

7.3 Management of Fetal Anesthesia

The survival and long-term health of both the mother and fetus should be considered by the anesthesiologist when planning fetal surgery with the aims of reducing/eliminating fetal pain and protecting the mother from surgical complications.

Fetal surgery is based on the following principles: inhibit fetal movement during a procedure; induce uterine atony, as a prerequisite to allow surgical access to the fetus and to limit the risk of contractions and placental separation; prevent hormonal stress response with adequate fetal anesthesia and analgesia; and guarantee analgesic sedation and maternal hemodynamic stability [6, 17, 41–43].

Management of fetal anesthesia also requires adequate knowledge of maternal-fetal hemodynamics, as reported in recent literature.

The maternal heart rate in mid-gestational pregnant ewes seems to provide regulation of placental flow during fetal surgery [44]. Due to placental transfer exchange, both the fetus and mother are protected from the effects of various substances which could cause damage. Pharmacokinetics change during pregnancy and possibly also influence maternal liver blood flow. During anesthesia delivery, some substances, propofol included, are strongly influenced by the placental passage; the low concentration reaching the fetus could partially explain the minimal fetal cardiac depression and limited anesthesia exposure in the fetal brain [43, 45].

Based on the abovementioned considerations, evidence of fetal pain is not required to justify dedicated fetal anesthesia and analgesia during surgery. Moreover, a dedicated prenatal anesthesiological approach is not based solely on pain reduction. In fact, the central consideration in fetal surgical management is more complex and includes the concept of "long-term fetal well-being" [46–50]. Anesthesia and analgesia management in the fetus, a special patient class, require a dedicated modality. Furthermore, an adequate anesthesiological approach regarding the mother will help shorten surgical times by improving surgical technical conditions.

General and regional anesthesia can be utilized, depending on the type of procedure [4, 17, 41–43, 51–55] (Table 7.2).

As opposed to anesthesia in the mother during cesarean section, fetal anesthesia should provide uterine relaxation. Due to the complexity of open fetal procedures, the duration of anesthesia is longer than that administered during a cesarean section, and the anesthetic requirements during open fetal surgery are greater. Nonetheless, we cannot assume that uniquely maternal anesthesia is sufficient to provide adequate anesthesia to the fetus too. This is evident in the case of cesarean sections performed with maternal general anesthesia, hwhere fetuses are delivered awake and surely not anesthesized.

During fetal surgery, propofol administration provides adequate maternal anesthesia and uterine relaxation without the direct fetal cardio-depressive effects observed with high-dose inhaled anesthesia.

7.4 Fetal Surgical Intervention and Anesthetic Goals

Fetal surgical interventions include the following procedures [51, 56]:

7.4.1 Minimally Invasive Fetoscopic Treatment

Minimally invasive procedures include ultrasound-guided fetal blood sampling, intrauterine transfusion, selective feticide, radio-frequency ablation of a nonviable twin, and fetal cardiac puncture for laser atrial septostomy [56]. Minimally invasive fetal procedures and fetoscopy do not require maternal laparotomy or hysterotomy

and instead use needles or endoscopy to access the fetus. These procedures are generally performed under locoregional anesthesia [4, 17, 41–43, 51–55].

7.4.2 Fetoscopic Procedures

Intrauterine endoscopic surgery is performed for laser coagulation of connecting vessels in twin-to-twin transfusion syndrome, selective cord occlusion, fetal endoscopic tracheal balloon occlusion, and the subsequent removal of the tracheal balloon or the resection of urethral valves [4, 17, 41–43, 51–55].

7.4.3 Open Procedures

Open fetal surgery includes an hysterotomy of the mother, with or without fetal exposure, for the surgical treatment of myelomeningocele, cystic adenomatoid malformation of the lung, and selected cases of sacrococcygeal teratoma [56].

Laparotomy and hysterotomy of the mother require general and/or regional anesthesia with the aim of inducing uterine atony and fetal immobilization. Inhaled anesthesia typically includes isoflurane or desflurane to maintain uterine relaxation [51, 52].

7.4.3.1 EXIT Procedure (Ex Utero Intrapartum Procedure)/OOPS (Operations on Placental Support)

Intrapartum in utero therapy was initially described in cases in which the airway was secured and surfactant was administered to patients in whom clip tracheal occlusion was practiced for managing congenital diaphragmatic hernia during the cesarean section under placental support. This therapy was later adapted to manage patients with giant neck masses or congenital upper airway obstruction (CHAOS syndrome). Other indications for EXIT/OOPS include: giant chest masses, pulmonary agenesis, and situations in which neonatal resuscitation is very complex [17].

EXIT procedures are usually performed under general anesthesia similar to open fetal surgery. In this case, the goals of anesthesia are to ensure adequate uterine relaxation to externalize the fetal head and trunk and avoid premature placental abruption, in addition to maintaining uterine volume, placental support, and maternal hemodynamic stability, while securing the airway in a controlled manner in an anesthetized fetus [57].

As reported by Cheek [57], analgesia, anesthesia, and fetal immobility may be provided by several means.

The most common methods include: administration of agents to the mother that take advantage of their high transplacental passage (inhaled agents, remifentanil-type opiates), intramuscular or intravenous administration directly to the fetus or through the umbilical cord (neuromuscular relaxants, fentanyl-type opiates), and, in some cases, administration of intra-amniotic anesthetics.

Table 7.2 Anesthetic management (analgesia and anesthesia) [56]

Surgical intervention	Fetal anesthesia
Mini-invasive surgery in the fetus	Fetal (i.m. or cord) opioids (e.g., fentanyl 10 μg/kg) and muscle relaxants (e.g., pancuronium 0.3 mg/kg) or maternal i.v. remifentanil 0.1–0.2 μg/kg/min
Mini-invasive surgery through the placenta and cord	Maternal i.v. remifentanil 0.1–0.2 μg/kg/min
Open surgery	Fetal anesthesia delivered through the placental passage, additional anesthesia can be given to the fetus (i.m. or cord), e.g., opioids (fentanyl 10 μg/kg), as well as muscle relaxants (e.g., pancuronium 0.3 mg/kg)
EXIT	Fetal (i.m. or cord) opioids (e.g., fentanyl 10 μg/kg) and muscle relaxants (e.g., pancuronium 0.3 mg/kg) or maternal i.v. remifentanil 0.1–0.2 μg/kg/min

Conclusions

New studies with innovative approaches are needed to define fetal pain relief during the second and third trimester of pregnancy. These investigations would better define the modality of fetal pain perception. Importantly, the absence of fetal pain evidence does not resolve the question of when pain perception begins. To date, evidence-based guidance for invasive procedures during pregnancy has underscored that injuries occurring in utero or in early life may contribute to negative long-term postnatal outcomes.

These data alone sufficiently establish the need to prevent fetal stress; thus, every effort should be made to avoid this occurrence during invasive surgical procedures.

New fields of research should consider the fetus as a special patient class requiring focused research studies and a dedicated multidisciplinary team to respect his/her neuropsychological development and long-term well-being.

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New Insights into Prenatal Stress: Immediate- and Long-Term Effects on the Fetus and Their Timing

8

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8.1 Introduction

A burgeoning literature emphasizes the importance of the in utero environment for a range of fetal, neonatal, infant and adult health-related outcomes [1]. This period of prenatal development, characterized by rapid growth and development, is a time of increased vulnerability, when intrauterine insults can have deleterious effects on emerging systems and structures. One important factor which affects the in utero environment is maternal stress.

The immediate effects of prenatal stress relate to fetal well-being and the possible experience of the fetus. In this chapter we will discuss fetal stress responses, the potential for the fetus to have experiences, including feeling pain, and the immediate- and long-term effects of maternal stress on the fetus.

8.2 Fetal Stress Responses

Human fetal endocrine responses to stress have been demonstrated from 18 weeks' gestation. Our group first demonstrated increases in fetal plasma concentrations of cortisol and β-endorphin in response to needling of the intrahepatic vein (IHV)

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for intrauterine transfusion [2]. Fetuses receiving the same procedure of transfusion, but via the non-innervated placental cord insertion, failed to show these hormonal responses [3]. The fetal cortisol response, independent of the mother's, was observed from 20 weeks' gestation but increased with gestational age. A similar but faster response is seen in fetal plasma noradrenaline (norepinephrine) levels to IHV needling. This, too, is observed in fetuses from at least 18 weeks and is independent of the maternal response [4]. Human intrauterine needling studies that involve transgression of the fetal trunk have shown that brain sparing, as assessed by colour Doppler ultrasonography, affects the human fetus from at least 16 weeks' gestation, with a decrease in the pulsatility index of the middle cerebral artery, indicative of increased blood flow to the brain [5], and an increase in pulsatility index of the femoral artery [6].

Thus, from these studies, one can conclude that the human blood flow redistribution response to an invasive stimulus is functional from at least 16 weeks' gestation, that the sympathetic system with the release of noradrenaline is functional from at least 18 weeks and that the hypothalamic–pituitary–adrenal (HPA) axis is functionally mature enough to produce a β-endorphin response by 18 weeks and a cortisol response from 20 weeks' gestation.

8.3 Fetal Experience and Fetal Pain

Stress responses and blood flow redistribution do not show that the fetus is feeling pain. Production and release of stress hormones such as cortisol can be mediated by the hypothalamus, without involvement of the cortex or other higher brain regions involved in sentience. Although stress hormones are increased when an individual suffers pain, many other situations which are not painful, such as exercise, also increase levels.

We still do not know when the fetus starts to feel pain or when it starts to become conscious [7]. This subject is particularly difficult because the understanding of the physical basis of conscious awareness in the human adult is still limited. There may not be a single moment when consciousness, or the potential to experience pain, is turned on; it may come on gradually like a light on a dimmer switch [8]. Conscious experience is associated with the activation and inactivation of populations of neurons that are widely distributed in the thalamocortical system. The driving force of the activations of the thalamic cortical connections comes from lower in the brain stem, in the reticular activating system, which is located in the evolutionarily older part of the brain [9]. Waking consciousness is associated with low-level irregular activity in the electroencephalogram (EEG) ranging from 12 to 70 Hz [10]. However, sufficient conditions for consciousness are hard to establish. Edelman and colleagues have discussed evidence for consciousness in different animal species and concluded that birds may well be conscious, and it is even possible that the octopus is also [10, 11]. Both birds and octopuses have brains as different from an adult human as does a mid-trimester fetus, and their arguments suggest that we cannot be sure that for conscious experience, it is necessary to have a functional cerebral cortex similar to that in the human adult.

For the fetus to feel pain, there must be functional connections between the peripheral nociceptive receptors and the sites in the brain necessary for conscious experience of pain. There is considerable evidence, from positron emission tomographic studies in adults, that when pain is experienced as unpleasant, there is activation of a thalamic pathway which projects to areas of the cortex including the anterior insula, the anterior cingulate and the prefrontal cortex [12]. When these pathways are functional, it is likely that the fetus or baby will feel pain. At earlier stages, we have to make an informed guess.

The nervous system of the human fetus develops gradually throughout gestation, with the anatomical pathways and synapses forming first in the periphery and spinal cord and then moving upwards into the brain. In the brain, the lower structures are connected first, and then anatomical pathways are formed outwards towards the thalamus, the subplate zone (a region specific to the fetus which lies underneath the cortex) and finally the cerebral cortex.

The first essential requirement for nociception is the presence of sensory receptors, which start to develop in the perioral area at around 7 weeks' gestation [13]. Sensory receptors then develop in the rest of the face and in the palmar surfaces of the hands and soles of the feet from 11 weeks. By 20 weeks, they are present throughout the skin and mucosal surfaces. In the first trimester, stimulation of sensory receptors can result in local reflex movements involving the spinal cord, but not the brain. Thus the fact that a fetus of 12 weeks will move away if touched is most unlikely to be associated with any conscious experience. As neurodevelopment continues these reflex pathways connect with the brain stem, and sensory stimulation can cause other responses such as increases in heart rate and blood pressure.

Assuming that activity in the cerebral cortex or subplate zone is necessary for consciousness, then for the fetus to be conscious of an external stimulus, these regions need to be connected with incoming nervous activity. Most incoming pathways, including nociceptive ones, are routed through the thalamus and start to penetrate the subplate zone from about 17 weeks. However, no human studies have examined the development of thalamocortical circuits specifically associated with pain perception.

Physiological evidence concerning the function of these pathways is even more limited than their anatomical development. There is evidence for a primitive EEG from 19 to 20 weeks. Sustained EEGs are obtainable from preterm infants of 23 weeks' gestation. Studies of evoked responses in preterm infants show that both visual and somatosensory potentials can be elicited from 24 weeks and are well developed by 27 weeks [14]. Clinical observations with preterm babies suggest that the nociceptive system is functional at 24–26 weeks [15], but when exactly it starts to function prior to this is not known.

Lee et al. [16] have stated that the capacity "for conscious perception of pain can arise only after thalamocortical pathways begin to function, which may occur in the third trimester around 29–30 weeks' gestational age." As discussed above, given the limitations of our current knowledge, this is unduly definite. Pain perception in the fetus may not use the same pathways as in the human adult, just as it may not in other species, such as the octopus [11]. Many fetal structures are different from those in the adult and may function in a different way. We do not know that in the

fetus thalamocortical pathways are essential for any perception of pain. Connections from the thalamus to the subplate zone may be sufficient, for example. If Lee et al.'s reasoning were correct, it would imply that many premature babies in intensive care do not feel pain either.

We suggest that the current evidence, although still limited, makes it quite likely that the fetus can feel pain from 26 weeks and very unlikely that it can feel pain before 17 weeks. It is possible that some sensory experience of pain may start by about 20 weeks.

8.4 Immediate Effects of Prenatal Maternal Experience on the Fetus

By mid-gestation, a fetus is rapidly responsive to a diverse array of environmental stimuli [17]. Animal studies have demonstrated a myriad of negative effects upon the fetus, including changes in heart rate, blood pressure and reduced arterial oxygenation, providing evidence for direct effects of maternal stress reactivity on fetal physiology [18, 19]. More recent studies demonstrate that distinct fetal behaviours, such as changes in fetal heart rate in response to maternal psychological functioning, begin to emerge from 24 weeks [20].

Increases in fetal heart rate in the final trimester, following exposure to a cognitive stressor such as the Stroop test, were found to be greatest in mothers reporting increased depressive symptoms [21] and increased with gestational age. Paradoxically, this increase in the fetal response is in contrast to the progressive blunting of the maternal stress response to such stressors [22]. Both the maternal HPA axis and sympathetic system are found to become hyporesponsive to acute physical and psychological stressors toward the latter stages of pregnancy [22, 23]. But as gestation advances, and the fetus matures, an increased sensitivity to maternal input is observed.

8.5 Evidence from 4D Scanning

Much of our understanding on the fetal stress response has been informed by invasive procedures, which are no longer used in routine clinical practice, e.g. fetal blood sampling from the hepatic vein. However, technological advances in fetal four dimensional (4D) imaging permit the direct assessment of fetal behaviour, which may provide new insights into the fetal response to pain.

Fetal facial movements [24] and fetal touch behaviours [25, 26] observed in ultrasound scans are increasingly being used as a non-invasive method to observe the developing nervous system prenatally. This method according to Butterworth and Hopkins [27] can be used to chart the functional development of the fetal nervous system but also identify cognitive development. They argued, for example, that the fetus touching the mouth with a hand is evidence for goal-directed behaviour. Fetuses may learn through accidental movements which develop into patterns and might help normal postnatal development [28].

Depending on the type of touch from reflex reactive movements observed in the first trimester [29] to anticipatory touch necessitating an increased level of motor control [30], progressively more cognitive involvement may be involved. In one study [26], fetuses were scanned using 4D ultrasound recordings from 24 to 36 weeks' gestation. An analysis of the changes in the proportion of different sequences of touch events by fetal age showed an increasing trend in the trajectory in "anticipated touch," namely, mouth movement before touch occurred and a decreasing trend in reactive mouth movements, namely, mouth movement following touch. The results showed a significant increase of around 8% with each week of gestational age in the proportion of number of mouth movements by fetuses anticipating a touch rather than reacting to a touch sensation. Furthermore, the proportion of reactive mouth movements decreased by around 3% for each week of gestational age. This potential for cortical learning via sensory motor experience in the fetal period has also been studied by Yamada et al. [31]. The authors modelled a number of fetal movements, including touch behaviours, at 32 weeks' gestation and argued that specific intrauterine movements induced somatosensory feedback, facilitating cortical learning of body representations.

Reissland and colleagues have studied a range of fetal facial expressions including those that in children or adults would be associated with pain or distress. Some of these different expressions are shown in Fig. 8.1. In Fig. 8.1a, the fetus is touching its mouth with its hand. In Fig. 8.1b, the expression resembles a smile in a baby. The pain/distress gestalt was defined as needing at least 4 co-occurring facial movements (see Fig. 8.1c). This study demonstrated that as the fetus matures we can see more of these facial configurations occurring spontaneously. In the light of research indicating that pathways mediating nociception appear to be functional at least from the third trimester, it is of interest that fetal facial expressions from around 28–30 weeks also show facial expressions which correspond to the experience of pain.

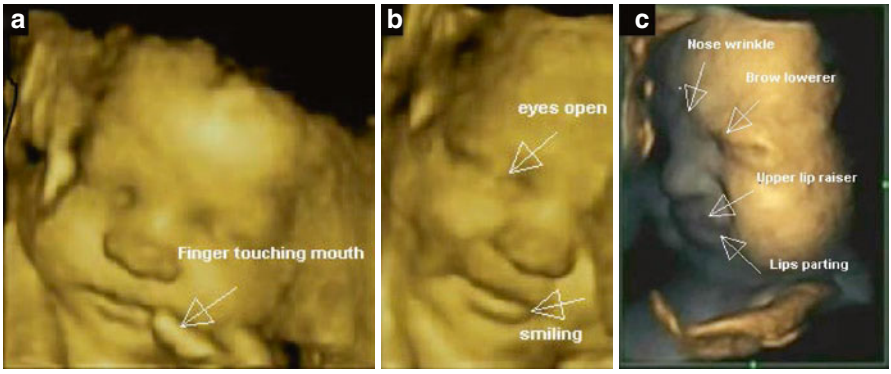

Fig. 8.1 Four-dimensional imaging of the fetus reveals a repertoire of behaviours evident from 28 weeks gestational age. Behaviours include facial touching (**a**), facial expressions which resemble smiling (**b**) and expressions typical of pain/distress (**c**) defined by four co-ordinated movements (lips parting, upper lip raiser, nose wrinkle and brow lower)

It would be interesting in the future to determine whether fetuses undergoing potentially painful invasive procedures show more pain/distress facial expressions during the intervention.

The ability to express facial gestalts of pain might be adaptive, preparing the fetus for postnatal life, and the need to alert carers to pain experiences by the infant. Given that it is well established that fetal prenatal exposure to sounds and language (e.g. [32]) are remembered postnatally, it is possible that the infant also remembers prenatal facial patterns after birth.

8.6 Long-Term Effects of Prenatal Maternal Stress on the Fetus: Fetal Programming

The long-term effects of the fetal period have been highlighted by epidemiological studies carried out by Barker and colleagues [33, 34]. They have shown that poorer fetal growth is associated with increased mortality due to coronary heart disease, together with other aspects of the metabolic syndrome. A separate strand of research has examined the long-term effects of prenatal stress on neurodevelopmental outcomes [35, 36]. There is now good evidence that if the mother is stressed, anxious or depressed while pregnant, this increases the risk of her child having a range of behavioural, emotional or cognitive problems. The most consistently observed adverse outcome are symptoms of attention deficit hyperactivity disorder, which have been found in children between 4 and 15 years of age [37, 38]. However, other effects have also been described, such as increases in anxiety symptoms [37] and externalizing problems [39]. Separate studies have shown an effect of prenatal stress on the cognitive development of the child or its performance at school. These studies have focused primarily on infants and young children, although one study found an association between maternal antenatal stress and school marks at 6 years [40]. Huizink and colleagues [41] reported an association between maternal reports of daily hassles, pregnancy-related concerns and performance on the Mental Developmental Index (MDI) at 8 months of age. Bergman et al. [42] have also found that exposure to life events during pregnancy was associated with a significant reduction in the same scale in children of 14–19 months; there was no such link with postnatal life events scores.

Maternal exposure to traumatic events during pregnancy has also been associated with children's cognitive outcomes. Toddlers whose mothers were pregnant during the 1998 ice storm in Quebec—a disaster that resulted in the loss of electricity and water for up to 5 weeks—displayed lower MDI and language development scores compared to standardized norms [43]. Investigating cognitive development at later stages in childhood using assessments with greater predictive reliability remains an important next step.

It is also of interest that in one recent study of financially and emotionally stable women, there was a small but significant positive association between antenatal stress and both the MDI and physical developmental index (PDI) of the Bayley scales of infant development [44], suggesting beneficial effects of exposure to small to moderate levels of antenatal stress on child developmental outcomes.

Antenatal stress has also been associated with altered adult outcomes, although here studies have focused almost exclusively on psychopathology. Maternal exposure to traumatic events during pregnancy, for example, has been associated with increased lifetime risk of developing psychiatric disorders. In a retrospective cohort study, Van Os and Selten [45] demonstrated that the offspring of women who were pregnant during the German invasion of the Netherlands in 1940 were at significantly increased risk for developing schizophrenia. These results were replicated among a sample of Dutch adults whose mothers were pregnant during a devastating flood in 1953 [46]. An increased incidence of affective disorders has also been observed in individuals exposed in utero to the effects of the Dutch winter famine 1944–1945 [47, 48].

The studies described have reported associations between antenatal stress and a range of negative sequelae spanning infancy through to adulthood. However, other factors impinging on the development of emotional/behavioural problems must also be considered, ranging from shared genetic variance to indirect behavioural mechanisms of influence. The best evidence for an antenatal effect of psychological stress comes from large population studies in which the association between prenatal stress and negative outcomes remains even after controlling for maternal postnatal anxiety or depression and a wide range of other possible confounders [37, 49]. The periods of greatest vulnerability for fetal programming effects are not clear and are likely to differ for different outcomes.

8.7 Underlying Mechanisms: HPA Axis and Associated Biological Processes

Investigators have focused primarily on the HPA axis in both mother and child as the primary biological mechanisms underlying the long-term effects of prenatal stress. In animal models, both rodent and non-human primate, the central role of the HPA axis in mediating prenatal stress effects in both mother and offspring is well established [50, 51]. Studies with non-human primates have provided convincing evidence that prenatal stress, and its associated increase in maternal HPA activity, is related to both short- and long-term negative sequelae in the offspring [52], including impairments in attention as well as heightened levels of anxiety. The central role of the maternal HPA axis has been demonstrated by showing that behavioural effects can be replicated by administering ACTH to the pregnant monkey [50] and abolished by adrenalectomy [53].

There is much less understanding of the mechanisms underlying the apparent effects of antenatal stress in humans, including the role of the HPA axis in mother or child [54]. In one study, O'Connor et al. [55] found maternal antenatal anxiety at 32 weeks predicted children's morning cortisol concentrations after allowance was made for obstetric and sociodemographic factors. No links between children's cortisol and maternal anxiety or depression earlier in pregnancy or postnatally were observed [55]. However in adolescent children, the association with alterations in the diurnal cortisol output was much less marked [56].

There is evidence of a strong correlation between maternal plasma and fetal plasma cortisol levels [57], although fetal levels are about 10 times lower than maternal levels. Recent results demonstrate that the correlation between maternal and fetal cortisol only becomes significant by mid-gestation [58].

Based on the animal literature, a primary hypothesis would be that if the mother is stressed, her cortisol rises and this in turn crosses the placenta in sufficient concentrations to affect fetal development. However, problems remain with this proposed mechanism in humans. In particular, maternal cortisol responses to stress decrease markedly across gestation, such that by late pregnancy, the maternal HPA axis can be quite unresponsive [23, 59, 60]. Thus, at the time in pregnancy when there appears to be the strongest link between maternal and fetal cortisol, the maternal HPA axis becomes less sensitive to stress. However there is evidence that if the mother is more anxious, there is a downregulation of the placental enzyme that metabolizes cortisol, thus potentially allowing more cortisol to pass from mother to fetus [61]. However, other mediating mechanisms are also possible; they just have not been extensively studied in humans. For example, stress and anxiety cause substantial activation of the sympathetic–adrenal system, and this could also be important. Noradrenaline does not appear to cross the placenta [4] but could have indirect effects upon the fetus by acting to cause contractions of the myometrium or by reducing uterine blood flow by affecting trophoblastic invasion. The role of immune activation (e.g., pro-inflammatory cytokines) and monoamines (e.g., serotonin) remains to be explored more fully.

Conclusions

It is suggested that extra vigilance or anxiety or readily distracted attention may have been adaptive in a stressful environment during evolution but exists today at the cost of vulnerability to neurodevelopmental disorders [62]. The effects of prenatal stress on child outcome should be a major public health concern.

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Part III

Neonatal Pain

Understanding Infant Pain Responding Within a Relational Context

9

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9.1 The Unique Nature of Infant Pain Responding

Unlike most other animals, human infants are born completely defenseless. It has been estimated that a human infant would need to be gestated for 18–21 months to have the level of development equal to a newborn chimpanzee [1]. Instead, with an average gestation of only 9 months, human infants are born completely dependent on a primary caregiver. It can be argued that the most powerful ability inherent to human infants is their capacity to signal the caregiver when distressed. Human infants are innately programed to signal an "other," reflecting from birth the foundational importance of social interrelatedness when handling distress. Interestingly, despite this fundamental evolutionary evidence about the primacy of social networks during distress, humans are born without the core ability that facilitates advanced understanding of another's subjective pain experience—language.

Without symbolic representations (i.e., words) to frame and understand pain, human infants are initially dependent on an "other" to be an external interpreter of the world around them and to help them build their own internal cognitive

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representations. The situation of being so dependent on a caregiver to not only scaffold life's physical experiences but to also help build one's first mental representations of life makes the first year of life a unique developmental context in which to study pain. This chapter posits that infant pain cannot be understood outside the context of caregivers because the complex interplay between caregivers and infants shapes the sensation, perception, and expression of an infant's pain experience.

9.2 A Primer in Attachment Theory and Its Applicability to Infant Pain Responding

The infant's unique connection to their primary caregiver has been emphasized in a foundational ethnological theory of child development initially posited by John Bowlby [2]. Attachment theory denotes that an optimal infant-caregiver relationship typically results from sensitive and responsive caregiving. Moreover, this theory posits that innate human behaviors when distressed (such as crying) all serve the primary goal of achieving physical proximity to the caregiver. Proximity not only facilitates a caregiver's ability to serve the infant's basic physical drives, such as hunger, but also the strong emotional drive for close physical comforting [3]. Because infants are largely dependent on their caregivers for survival, they are innately driven to enact behaviors (e.g., crying) that will elicit proximity to and ideally trigger distress-reducing actions from the caregiver [4, 5].

Conversely, caregivers have been posited to have an innate system activated by infant distress behaviors, which will generally drive caregivers to achieve proximity and soothe an infant in a way that will regulate the infant (i.e., reduce the infant's distress and therefore reduce the infant's drive to achieve proximity to the caregiver). This reciprocal relationship between the caregiver and infant allows the caregiver to function as an external regulator of the infant's emotion and distress, thus providing an infant with a sense of security when threatened. As children develop and become more autonomous, they internalize their caregiver's behaviors and learn how to self-regulate their distress based on these early experiences [6, 7]. This early process of behavioral patterning between an infant signaling distress and a caregiver's response to distress is called "attachment in the making."

Multiple pairings of infant distress signaling and caregiver responses over the first year of life develop into reliable cognitive distress regulation representations or schemas in the infant that are known as attachment patterns [8]. Attachment classifications or patterns stabilize at about 1 year of age and come to represent the pattern of how the infant and parent have repeatedly interacted during distressing situations. There are four main attachment patterns, secure, avoidant, resistant, and disorganized, that were created through observing dyads in a separation procedure (Strange Situation Procedure) [8]. When facing a reunion with their caregiver

after experiencing separation distress, secure infants signal distress vigorously and soothe quickly in the presence of their parent, suggesting that parents have regularly responded contingently to their infant's distress with effective ways to reduce distress. Avoidant infants minimize the overt expression of distress and often ignore the caregiver upon reunion. Parents of these infants often respond such that distress displays are not encouraged and may actually increase physical distance in the dyad. Resistant infants display an opposite pattern characterized by high distress signaling and a resistance to settling with parent, believed to develop from inconsistent contingent soothing by parents. Often these parents oscillate between sensitive and less sensitive responses when attending to the child's distress so that a predictable pattern of behavior to ensure caregiver proximity is not discernible to the child. Disorganized infants are challenging to typify in their reactions, but their behaviors are contralogical to the primary goal of achieving proximity, even when overt indicators suggest this is what the infant wants (e.g., walking backward away from the mother upon reunion despite lifting arms and saying "Mama") [9]. Parents of these infants often act in ways that exacerbate distress in the already distressed child. These four attachment classifications can also be classified dichotomously as either secure versus insecure (avoidant, resistant, disorganized) or organized (secure, avoidant, resistant) versus disorganized.

Attachment patterns are considered one of the strongest psychosocial predictors of future development from early childhood stage [10]. Thus, outside the field of infant pain, it has been well established that infant distress responding is a complex equation involving not only the child's internal factors (e.g., sensory thresholds, innate neural circuitry, temperament), the in situ infant distress (e.g., infant crying), and caregiver soothing behaviors (e.g., parent rocking) but also a function of the established schema or representation that the distressed infant holds about how the caregiver will respond. Thus, it is critical to understand that the infant's distress responses may vary depending on the caregiver present. This fundamental premise must be acknowledged when studying infant expressions of pain-related distress in any context.

Separation is the most commonly used experimental paradigm to examine the infant attachment relationship in developmental psychology (e.g., the Strange Situation Procedure), but Bowlby also initially mentions pain as a key attachment context (Bowlby 1969/1982). The next section will describe the Development of Infant Acute Pain Responding (DIAPR) model—a model structured to postulate about unique mechanisms surrounding infant pain over the first year of life that incorporates the caregiver as a central component. Grounded in a fundamental proposition of attachment theory that purports the central importance of the caregiver to understanding pain-related distress responding, the DIAPR model ([11], see Fig. 9.1) was built from a program of research that followed a large cohort of infants and caregivers during vaccinations over the first year of life (the Opportunities to Understand Childhood Hurt or OUCH cohort).

9.3 Understanding the Development of Infant Acute Pain Responding: The DIAPR Model

To understand the infant in pain, it is imperative to have an understanding of the dyadic relationship between the infant and the caregiver [12, 13]. The integral role of the caregiver in regulating an infant's distress was highlighted in a review by Pillai Riddell and Racine [13], in which the authors highlighted the paucity of research in the assessment and management of infant pain, especially focusing on infant pain in the context of the caregiver. Grounded in attachment theory but structured around findings from the OUCH cohort, the DIAPR model [11] was created to provide a framework for understanding the psychosocial development of infant acute pain behaviors over the first year of life. The OUCH cohort was a cohort-sequential sample comprised of 760 parent-child dyads in Toronto, Canada, who were followed through vaccinations during the first year of life across three pediatric clinics.

The DIAPR model acknowledges that in the immediate days and weeks following birth, pain behaviors are based mainly on intra-infant biological factors associated with the nociceptive processing of the central and peripheral nervous system pathways that had foundations laid prenatally (e.g., cortical pathways, sensory thresholds) [14] and early substantive pain experiences (e.g., repetitive needle pokes) [15]. However, over time, it is speculated that the dynamics between the caregiver and child during painful and distressing events comes to impact the infant's pain sensation, perception, and behavioral expression through different feedback loops.

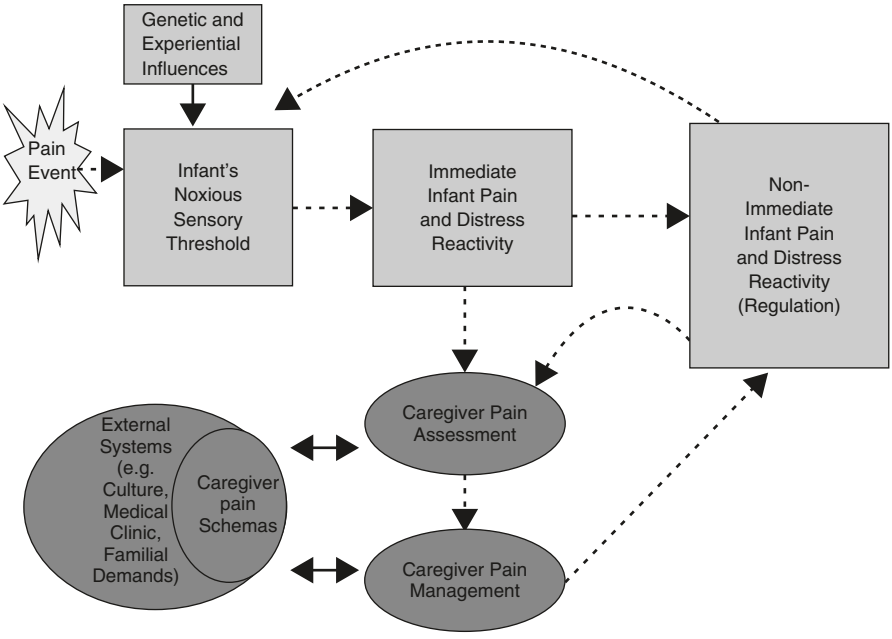

Fig. 9.1 The development of infant acute pain responses model

Three feedback loops (infant loop, parent loop, parent-child loop) are posited to occur concurrently over time in the model to help explain the complexity of an infant's behavioral pain responding over the first year of life. The infant feedback loop refers to the initially linear progression of events that starts with the acutely painful stimuli, to the painful stimuli exceeding the infant's nociception threshold, to the infant's immediate peak pain reaction, to the infant's regulatory pain responses, which then in turn could modify the infant's future pain responding over time through either peripheral or central mechanisms depending on the frequency, duration, and/or intensity of the pain experience and thus impact the beginning of the loop. Our research has shown that one of the strongest predictors of infant pain responding over the first year of life is past pain responding [16, 17]. While this loop is occurring in the infant, a complementary cycle of events is ongoing in the caregiver. A parent will witness their infant's pain behavior and then synthesize an initial pain assessment based on their own pain schemas or representations built from their own past experiences, their past experiences with their child's pain and distress, their knowledge of pain contexts, etc. This initial pain assessment can lead to both immediate (e.g., pick up the child) and less immediate (e.g., go get a topical teething gel) pain management behaviors. Both the initial parental assessment and pain management strategy choices will change through mutual influence between infant and caregiver over the course of a painful event. Moreover, over time, these interactions between assessment and management may also further influence core pain schemas in the parent (e.g., "my baby has a high pain threshold," "breastfeeding really helps lower my baby's pain") which would change pain schemas or representations for when the loop starts again for a subsequent infant pain event. Finally, the infant-caregiver feedback loop asserts that while the infant's immediate pain reactivity (responses right after an acute pain stimulus) will affect the caregiver's initial assessment and management of an infant's acute pain, the infant's regulatory pain response (after the infant's peak response when infant is moving toward homeostasis after the painful insult) will also independently predict parent's pain assessment and management [18–20]. This will lead to an ongoing loop that includes parental assessment and management and infant's pain behaviors which end when the infant pain-related distress is no longer evident.

An important key element of the DIAPR model posits that during the first year of life, larger systemic influences (such as mainstream culture of the country of birth, heritage culture(s) of the parents, hospital policies, etc.) do not exert a direct influence on the child's pain behaviors but rather are mediated through the parent and/or other caregivers [21]. We postulate that this is a distinct mechanism of the newborn and young infant (as opposed to other stages in the lifespan) and holds until the infant is able to have direct purposeful interactions with larger spheres of influence such as the family, the school, etc.

Concluding this section, the DIAPR model offers cognitive-behavioral perspectives of the infant, the caregiver, and the infant-caregiver interaction when understanding an infant's behavioral response to pain. The chapter will now present data on the relationship of attachment theory to the pain context through discussing parallels in findings in the attachment and pain literatures. To emphasize the relevance of the relational lens, aspects of attachment theory will be examined in the acute

pain setting. These aspects include the strengthening of the relationship between infant pain-related distress with both caregiver behaviors and parental sensitivity over the first year, the importance of early parent-infant interactions in predicting infant pain-related distress regulation, and finally links between attachment classifications and infant pain behaviors.

9.4 Linking Infant Pain Responding to Tenets of Attachment Theory

9.4.1 Attachment in the Making over the First Year of Life

Attachment theory posits that the relationship between the infant and parent undergoes a steep trajectory of development during the first year of life, and it is has been found that the relationship between caregiver behaviors on infant distress behaviors is only reliably measured after approximately 12 months of age (Bowlby 1969/1982) [8]. Similarly, we found parallel findings for our OUCH cohort dyads aged over the first year of life. As the year progressed, parent caregiving behaviors (e.g., proximal soothing, distraction, verbal reassurance) accounted for increasing variance in observed childhood pain-related distress signaling behaviors [16, 20, 22]. These complementary temporal findings suggest that the strengthened attachment relationships relate to the increased relationship of caregiver behaviors to infant pain behaviors at 12 months of age.

9.4.2 The Importance of Caregiver Sensitivity

It is important to recognize when studying caregiver behaviors that not only is the action itself important but also the caregiver's awareness of the impact of their action on the infant. Rocking a baby is recognized as a behavior that reduces infant pain when an infant is distressed [22]. However, if that baby simply wants to be held still, that same behavior has a very different impact. A key mechanism postulated by attachment theory relates to the importance of a caregiver's sensitivity. In the pain context, caregiver sensitivity can be described as the quality of how a caregiver attends to and contingently addresses the infant's pain-related distress. Meta-analysis of classic attachment research suggests significant but moderate magnitude relationships between caregiver sensitivity and attachment [23]. It is thought that through contingent emotional responsiveness from a parent (i.e., sensitivity), infants develop an understanding of their own emotional states and how to regulate from distressing events like pain (Bowlby 1969/1982). These findings have also been paralleled in the pain context.

There are many different ways to operationalize caregiver sensitivity. For our cohort work, our lab used the Emotional Availability Scales [24], which examines four subscales, including sensitivity (e.g., the ability to "read" an infant's cues and display appropriate affect), structuring (e.g., parent's ability to appropriately

structure the context for the infant during the immunization), non-intrusiveness (e.g., caregiver's ability to be available and avoid intrusive, direct, overstimulating, or overpowering behaviors), and non-hostility (e.g., parent's ability to refrain from antagonistic or impatient behaviors). As predicted by attachment theory, all significant concurrent relationships between caregiver emotional availability and infant pain responding over the first year of life were negative; greater emotional availability was linked to less infant pain response [17]. Moreover, the strength of associations among different infant pain scores (e.g., immediate reactivity versus regulation pain scores at each age) and caregiver emotional availability also showed increasing strength as the child aged, paralleling what was reported earlier with respect to parent pain management behaviors [16, 20].

Further explicating the relationship between emotional availability and parental acute pain soothing behaviors, Atkinson et al. [18] investigated the extremes of emotional availability to examine differences in high and low emotionally available parents on parent soothing behaviors and infant pain behaviors across the first year of life. Not surprisingly, the authors found that infants of caregivers with higher emotional availability scores had lower pain scores across the vaccination appointments. However, an interesting finding suggested that when an infant is experiencing high pain-related distress (i.e., the immediate pain response), the association with caregiver emotional availability is lower in magnitude, but increases as the initial high pain of the needle abates (i.e., pain regulation scores). In addition, high and low emotionally available parents differed in their use of soothing strategies during the vaccination appointment, most notably in the first minute postvaccination. Specifically, rocking and physical comforting in the first 2 minutes immediately after needle were most able to distinguish between the caregivers who were high or low on emotional availability across the first year of life (as opposed to behaviors such as verbal reassurance or distraction). Attachment theory would predict that the caregiver behaviors most able to bring the caregiver and child close together would distinguish sensitive and less sensitive parents in the pain context. Aligned with these predictions, the caregiver behaviors most strongly linked to increasing proximity of the infant to the caregiver (i.e., rocking and physical comforting) best distinguished the highly sensitive caregivers and the lowest distressed babies. Thus, we speculate that one of the main mechanisms by which higher caregiver emotional availability lowers infant pain behavior is via achieving the infant's primary attachment goal—maintaining proximity to the caregiver.

9.4.3 Early Parent-Child Interactions in Distress Impact Future Distress Regulation

Previous attachment research reviewed by Streeck-Fischer and van der Kolk [25] also demonstrated that emotionally available mothers provided more proximal soothing, which allowed infants to regulate their physiological arousal and modulate stress not just in the present but also in future stressful situations. Din Osmun and colleagues [19] provided further confirmation of the importance of early caregiver

behaviors by elucidating a series of findings relating emotional availability to future pain regulation using the OUCH cohort. Higher mean levels of caregiver emotional availability (averaged across 2, 4, and 6 months) were related to larger decreases in the duration of infant pain-related negative affect during the first 6 months of life (i.e., infants regulated pain-related distress faster). In addition, the infants who had smaller decreases in negative affect regulation over the first 6 months of life (i.e., did not show a decreased duration of pain responding as the child aged) also had caregivers with lower emotional availability scores at each age point.

However, statistical modeling showed that early caregiver emotional availability (i.e., over the first 6 months) did not have a direct effect on 12-month negative affect regulation. Rather, the model suggested that early emotional availability had an indirect effect by significantly predicting better early negative affect regulation over the first 6 months of life, which in turn then directly predicted 12-month negative affect regulation. The authors posited that caregivers who were more emotionally available early on had infants who were better able to regulate negative affect quicker (i.e., express less distress post-needle) and that it was this increase in negative affect regulation early on that predicted greater success in negative affect regulation development at 12 months of age. Taken together, these results support that even if associations between emotional availability and infant distress are weaker early on in the first year of life, this early link demonstrates gains in importance when predicting future infant pain regulation.

9.4.4 Infant Attachment Patterns

The infant attachment classification or pattern (i.e., secure, disorganized, avoidant, resistant) is postulated to represent a preliminary but reliable cognitive schema or representation that shapes how the developing person learns to self-regulate distress in social contexts. While based in the infant-caregiver relationship, this representation is believed to later evolve into how one regulates from distress in relation to others across the lifespan (Bowlby 1969/1982) [26, 27]. We have already discussed the developmental psychology concept of caregiver sensitivity as being critical in understanding the attachment relationship between an infant and their caregiver and relayed that sensitivity involves the caregiver accurately and contingently interpreting infant cues and responding accordingly [8, 26]. However, to further underscore the importance of the caregiver to understanding the infant in pain, the final section will review some of our findings that link actual attachment classifications (derived from the Strange Situation Procedure) to 12-month vaccination behaviors.

Horton and colleagues [28] empirically demonstrated how specific infant behaviors during routine vaccination appointments at 12 months were associated with attachment using the four-level comparison of secure, avoidant, resistant, and disorganized behavior patterns. These authors reported that infant proximity-seeking post-needle significantly discriminated attachment classifications, with secure infants being more likely to seek proximity to caregivers post-needle in comparison

to avoidant and disorganized infants. The authors posit that the attachment system is triggered following a painful stimulus and that secure infants actively regulate pain-related distress by initiating close physical comfort with their caregivers. Of note, an important divergence between understanding attachment behaviors in a pain context versus a separation context was suggested by findings in this paper.

During the time period under investigation (i.e., the immediate minutes postvaccination), the resistant group of infants appeared to proximity seek and attempt to maintain contact with caregiver in a manner that was not wholly different from the secure group infants. In the Strange Situation Procedure (from which the infant attachment classifications were based on), infant distress is generally moderate and quickly alleviated with the presentation of the caregiver upon reunion. In contrast, within the vaccination context, infant distress is typically initially severe and is not simply alleviated by the presence of the caregiver [29]. The ongoing distress during the vaccination context would cause a secure baby to continue to signal, even in the arms of a caregiver, thus mimicking the behavior typical of a resistantly attached infant. Moreover, in the vaccination context when faced with ongoing distress being held in a parent's arms or lap, it would be expected that avoidant infants would mute signaling. However, we also found that disorganized infants also had a similar behavioral response. Thus, in the time period under consideration for the pain context (i.e., the few minutes postvaccination), researchers and clinicians may have challenges distinguishing secure from resistant infants and avoidant from disorganized infants based on infant behavior alone.

These results were mirrored by Hillgrove Stuart and colleagues [30], which explored the link between caregiver behaviors during routine pediatric vaccinations and infant attachment with the OUCH cohort. The authors found that higher frequencies of caregiver proximal soothing (e.g., holding, rocking) at 12 months were related to infants' organized attachment (i.e., secure, avoidant, resistant), whereas steeper decreases in proximal soothing across the first year of life were associated with disorganized infant attachment at 12–14 months. Moreover, parental proximal soothing was significantly positively related to secure attachment and negatively related to disorganized attachment. In essence, parents who proximally soothed their infants longer during the 12-month vaccination appointment had more optimal attachment outcomes. Based on these findings together, it is postulated that, by the 12-month vaccination, infants with more optimal attachment classifications (secure, organized) engage in more proximity-seeking during distress and have parents who respond with close-contact soothing, both of which result in less pain responding in the acute pain context. An important practical juxtaposition of attachment theory and infant pain will conclude this section.

Thus far in the chapter, we have reviewed that infant-caregiver interactions during periods of distress over the first year of life contribute to lasting cognitive schemas (attachment representations) that impact how the infant expresses and regulates distress throughout the lifespan. Practically, this means that to understand infant pain responding, one often needs to understand the direct link to the caregiver actually present during the painful stimulus. An infant who receives a vaccination in the arms of their primary caregiver versus the arms of a stranger will regulate from

pain-related distress differently. We acknowledge across the lifespan that pain is a sensory, affective, and social experience [31]; however, at other ages we can use self-report to understand the physical, emotional, and interpersonal nuances subsuming one's overt pain responding. In contrast, an infant cannot offer a verbal breakdown of the affective and sensory components of their pain. But both researchers and clinicians can access important proxy information about an infant's affective experience of pain by factoring in the infant's caregiver through measures such as those discussed thus far (e.g., attachment relationship, parental sensitivity, and discrete soothing behaviors). It is our contention that understanding infant pain sensation or perception, measuring an infant's acute pain behaviors, or even judging the efficacy of a drug without taking into account caregiver behavior and caregiver-infant factors runs the high risk of findings that do not fully represent the infant's experience of pain or pain relief.

The final section of this chapter takes a more practical focus and a different view about emphasizing the importance of understanding the caregiver in an infant acute pain context. Instead of focusing on how the infant's pain experience is partially defined by caregivers and the infant-caregiver relationship, the discussion will be turned to caregivers and how they perceive the infant's pain experience. Research is showing that the infant's pain responding may not be central to caregiver assessments of infant pain, leaving the question behind as to what are judgments of infant pain actually telling us.

9.5 Caregiver Judgments of Infant Pain: Infant Pain Is in the Eye of the Beholder

In primary healthcare, where most healthy infants receive the majority of their most painful experiences, parental caregivers are the main conduit to information about the infant used by health professionals to diagnose and treat. Because the health professional is not in the home with the infant, it is put upon the parental caregivers to reliably relay the infant's symptoms or behaviors. Routine vaccinations provide a valuable paradigm to understand caregiver reporting of infant symptomology, because the subjective symptom (i.e., pain) can be more easily observed through infant behaviors by parents and other professionals (clinicians, researchers).

Based on infants' inability to self-report their subjective experience of pain, we must rely on proxy indicators of infant pain based on caregiver judgments [32]. As such, it is important to acknowledge that there will be bias in the judgments of infant pain based on a variety of individual factors related to the caregiver in question. Indeed, in the DIAPR model, both distal factors (e.g., culture, parent's own pain experiences) and proximal factors (e.g., infant pain behaviors, caregiver management behaviors) are hypothesized to influence parental pain judgments [29]. Below we will summarize the available literature from other infant pain contexts, as well as work done by our group, that focus on different pain attributions for not just parents but also health professionals when possible (i.e., parents, nurses, pediatricians).

9.5.1 Differences in Caregiver Assessment and Management of Infant Pain

Despite the paucity of research, consistent differences in the assessment of infant pain have been found among caregiver groups [33, 34] (Pillai Riddell et al. 2008). When exposed to the same quasi-experimental methodology (watching videos of infants who all demonstrated, unbeknownst to the assessing caregivers, the same level of objectively coded pain behaviors), physicians tended to attribute lower levels of pain than parents, while nurses were intermediate to the other groups, not significantly differing from either group [34] (Pillai Riddell et al. 2008).

These differences in pain attributions in caregivers of infants have also led to differences in pain management techniques such that parents may have higher pain ratings but they are more hesitant to administer pain medication. Indeed, researchers report a positive association between physicians' and nurses' belief that infants experience pain and analgesic administration use [35, 36]. However, parents are generally reluctant to provide adequate analgesia to alleviate pain in infants and children [37, 38], a tendency that may reflect the belief that the use of analgesics is dangerous [39] and their general lack of experience with pharmacological agents.

9.5.2 Caregivers' Pain Beliefs as Factors Underlying Pain Judgments

Much work in the broader field of infant pain has focused on differences in caregiver pain beliefs and how this may affect caregiver pain judgments [35, 40, 41, 42]. Specifically, caregivers differ in their knowledge, skills, and experiences with infants in pain, which likely leads to different cognitive appraisals when assessing pain [38].

With regard to parents, many theories behind increased pain attributions have been postulated. For example, parents share a biological connection to their child and are likely the primary caregiver for the infant in question [43]. As such, they are more attuned to the idiosyncrasies of their child in pain, but because they lack formal training, they may also rely on personal, familial, and cultural experiences [33, 34] to rate their infant's level of pain. Additionally, given caregivers' lack of training and experience with other children in pain, infant pain may be more ambiguous, distressing, or challenging for primary caregivers to assess and manage, compared to healthcare professionals [5]. Certainly, pediatricians have the most extensive training and specialized medical knowledge, but often spend less time with their patients compared to nurses. Nurses are the professional group spending the most time with individual patients, yet still have a considerable amount of medical knowledge and training [44, 45]. As such, it is not surprising that physicians and nurses may have some commonalities when assessing and treating infant pain and that nurses and parents also show similarities in this domain [34, 44] (Pillai Riddell et al. 2008).

Pillai Riddell and colleagues [33] investigated the role of parental beliefs regarding an infant's cognitive capacity to understand and remember pain, on perceived infant pain. When parents viewed videotapes of healthy, unrelated infants aged 2, 4, 6, 12, and 18 months receiving routine vaccinations, parents had little difficulty identifying significant pain in even the youngest age group. Additionally, parents recognized that the cognitive capacities of understanding and memory for pain unfolded with increasing age. However, despite this increased understanding that an infant's cognitive capacity and memory for pain increase over time, parents did not consider cognitive capabilities to be important factors in their pain judgments.

Interestingly, in another analysis from the same study, Pillai Riddell and Craig [34] found that all caregivers believed vaccine injections instigated significant pain for infants of all ages, but attributed more pain to older babies than younger babies. This is despite the video footage being controlled such that all babies expressed the same level of pain behavior. Therefore, it was hypothesized that, despite not being reported highly in importance, perceived age-related developmental maturity may be a key determinant of infant pain judgments for both professional and nonprofessional caregivers [34].

9.5.2.1 Infant Pain Cues as Factors Underlying Differences in Infant Pain Judgment

Seminal research in the area of variation in judgments of infant pain investigated nurses and found that they use various infant states and facial and body movements to assess pain (e.g., [42, 46, 47, 48]). Moreover, research on nurses and physician judgments of infant pain revealed that more experienced healthcare practitioners were more selective in the number of cues they considered important for making pain judgments, but that similar types of cues were used [34, 35, 49].

When compared head to head in the same study with a quasi-experimental video judgment paradigm described earlier, parents, pediatricians and nurses were found to utilize the same cues but used them differently to make infant pain judgments [38]. These authors reported that although pediatricians, nurses, and parents rated "sounds," "facial expressions," "body movements," and the fact that the "infants had just received a needle" as being of highest importance in their assessment of infant pain experience, pediatricians reported utilizing these cues exclusively, regardless of age, while nurses and parents reported using an increased repertoire of cues as important to their pain judgments for older infants (i.e., 18-month-olds) compared to younger infants (i.e., 2-month-olds). Following these findings, it was suggested that pediatricians may have a more economical heuristic when judging infant pain, compared to nurses and parents, reflective of the nature of the pediatricians' shorter clinical interactions with patients (Pillai Riddell et al. 2008). Additionally, when the importance of cues was compared between parents and healthcare professionals, parents tended to rate infant behavioral cues (i.e., sounds, age, facial expression) as more important at 2 months, while rating subjective cues (i.e., infant's cognitive abilities, remembering pain) as more important at 18 months.

9.5.2.2 What Is Going into Infant Pain Judgments?

The previous sections outlined research examining how caregivers make judgments about infants in acute pain. This research was often conducted in studies that had caregivers judge infants' pain from video or vignette. The final area for review switches from the 'how' to the 'how much' by examining parents making pain judgments about their own children and examining how much these reported factors predicted their pain judgments.

More recent research has begun to investigate the relative impacts of both caregiver (i.e., cultural, community, familial, and individual) and infant (infant age, infant behavioral reactivity) variables on mother's infant pain ratings. Pillai Riddell and colleagues [50] found that, after controlling for infant distress behavior, a significant amount of variance in mothers' infant pain judgments was predicted by factors distal to the infant's actual pain from vaccination. General level of maternal psychopathology and identification with North American culture was related to maternal recall of their infant's pain postvaccination, with higher levels of psychopathology and lower levels of engagement with mainstream culture being associated with greater recall of infant pain after vaccination.

Following up on this study, our group utilized the OUCH cohort to study the relative contribution of both top-down variables (i.e., caregiver emotional availability, parent demographics) and bottom-up factors (i.e., behavioral pain responses, infant demographics) to predict parental ratings of their infant's vaccination pain at 2-, 4-, 6-, and 12-month appointments [51]. Despite incorporating objectively coded infant behaviors (purported to be most important to their pain judgments from earlier work), the vast majority of the variance in parental pain assessments remained unexplained. Only about 18% of the variance in pain assessments was predicted when judging younger infants (2 and 4 months), and about 33% of the variance was predicted with older infants (6 and 12 months). In addition, several demographic factors (i.e., having multiple children, female sex of child) also showed small, significant direct relationships with pain judgments at 12 months. This meant that the vast majority of variability in parental pain ratings was not accounted for by infant pain behaviors.

Synthesizing results from this section on understanding caregiver attributions of pain suggests that factors distal to the infant's pain experience and expression are being heavily factored into caregivers' pain judgments. Cultural beliefs, psychosocial stressors, health profession, and the number of children in the family all have also demonstrated small but significant predictive relationships with caregiver pain judgments. This is concerning because infants in pain cannot speak for themselves and they depend entirely on adult caregivers to understand their pain-related behavioral signaling to have caregivers sensitively act in ways to reduce their pain.

Given that the vast majority of variability in infant pain judgments has been consistently shown not to be related to the infant's only method of pain communication (i.e., behaviors), it is of critical importance to better understand what is influencing caregiver pain judgments to improve our ability to create a proxy appraisal of the infant's experience. More research must be conducted to better understand what exactly is going into caregiver pain judgments if not pain signals from the infant themselves.

9.6 Conclusions and Future Directions

The unique complexity of infant pain responding has been highlighted in this review through an overview of the importance of understanding infant caregivers, and the relationship an infant has with their caregivers, as a critical piece to understanding infant pain. As set forth by the DIAPR model and grounded in an attachment perspective, we have presented research from our OUCH cohort and other research groups that confirms infant pain-related distress is linked not only with infant (e.g., temperament, negative affect) and caregiver factors (e.g, caregiver pain management, psychopathology, acculturation, caregiver emotional availability) but also with dimensions of the caregiver-infant relationship (e.g., infant attachment). Additionally, factors wholly beyond infant behavioral pain signaling appear to be accounting for a large proportion of caregiver-infant pain judgments, making infants highly vulnerable to having their pain over- or underestimated.

Successful regulation from infant pain-related distress is heavily impacted by patterns of contingent and sensitive caregiver behavior that have been built since birth. Moreover, it is important to acknowledge that both parental and health professional assessments of infant pain are influenced by factors that as of yet have to be defined. Future research needs to focus on caregiver factors that may impact proper infant pain assessment and management, such as maternal or health professional mental health.

As a field coming out of its own infancy period, infant pain researchers and clinicians need to next determine how to best incorporate the dyadic relationship between infant and primary caregiver into both practical and theoretical pursuits.

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Detecting Acute Pain is Enough: The Conundrum of Pain Assessment

10

C.V. Bellieni and G. Buonocore

Is scoring pain mandatory in newborns? Sometimes it is, and some others it is not [1]. We shall describe when and how in this chapter.

More than 30 neonatal pain scales exist, but almost none is actually used in clinical settings. Many of them are multifactorial, i.e. they simultaneously take account of fluctuations in oxygen saturation, blood pressure and facial expression but also score gestational age, behaviour and so on [2–9]. The more complex scales are good for research purposes, but only if we record the procedure in order to give the scorers the opportunity to assess the requested items in a later session. The most widely used are the PIPP (Premature Infant Pain Profile), NIPS (Neonatal Infant Pain Scale) and DAN (Douleur Aiguë du Nouveau-né) (see Chap. 9).

10.1 Limitations of Current Scales

Most current scales are specific and sensitive but scarcely functional: caregivers are unable to take a blood sample or do some other painful operation and at the same time evaluate and time three or four physiological parameters, as required by certain scales. We recently published a work in which we studied the reliability of two of the most used pain scales for newborns [10]. We studied a group of babies who underwent a routine heel prick and compared the scores for the babies' pain given by three different operators. Operator 1 was the nurse who was actually performing the heel prick; operator 2 was another nurse who did not perform the heel prick and was free to watch the baby and the saturometer closely; and operator 3 was a third

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scorer who recorded the procedure through a video camera and scored the pain later. We studied two groups of babies, one made up of preterm babies, to whom scores were given using the PIPP, and another of term babies, for whom we used the NIPS. We used the score given by operator 3 as a reference score, because she could give the scores in a calm frame of mind and with the possibility of watching the video clip more than once. We found that, in both groups, both scorers 1 and 2 gave results different from those given by scorer 3; in the case of PIPP, these differences were higher than in the case of NIPS. This difference may be due to the higher score range in PIPP, but could also be due to the greater complexity of the scale.

At all events, nobody can measure changes in heart rate and oxygen saturation, make the percentages of these variations or record to the split second the time babies spend frowning and simultaneously perform an invasive procedure. The need for an easy tool is clear.

Unifactorial assessment of acute pain through the observation of only one parameter (e.g. measuring crying length or heart rate variation) is unreliable, having low specificity and sensitivity. Post-surgery assessment of pain (assessment of chronic pain), by contrast, is easy and reliable using scales which are intended to evaluate the pain level every 4–6 h: the most used are the CRIES scale (*C*, crying; *R*, requires oxygen to maintain saturation greater than 95%; *I*, increased vital signs; *E*, expression; *S*, sleeplessness) and the EDIN scale (*É*chelle de *d*ouleur et d'*i*nconfort du *n*ouveau-né).

10.2 Acoustic Analysis of Crying

Can we find an easier and more reliable way to measure acute pain in newborns? Is crying analysis a possible path for this? Crying is simultaneously a sign, a symptom and a signal and is the infant's earliest form of communication [11], but the significance and meaning of neonatal crying are still unclear [12] because different crying features reflect not different causes (e.g. hunger, pain, fussing) [13] but the amount of distress caused [14–16]. Thus, gradations of crying may help a listener to narrow down the range of possible causes only with the help of contextual information [14, 16–19]. In the last few years, pain scales have been developed to discriminate levels of pain suffered by newborns [2, 4, 5, 20–22], but in analyses of crying, the level of pain that has provoked it is rarely considered [23]. Simple assessment of pain through measuring the duration of crying or other isolated parameters has been criticised for being neither sensitive nor specific [24, 25]. In the 1960s, crying was thought to be cause-specific (hunger, pain) [26], but recent reports have not found such a close correspondence, and this lack of specificity would prevent its use as a reliable pain indicator when crying is produced without contextual indications [27].

In 2003 our research group began analysing features of pain crying. We thoroughly assessed the characteristics of crying for different levels of pain: previous works had analysed pain crying, but without any regard to the different degrees of pain the baby was experiencing, with the single exception of one preliminary study [28]. We analysed a group of 56 healthy term babies during a common heel prick

to obtain blood for routine analyses. We scored pain through a validated pain scale (DAN scale) and studied how the features of the crying varied according to the pain the babies were experiencing [29]. We studied three features: the pitch of the first cry emitted by the babies, the shape of the wave throughout the procedure and, especially, the rhythmicity and constancy in time of the sound level. We chose these parameters because they are modulated by different parts of the nervous system. Cry pitch is due to vagus nerve tone, i.e. to the parasympathetic system: stress causes a decrease in tone, causing increased tension in the vocal cords innervated by the vagus nerve [30]. The rhythmic organisation of infant crying is a complex phenomenon. Like other rhythmic patterns (sucking, walking), it has been correlated with central pattern generators [31], which are neural networks that produce rhythmic patterned outputs endogenously (i.e. without rhythmic sensory or central input). Last, the constancy of cry intensity is a sign of the persistence of the painful stimulus [17].

We saw that constancy of intensity increased with increasing pain. The basic frequency of the first cry did not increase until a certain pain threshold was reached (DAN > 8), after which the first burst was very acute. Rhythmic crying was absent up to a certain pain threshold (the same as for the above-mentioned basic frequency). We can therefore say that when pain passes a certain threshold, the characteristics of crying change: the crying becomes rhythmic and the first sound becomes acute, as if to express unbearable pain. We saw that, while crying constancy in time increases in accordance with the increase of pain, the other two parameters varied abruptly when pain exceeded a certain threshold. We supposed that this was a sort of protolanguage; it is unconscious, but finalised to express a state of extreme pain.

This was the premise of the development of the pain scale. We tried to verify whether these three items could be useful to score pain. We have already said that crying duration is not specific nor sensitive, but in this case we used not crying duration but some features of crying and integrated the three items to form a scale [32], of which we assessed the specificity, sensitivity, concurrent validity with another scale, interrater reliability and clinical utility. We called it the ABC scale (Table 10.1), because it used the "acuteness" of the first cry, the "burst rhythmicity" and the

Table 10.1 ABC scale for pain in newborns

Item	Score
Is the first cry acute?	
No	0
Yes	2
Are bursts rhythmic?	
No	0
Yes	2
Is crying constant in time?	
No (brief moan rather than crying)	0
No (more than brief moan, but not constant)	1
Yes	2

"constancy" in time of the crying. J. Schollin in a recent issue of *Acta Paediatrica* [33] said that this scale was a good step in the field of pain assessment, because it was both easy and reliable. Examples of the different types of crying are available at the following URL: http://www.euraibi.com.

The last step in our research was validation of the ABC scale in premature babies [34]. In this case, too, we studied the specificity, sensitivity and reliability of the scale using statistical parameters. To make it easier to use the ABC scale, we developed a software to measure pain automatically. This software uses the ABC scale and automatically analyses crying that arrives in the computer via a microphone. We have verified its validity and have published our data of our patented tool that we called "ABC analyser" [35]. It can be used to assess pain in nurseries and to train nurses who want to learn how to avoid pain in newborns.

10.3 Is It Really Useful to Score Acute Pain?

Caregivers should be able to recognise the main physical signs associated with pain. Most are non-specific: babies can cry for reasons other than pain, and pain is expressed through complex behaviour. This does not mean that a crying baby should be ignored "because we are not sure" that he/she is crying for pain. On the contrary, this should alarm us as if it were pain: our duty is to exclude it and/or to treat it. Newborns show a distinct pattern of behaviour to painful stimuli. This includes a wide range of expressions including screwing up the eyes, frowning, opening the mouth, extending the fingers, kicking as well as clenching. For a thorough assessment, pain scales exist.

10.3.1 Chronic Pain Scales

It is mandatory to monitor pain in order to prevent its occurrence, mainly after surgery or in ventilated babies. For this purpose, scales exist to evaluate the level of pain and stress in intubated babies or after surgery, in order to adjust or introduce an effective analgesia.

10.3.2 Acute Pain Scales

Many scales exist in this field and are scarcely used. There are two reasons for this. First, the difficulty of applying scales where many items have to be assessed simultaneously [36]. Second, the lack of any point in assessing pain after it has occurred. Acute pain scales are useful for research purposes. It is not always useful for clinical practice to assess the actual level of pain; it is more useful to be aware of whether we are actually provoking pain or rather if we might provoke it, before we start. Acute pain scales are retrospective and assess pain only after it has been provoked,

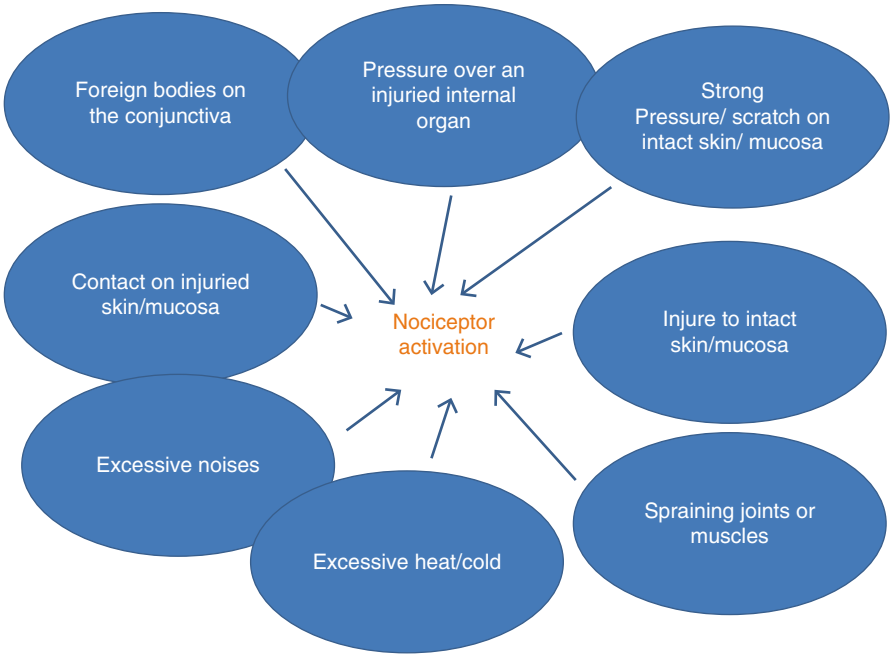

Fig. 10.1 Red flag stimuli that can induce pain in newborns

so it has been proposed to assess pain preventively wondering if we risk to perform pain (Fig. 10.1) and detecting it trough a sole sign. How? Acute pain scales include many data, but the type of the stimulus and the region of the body (with or without nociceptors) where it is applied are decontextualized information. It has been proposed [37] to foresee pain using a contextual model. The context of the stimulus is crucial: stimuli that might provoke pain applied to an innervated area (Fig. 10.1) will not be painful if applied where nociceptors are absent; and the invasiveness of a procedure often is proportional to the pain it provokes. Thus we should proceed first assessing if the site and the stimulus are adequate to provoke pain and then assessing the reaction to this stimulus to detect if pain has been felt. When we may provoke pain-activating nociceptors, we should use easily noticeable signs such as crying or heart rate to detect pain. Crying and increased heart rate both show a high sensitivity for pain in studies made for the validation of pain scales: neither crying nor heart rate are specific to pain [38], but the sudden appearance of a reaction (crying or increase of heart rate) overcomes this limit.

In conclusion, pain scoring is very important in long-term treatment, to modulate analgesic therapy; it has less sense in acute sudden procedures, when pain is scored when the procedure is over. In the latter case, should we retain to do anything? Of course no: we should prevent pain contextualising the procedure, wondering if this procedure can actually provoke pain and starting an antalgic treatment before the procedure begins and throughout it (see Chap. 13).

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Analgesic Procedures in Newborns

11

Laura Giuntini, Cristina Navarra, Rossella Angotti, and Giovanna Amato

11.1 Fetal Period

Stress is already present in the fetal period and may be physical or psychological.

11.1.1 Physical Stress

Alcohol, smoking and drugs may cause behavioural disorganisation, up to temporary suppression of breathing in the case of maternal ingestion of alcohol. Ultrasound examination may also disorganise growth, as shown by studies of Newnham 1993 [1] and Evans 1996 [2]. According to the American Academy of Pediatrics, even noise may damage the fetus. Babies of mothers exposed to occupational noise during pregnancy showed statistically significant hearing deficit when they reached school age. It has also been shown that fetuses feel pain from week 18. This has given rise to the practice of using fetal anaesthesia for surgery or invasive diagnostic procedures in utero.

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11.1.2 Psychological Stress

Psychological stress is as important as physical stress. Acute stress, such as grieving, can cause long-term psychological damage, and chronic stress has been shown to affect fetuses, being associated with premature birth, birth complications or breast-feeding problems in babies whose mothers were unbalanced or had major psychoaffective disorders [3, 4]. All interruptions of affectivity during pregnancy with rejection by parents (e.g. awaiting diagnosis of pathological conditions) have repercussions on the fetus and its physiological parameters [5, 6].

11.2 Neonatal Period

After birth, stimuli vary, but if they are inadequate they can still affect development. Anand formulated a theory of hyperstimulation and hypostimulation [7].

According to Anand and Carr, excessive neonatal stimulation leads to excitotoxic *N*-methyl-d-aspartate (NMDA) in many areas of the developing brain. The classic example is acute pain due to multiple invasive procedures, which may even cause intraventricular haemorrhage and leucomalacia due to intracranial pressure [7]. It has also been demonstrated that babies who were born prematurely, and were therefore subjected to many painful invasive procedures, have an anomalously low response to pain during infancy and greater somatisation in childhood [5]. The message is therefore that the clinical benefits of analgesia persist well beyond the duration of the therapeutic effect.

Prolonged separation of mother and newborn leads to anomalously exaggerated responses and alteration of neurotransmitter production [8]. The absence of stimuli increases apoptosis in the neonatal brain. If this is true, the clinical importance of preventing early insult and of developing appropriate analgesia and measures against neonatal stress (limitation of excitotoxicity and apoptosis) is evident. Pain experienced by babies has been underestimated throughout the world, not only in Italy. Before the above theories achieved scientific and clinical recognition, not only was neonatal pain ignored, but it was even denied. It was thought that newborns did not feel pain, and this idea was sustained with scientific arguments: the immaturity of the neonatal central nervous system, the difficulty doctors had in clinical recognition of pain and the assumption that the side effects of analgesia and anaesthesia outweighed their benefits.

In fact, the neuroexcitatory pain system controlled by NMDA (glutamate) and neuroquinine (neuropeptides) receptors develops in early fetal life, whereas the inhibitory system matures much more slowly and beyond birth. The inhibitory system consists of C fibres, descending nerve inhibitors, branches of which project onto the spinal cord only after birth and neurotransmitters (GABA and glycine) that cause inhibition in adults but excitation in the immature nervous system. As a result, pain transmission through the spinal cord is amplified in newborns, and the control system develops weeks later. On the other hand, opioid receptors are already active

in fetal life. It can therefore be deduced that newborns feel pain more intensely than adults. The differences make babies even more vulnerable to nerve stimulation, and, as we saw, continuous painful stimulation in a developing newborn has been demonstrated to modify its central nervous system [9, 10].

11.3 Pain Control

Pain control techniques should be used whenever possible before painful stimulation.

The prevention and alleviation of pain in neonates, particularly preterm infants, is important not only because it is ethical but also because exposure to repeated painful stimuli is known to have short- and long-term adverse sequelae [11].

This is the basis of preventive analgesia. The aims are to:

- Minimise emotional problems
- Prevent central nervous system (CNS) sensitisation
- Minimise release of pain mediators in tissues
- Decrease stress response

Preventive analgesia also makes it possible to reduce the need for intrasurgical anaesthetic and also the demand for postoperative analgesia [12]. There are risks of various problems, psychological and physical, associated with not treating pain.

Psychological problems:

- Fear and anxiety
- Behavioural and personality changes
- Vicious circle of chronic pain

Physical problems:

- Increased mortality and morbidity after surgery
- Respiratory problems (hypoxaemia, reduction of coughing reflex, accumulation of secretions, infections)
- Cardiovascular problems (increased heart rate and blood pressure, vasoconstriction, increased oxygen consumption)
- Cerebral problems (increased intracerebral pressure, leading to risk of intraventricular haemorrhage and ischaemia)
- Skeletal muscle problems (muscle spasm, delayed mobilisation)
- Visceral problems (slowed gastrointestinal and urinary function)
- Delayed cicatrisation
- Stress response characterised by alteration of electrical signals and water balance (hyperglycaemia, osmotic diuresis)
- Immune depression

11.4 Multimodal Treatment

The multimodal treatment of pain combines pharmacological and non-pharmacological techniques in a safe, planned according to personal need. It therefore requires observation of the baby's physiological parameters and behaviour [13]. To this aim specific pain scores have been created for newborns. In this age group, pain is evaluated through behavioural and physiological parameters and in some cases stress hormone levels (Table 11.1). However there are many neonatal pain scales according to the age of the patients, e.g. the Premature Infant Pain Profile (PIPP) 28–40 weeks of gestational age (GA) (Table 11.2) [14] and EDIN scale [15] 25–36 weeks of GA (Table 11.3) for prolonged pain. During the compilation of the PIPP, one should evaluate gestational age, score behaviour before potential pain (observe for 15 s), measure heart rate and basal oxygen saturation and observe the baby and score the behaviour in 30 s immediately after the painful event.

Table 11.1 Behavioural and physiological parameters used for the evaluation of pain in newborns by a physician

Behavioural (observational)	Physiological	Psychological	
Position of the body	Reflexes	Projectional	Self-report
Facial expression	Heart rate	Colours	Interview
Vocalisation pattern	Breathing frequency	Forms	Questionnaires
	Index fatigue		
Crying	Endorphin levels	Illustrations	Thermometer
		Drawings	Facial scales
		Visual analogue scales	

Table 11.2 Premature infant pain profile (PIPP)

Indicator	Finding	Points
Gestational age	≤36 weeks	0
	32 weeks to 35 weeks 6 days	1
	28 weeks to 31 weeks 6 days	2
	<28 weeks	3
Behavioural state	Active/awake, eyes open, facial movements	0
	Quiet/awake, eyes open, no facial movements	1
	Active/asleep, eyes closed, facial movements	2
	Quiet/asleep, eyes closed, no facial movements	3
Heart rate maximum	0–4 beats/min increase	0
	5–14 beats/min increase	1
	15–24 beats/min increase	2
	≥25 beats/min increase	3

Table 11.2 (continued)

Indicator	Finding	Points
Oxygen saturation minimum	0–2.4% decrease	0
	2.5–4.9% decrease	1
	5.0–7.4% decrease	2
	7.5% decrease or more	3
Brow bulge	None (≤9% of time)	0
	Minimum (10–39% of time)	1
	Moderate (40–69% of time)	2
	Maximum (≥70% of time)	3
Eye squeeze	None (≤9% of time)	0
	Minimum (10–39% of time)	1
	Moderate (40–69% of time)	2
	Maximum (≥70% of time)	3
Nasolabial furrow	None (≤9% of time)	0
	Minimum (10–39% of time)	1
	Moderate (40–69% of time)	2
	Maximum (≥70% of time)	3

Table 11.3 Échelle de douleur et d'inconfort du nouveau-né, neonatal pain discomfort scale (EDIN) [15]

Indicator	Description
Facial activity	0 Relaxed facial activity 1 Transient grimaces with frowning, lip purse and chin quiver or tautness 2 Frequent grimaces, lasting grimaces 3 Permanent grimaces resembling crying or blank face
Body movements	0 Relaxed body movements 1 Transient agitation, often quiet 2 Frequent agitation but can be calmed down 3 Permanent agitation with contraction of fingers and toes and hypertonia of limbs or infrequent, slow movements and prostration
Quality of sleep	0 Falls asleep easily 1 Falls asleep with difficulty 2 Frequent, spontaneous arousals, independent of nursing, restless sleep 3 Sleepless
Quality of contact	0 Smiles, attentive to voice 1 Transient apprehension during interactions with nurse 2 Difficulty communicating with nurse, cries in response to minor stimulation 3 Refuses to communicate with nurse. No interpersonal rapport, moans without stimulation
Consolability	0 Quiet, total relaxation 1 Calms down quickly in response to stroking or voice or with sucking 2 Calms down with difficulty 3 Disconsolate, sucks desperately
	Total score 0/15

11.5 Clinical Neonatal Pain Control

Many invasive procedures need pain control in neonatal intensive care unit (NICU):

- Heel lancing
- Venipuncture, arterial puncture and percutaneous central venous catheter insertion
- Intramuscular or subcutaneous injection
- Central venous catheter insertion by surgical cut-down
- Tracheal intubation
- Lumbar puncture
- Chest tube insertion
- Chest tube removal
- Screening for retinopathy of prematurity (ROP)

Pain control is possible with several methods, e.g. environmental measures, non-pharmacological measures, local pharmacological measures and systemic analgesia.

11.6 Analgesic Procedures in Newborns

11.6.1 Environmental Measures and Non-pharmacological Measures

Non-pharmacological interventions in neonates differ somewhat from those used in infants and children. Neonates respond well to sensory stimulation such as gentle stroking, rocking and nonnutritive sucking and to maternal interventions such as being breast-fed during procedures where practicable [16]. There are many non-pharmacological types of pain control, and they are of great significance for preventing suffering and reducing the use of analgesic agents [17]. They include having a favourable environment, combining procedures, planning routine procedures, avoiding heel prick, decreasing noise and bright light, respecting the sleep-wake cycle, satisfying the sucking reflex, placing the baby in a comfortable natural position, changing its position from time to time (this includes ventilated babies), maintaining physical contact (stroking, rocking, massage), giving glucose solution and sensorial saturation and feeding or giving the pacifier before painful procedures [16]. The optimal baseline state of quiet wakefulness should be obtained before starting the procedure, do not interrupt sleep and plan the procedure far from mealtimes and from any other painful invasive procedures for at least 2 h after the procedure.

The general principles of procedural pain management are summarised in Table 11.4 [18].

Table 11.4 General principles of procedural pain management

1. Infants and children of all ages, including preterm neonates, feel pain and require analgesia for painful procedures
2. Developmental differences in responses to pain and analgesics need to be considered when choosing analgesia
3. Consider if the planned procedure is necessary: • Avoid multiple procedures where possible • Consider how the information gained may influence care • Consider whether modification of procedure may reduce pain, e.g. venepuncture is less painful than heel lance
4. Consider whether sedation or general anaesthesia may be required for safe and satisfactory outcome
5. Ensure suitable environment: a quiet, calm location with suitable toys and distractions
6. Ensure appropriate personnel are available: enlist additional experienced help when necessary
7. Allow sufficient time for analgesic measures and medications to be effective
8. Formulate a clear plan of action should the procedure fail or pain becomes unmanageable using the techniques selected
Good Practice Point: Pain management for procedural pain should be planned, taking into account general principles, and should include both pharmacological and non-pharmacological strategies wherever possible

Topical local anaesthesia is widely used either on its own or in combination with other techniques. These agents have been found to decrease measures of pain during venipuncture, percutaneous central venous catheter insertion and peripheral arterial puncture [19].

EMLA and Ametop (tetracaine gel 4%) are the most commonly used topical local anaesthetic cream preparations in paediatric practice (Table 11.5) [18, 20].

EMLA application time is 1 h, the depth of action is 5 mm and the duration of effect is 30–60 min. There is an associated risk of methaemoglobinaemia (reduced methaemoglobin reductase, fetal haemoglobin). Precautions to be taken in term and preterm newborns are to limit the contact surface and give no more than one application per day. Dosages: premature babies <1500 g, 0.5 cm^2 (0.20 g); premature >1500 g, 1 cm^2 (0.30 g); and term babies 2 cm^2 (0.50 g).

Topical anesthetics have proven efficacy in reducing the needle insertion pain for intravenous cannulation and venepuncture, with Ametop being superior to EMLA [18, 20].

Lidocaine 1% and 2% lubricant gels are effective for urethral analgesia in urinary catheterisation. They also reduce discomfort during insertion of nasogastric tubes and can be used topically for analgesia following circumcision.

Table 11.5 Topical anaesthesia: BNFC guidance on properties and use of EMLA and Ametop topical anaesthesia [19]

	EMLA	Ametop
Formulation	Eutectic mix lignocaine 2.5% and prilocaine 2.5%	Tetracaine 4% gel
Time of onset to effective analgesia	60 min	30 min
Duration can be left applied to the skin	5 h	1 h
Duration of action after removal	1–2 h	4–6 h
Age limits	Under 1 year—not licensed Not recommended <1 month[a]	Not recommended in neonates <1 month or preterm infants
Dose	**Age**	**Dose**
	0–3 months	1 g
	3–12 months	2 g
	1–5 years	10 g
	6–11 years	20 g
Caution	G6PD deficiency anaemia Methaemoglobinaemia	Methaemoglobinaemia
Contraindications	Open wound, mucosae atopic dermatitis	Inflamed, traumatised areas

[a]EMLA is usually safe to use in neonates and infants without predisposition to methaemoglobinaemia, e.g. G4PD deficiency, haemoglobinopathies, BNFC (British National Formulary for Children)

11.6.2 Regional Anaesthesia

Regional techniques, such as peripheral nerve blocks and central neuraxis blockade (spinal, epidural), are sometimes used to provide anaesthesia and analgesia for procedures on the trunk or limbs, as an adjunct to general anaesthesia, and for postoperative analgesia. Examples of regional nerve blocks include ilioinguinal and iliohypogastric nerve blocks, penile block, digital block and local infiltration and intercostal nerve blocks. These techniques should be used carefully by healthcare professionals trained in their use and with appropriate and careful observation. In neonates, intermittent administration of dilute local anaesthetics with low-dose extradural opioids such as fentanyl offers less potential for the toxic effects of drugs than continuous infusion techniques with either drug alone. Accurate calculation is a particular concern in the care of preterm and term neonates, in whom differences in protein binding and metabolism can result in local anaesthetic drug accumulation and toxic effects [17].

11.6.3 Pharmacological Therapies

There are many potential routes of administration of drugs including sublingual and nasal, in addition to oral, rectal and intravenous. Routes available, time to onset and analgesic duration of action vary considerably between agents.

A knowledge of pharmacokinetics and pharmacodynamics in the neonatal period is necessary in order to use drugs. Major differences in this period involve distribution volume, limited fat reserve, hepatic immaturity, renal immaturity with decreased glomerular filtration and reduced tubule resorption (lower metabolism and elimination) and qualitative and quantitative differences in plasma proteins. This means that the dose of most drugs must be reduced and the administration interval increased, at least in the first week of life. After the first month, the situation changes, and there is an increase in metabolic capacity and distribution volume. Doses become equal to, if not higher than, those for adults.

11.6.4 Systemic Analgesia

The American Academy of Pediatrics (AAP) recommends routine pain management for persistent pain and during procedures such as circumcision, chest drain insertion and removal, nonemergency intubations and mechanical ventilation.

Analgesics and sedatives are known to be potent modulators of several G-protein-linked receptor signalling pathways in the developing brain that are implicated in the critical regulation of neural tissue proliferation, survival and differentiation. Studies of appropriate dosing and long-term effects of these analgesics given during the neonatal period are lacking and/or conflicting (Table 11.6) [21].

11.6.4.1 Non-steroidal Anti-inflammatory Drugs (NSAIDs)

Relatively few studies have been published on the use of NSAIDs in newborns. Except for paracetamol, these drugs should not be used until renal function is mature. NSAIDs act by peripheral inhibition of eicosanoids, especially prostaglandins (responsible for pain associated with inflammation, together with histamine, serotonin and free thromboxane, in tissue lesions) [18]. They also decrease the synthesis of free radicals, reduce the migration of macrophages and inhibit the synthesis of nitric oxide (NO). Unfortunately, they have a therapeutic threshold, their effect does not increase with increasing dose, and to obtain further effects they must be associated with opioids (oxycodone).

Table 11.6 Balance between pain treatment pros and cons

Reasons to treat pain	Concerns regarding pain treatment
Beneficial short-term effects (less ventilatorasynchrony, splinting, faster intubation, decreased morbidity especially after surgery)	Adverse short-term effects (hypotension, respiratory depression, prolonged ventilator dependence, intraventricular hemorrhage)
Beneficial long-term effects (improved response to pain, downregulation of the hypopituitary-adrenal axis)	Prolonged metabolism of opioids and benzodiazepine
Less stress	Hyperalgesia
Decreased neuronal cell	Enhanced neuronal cell death
Compassion	Unknown effects of commonly used drugs

Table 11.7 Recommended dose of IV paracetamol

	Term newborn infants, infants, toddlers and children weighing <10 kg
Dose of IV paracetamol (per infusion administration)	7.5 mg/kg
Maximum daily infusion dose	30 mg/kg

11.6.4.2 Paracetamol

Unlike NSAIDs, it does not have peripheral tissue anti-inflammatory properties but inhibits prostaglandins at hypothalamic level (pure analgesic and antipyretic). Its analgesic effect is directly proportional to its concentration in the blood. It is metabolised by the liver to active metabolites. Hepatic immaturity may be an advantage because production of toxic metabolites is believed to be lower.

Recommended dose of IV paracetamol [22]:

The dose of IV paracetamol recommended by the Medicines and Healthcare Products Regulatory Agency (MHRA) for children and adults is shown in the table below (Table 11.7). Paracetamol should be given by infusion over 15 min, and the minimum dose interval should not be less than 4 h (6 h in patients with renal impairment).

11.6.4.3 Opioids

Newborns are particularly vulnerable to side effects of opioids, such as respiratory depression. The greater sensitivity of newborns does not mean that opioids should not be used, but it is important to select babies carefully, prescribe and prepare correctly, monitor side effects and monitor the infusion systems.

11.6.4.4 Morphine

This is the best known opioid to be used in the neonatal period. In Italy it is rarely used, despite its low cost, because of its side effects, particularly in preterm babies [23].

Side effects are largely related to histamine release with bronchospasm and cardiocirculatory collapse due to vasodilation. Morphine causes less respiratory depression and thoracic rigidity than other opioids and may be used intravenously without respiratory assistance in the neonatal intensive care unit.

11.6.4.5 Fentanyl

Fentanyl is a synthetic derivative of morphine. It is a highly potent synthetic opioid, with more rapid onset and offset of action than morphine. Fentanyl has the following properties: it is a powerful analgesic, ten times stronger than morphine and fast-acting (1 min) and has short duration (35–45 min); it maintains haemodynamic stability and is a poor sedative; it has a bradycardiac effect and induces thoracic rigidity; it is liposoluble, binds protein plasma and is metabolised in the liver [24]. Its half-life is up to 32 h in premature babies and infants. It may therefore be preferred for short duration procedures. Fentanyl's lipophilicity means that it can be

Table 11.8 Schedule for administration of morphine to neonates (with ventilation support)

	Intravenous bolus	Continuous infusion
Newborns < 34 weeks gestational age	5–10 µg/kg in 10 min max. 20 µg/kg	5 µg/kg/h to increase if necessary at 2 µg/kg/h every 4 h. Maximum: 15 µg/kg/h
Term newborns	Max. 40 µg/kg	10 µg/kg/h to increase if necessary at 5 µg/kg/h every 4 h max. 20–25 µg/kg/h

Without ventilation: 10–15 µg/kg/h

Table 11.9 Schedule for administration of fentanyl to neonates

	Intravenous bolus	Continuous infusion
Newborns < 32 weeks gestational age	0.5 µg/kg in 10 min max. 1.5 µg/kg	0.5 µg/kg/h to increase if necessary 0.5 µg/kg/h every 4 h. Max. 2 µg/kg/h
Term newborns	0.5 µg/kg in 10 min max. 3 µg/kg	1 µg/kg/h to increase if necessary 0.5 µg/kg/h every 2–4 h. Max. 2–4 µg/kg/h

readily absorbed via transdermal, buccal and nasal routes, as well as being rapidly effective following intravenous administration.

Guidelines for the administration of morphine and fentanyl are shown in Tables 11.8 and 11.9.

11.7 Other Opiates

Other opiates include the short-acting drugs sufentanil, alfentanil and remifentanil. All are useful for short procedures, such as intubation. Sufentanil and alfentanil are metabolised by the liver, which is immature in preterm neonates resulting in increased levels with repeated infusions, especially in preterm neonates [25]. Remifentanil, however, is rapidly cleared by plasma esterases and is unaffected by the maturity of the liver enzyme system, making it attractive for short neonatal surgery or other procedures when rapid recovery is anticipated.

Furthermore, methadone, ketamine, propofol and dexmedetomidine have been proposed for pain management in neonates; however, few, if any, studies of these agents have been performed in this population, and caution should be exercised when considering them for use because of concerns about unanticipated adverse effects and potential neurotoxic effects [21].

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Nonpharmacological Treatment of Neonatal Pain

12

R. Carbajal

12.1 Introduction

The alleviation of pain is a basic and human right regardless of age. It therefore seems unbelievable how long it took the medical community to realize that newborns are able to feel pain. During the last 25 years, there has been a significant increase in our knowledge of pain in neonates, and broad areas of research have been addressed in the medical, nursing, psychological, neuroscientific, social, bioethical, and philosophical literature [1]. Despite these impressive gains, many of the previously identified and newer challenges remain, since we have not completely reversed the de-emphasis of infant pain [2], and no effective methods of preventing or treating pain for all infants in all clinical situations have been developed. However, the reason most of these challenges remain is because of the large gap that exists between published research results and routine clinical practice [3].

This article describes the sweet solutions and nonpharmacological treatments currently available to alleviate procedural pain in neonates.

12.2 The Burden of Procedural Pain

Newborns routinely undergo painful invasive procedures, even after an uncomplicated birth. For obvious reasons, these invasive procedures that cause pain or distress are most frequently performed on infants admitted to the neonatal intensive care unit (NICU). For sick babies, multiple studies have documented a high

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frequency of invasive procedures during neonatal intensive care, particularly in preterm neonates, most of which are performed in the first week after birth [3–6]. The most frequent procedures performed in NICUs are heel sticks, endotracheal suction, and intravenous line insertion [3, 5].

Despite increased awareness among clinicians about neonatal pain, management of procedural pain in neonates is not yet optimal, although recent surveys show that it is improving. In a multicenter prospective study carried out in French NICUs, 430 neonates experienced 60,969 first-attempt procedures, with 42,413 (69.6%) painful and 18,556 (30.4%) stressful procedures. Each neonate experienced a median of 115 (range, 4–613) procedures during the study period and 16 (range, 0–62) procedures per day of hospitalization. Of the 42,413 painful procedures, 2.1% were performed with pharmacological-only therapy; 18.2% with nonpharmacological-only interventions; 20.8% with pharmacological, nonpharmacological, or both types of therapy; 79.2% without specific analgesia; and 34.2% were performed while the neonate was receiving concurrent analgesic or anesthetic infusions for other reasons [3].

Studies using skin-breaking procedures as a proxy of nociception have shown that repeated neonatal pain leads to poorer cognition [7] and motor function [8], impaired brain development [9, 10], and altered pain responses [11].

12.3 Analgesic Treatment

Nonpharmacological techniques and pharmacological treatments are available for pain management in the neonate. Nonpharmacological interventions, which comprise environmental and behavioral interventions, have a wide applicability for neonatal pain management alone or in combinations with pharmacological treatments. Sweet solutions are often included among the nonpharmacological interventions, although they certainly have a pharmacological effect. Nonpharmacological interventions and sweet solutions are not necessarily substitutes or alternatives for pharmacological interventions, but rather are complementary [12]. Moreover, because painful procedures are extremely frequent in sick and preterm neonates and because concerns exist regarding potential adverse effects of pharmacological agents, a growing interest has recently been developing in nonpharmacological interventions for procedural pain. These interventions can reduce pain in neonates indirectly, by reducing the total amount of noxious stimuli to which they are exposed and, directly, by blocking nociceptive transduction or transmission, or by activation of descending inhibitory pathways or by activating attention and arousal systems that modulate pain [12].

12.3.1 Prevention

One of the most effective methods for reducing pain in neonates is to prevent it. Procedural pain can be minimized by efficient training of staff using indwelling lines for sampling, by planning procedures so that an analgesic approach can be

considered [13], and by using mechanical devices if heel lance is necessary [14–16]. Compared to manual lancets, the use of mechanical lancets for heel pricks resulted in decreased behavioral and physiological distress and fewer repeated punctures [14, 15], increased volumes of blood, shortened time intervals for blood collection, and reduced hemolysis [17]. Heel warming, however, has not been found to have an effect on pain response [18]. It has also been shown that venepuncture is less painful than heel lance [19, 20]. A Cochrane review that evaluated six studies comparing venepuncture versus heel lance for blood sampling in term neonates concluded that venepuncture, when performed by a skilled phlebotomist, appears to be the method of choice for blood sampling in term neonates. Further well-designed randomized controlled trials should be conducted, especially in preterm neonates, in settings where several individuals perform the procedures [21].

Another important prevention issue is to avoid systematic procedures; these must only be performed if they are absolutely necessary for the diagnostic and/or therapeutic management of neonates. In the French Epippain study, a 26 weeks' gestational age neonate underwent 95 heel sticks during the first 14 days after admission to the NICU, and several others underwent more than 300 painful procedures during the first 2 weeks of NICU admission [3]. Given the burden that these procedures imposed on the neonate, one may ask if all these heel sticks were absolutely necessary.

12.3.2 Sweet Solutions

12.3.2.1 Sucrose

Blass and Hoffmeyer reported in 1991 the effectiveness of sucrose as an analgesic agent for newborn infants during heel stick and circumcision [22]. Infants who drank 2 ml of a 12% sucrose solution (0.24 g) prior to blood collection cried 50% less during the blood collection procedure than did control infants who had received 2 ml of sterile water. Crying of infants who ingested sucrose returned to baseline levels within 30–60 s after blood collection, whereas control infants required 2.5–3.0 min to return to baseline. These findings provided the background for other studies that confirmed the analgesic properties of oral sucrose [23].

12.3.2.2 Evidence of Analgesic Efficacy

Oral sucrose has been the most frequently studied nonpharmacological intervention for relief of procedural pain in neonates [24]. A Cochrane systematic review of the literature published in 2013 in order to determine the efficacy, effect of dose, and safety of sucrose for relieving procedural pain found 57 studies enrolling 4730 infants who underwent painful procedures [24]. Sucrose was safe and effective for reducing procedural pain from single events (heel lance, venepuncture). Sucrose was effective in reducing crying, grimacing, vagal tone, and pain scores during heel lance in volumes and concentrations ranging from 0.5 to 2 ml of 12% to 50% solution. Some effectiveness of sucrose administration was evident during venepuncture with respect to reducing heart rate, and pain scores. Sucrose did not appear to be

effective for retinopathy of prematurity examination. For other painful procedures, such as bladder catheterization, subcutaneous injections, nasogastric tube insertions, and circumcision, there were few studies and conflicting results. For procedures of longer duration, multiple doses of sucrose or sucrose combined with other pharmacological and nonpharmacological interventions may be required to achieve an effect. Oral sucrose administration seems to be safe as few adverse effects have been reported; most were transient minor side effects (e.g., oxygen desaturation, choking), which resolved spontaneously without intervention [24]. One study has questioned sucrose analgesic efficacy [25]. In 20 term neonates given 0.5 ml of 24% sucrose before a heel lance, nociceptive brain activity—recorded with electroencephalography and identified by principal component analysis—as well as spinal nociceptive reflex withdrawal activity measured by electromyography did not differ significantly compared to 24 term neonates who received water. Nonetheless, a reduction in the pain score was observed in the sucrose group [25]. Some methodological issues related to this study need to be addressed before drawing conclusions [24].

12.4 Repeated Doses

Johnston et al. tested the efficacy of repeated doses versus a single dose of sucrose to decrease pain from routine heel sticks in 48 preterm neonates [26]. Infants in the first week of life with a mean gestational age of 31 weeks received 0.05 ml of 24% (0.012 g) sucrose solution or sterile water by mouth (1) 2 min prior to actual lancing of the heel, (2) just prior to lancing, and (3) 2 min after lancing. The single-dose group received sucrose for the first dose and water for the second and third dose, the repeated-dose group received sucrose three times, and the placebo group received only water. The pain scores (PIPP) group were obtained for five 30-s blocks from lancing. Both sucrose groups had lower PIPP scores (single sucrose pain scores: 6.8–8.2, $p = 0.07$; repeated sucrose pain scores: 5.3–6.2, $p < 0.01$) than water (pain scores: 7.9–9.1), and in the last block, the repeated dose had lower scores than the single dose (6.2 vs. 8.2, $p < 0.05$).

12.5 Doses, Age for Efficacy, and Recommendations

Regarding effective sucrose dosage, a meta-analysis published in 1997 and including primarily term neonates showed that the 0.18 g dose of sucrose was ineffective to reduce crying time [27]. Doses of 0.24 g (2 ml of 12% sucrose solution) were effective. A dose of 0.50 g provided no additional benefit [27]. In the 2013 Cochrane review, very small doses of 24% sucrose (0.01–0.02 g) were efficacious in reducing pain in very low birth weight infants while larger doses (0.24–0.50 g) reduced the proportion of time crying in term infants following a painful procedure [24]. The peak effect appears to occur at 2 min and lasts approximately 4 min [28]. Therefore, if the procedure exceeds this duration, another oral administration should be given [24].

Age parameters for efficacy are not very clear. Although sucrose continues to have an effect beyond the newborn period, some data show that this analgesic effect decreases with age, and it is very modest at 2 months [29, 30]. A trial showed that 2 ml of 24% sucrose was not effective in reducing pain in infants older than 30 days during bladder catheterization [31]. However, another study suggested that 2 ml of 75% sucrose was effective in relieving crying after immunization in infants aged 2–6 months [32]. Similarly, Ramenghi et al. found that 50% sucrose resulted in shorter crying times, compared with 25% sucrose, glucose, or sterile water, for 4-month-old children [33]. They also found a trend toward shorter crying times for 2- and 3-month-old children receiving 50% sucrose, but it did not reach statistical significance. In spite of these conflicting data, an expert conference on pain reduction during immunizations considered that there seemed to be sufficient data to recommend sucrose use as a routine part of immunization administration for infants less than 6 months of age [34]. A systematic review published in 2010 on the efficacy of sweet solutions for analgesia in infants between 1 and 12 months of age concluded that sucrose or glucose before immunization moderately reduced the incidence and duration of crying in this population and that healthcare professionals should consider using sucrose or glucose before and during immunization [35].

It has been shown that the responses to intraoral sucrose are neither specific to sucrose nor to the general class of carbohydrates and that these effects are more appropriately understood as "sweetness" effects, since other sweet solutions are also effective [36]. A systematic review and meta-analyses of nonsucrose sweet solutions for pain relief in neonates published in 2013 found 38 studies, of which 35 investigated glucose. An analgesic effect of 20–30% glucose solutions was found [37].

Since the early 2000s, the American and Canadian Pediatric Societies [38] as well as the Royal Australasian College of Physicians [39] have recommended the use of sucrose for such procedures as heel lances, injections, and intravenous line insertions. An update on the prevention and management of procedural pain in the neonate issued in 2016 by the American of Pediatrics stated that oral sucrose and/or glucose solutions can be effective in neonates undergoing mild to moderately painful procedures, either alone or in combination with other pain relief strategies [40]. These recommendations underlined that fact that when sucrose or glucose is used as a pain management strategy, it should be prescribed and tracked as a medication.

12.6 Glucose

As mentioned above, oral glucose has also been shown to be effective in reducing procedural pain in neonates during minor procedures; 30% glucose has been effective both in term neonates during heel stick [41] and venepunctures [42], and in preterm neonates during subcutaneous injections [43]. Deshmukh and Udani studied the analgesic effect of different concentrations of oral glucose in preterm infants during venepuncture in a double-blind, randomized controlled trial [44]. They randomized 60 infants to receive 2 ml of one of three solutions (sterile water, 10%

glucose, and 25% glucose) in the mouth 2 min before venepuncture. There was a significant reduction in duration of first cry in the babies given 25% glucose compared with controls and those given 10% glucose. There was no significant effect on heart rate, respiratory rate, or oxygen saturation. There was no difference between 10% glucose and sterile water. Eriksson and Finnström studied whether repeated doses of orally administered glucose would cause tolerance [45]. They found in term neonates that the efficacy of oral glucose was not modified when 1 ml of 30% glucose was given three times a day for 3–5 days.

Initial data on the comparison of the analgesic efficacy of sucrose and glucose were conflicting. In one study assessing pain with a behavioral pain score, the efficacies of 30% glucose and 30% sucrose were found to be similar [42]; in another study, changes in heart rate during heel sticks were similar among neonates receiving 33% and 50% glucose or sucrose [46]. However, in another study of 113 healthy term newborns, 30% sucrose was superior to 10% and 30% glucose solutions in reducing crying time [47]. Recently, a double-blind randomized controlled trial comparing the efficacy of oral 25% glucose with oral 24% sucrose for pain relief during heel lance in preterm neonates found that glucose and sucrose provided comparable analgesia [48]. Thus, glucose can be recommended as an alternative to sucrose for procedural pain reduction in healthy term and preterm neonates [37].

The coadministration of sucrose or glucose with a pacifier has been found to be synergistic [42, 49]. The association of a sweet solution and a pacifier provides a stronger analgesic effect than either one alone [42, 50].

12.7 Adverse Effects of Sweet Solutions

A Cochrane review published in 2013 found 16 studies that evaluated adverse effects of sucrose compared to placebo [24]; six of these studies observed minor brief and self-limiting side effects in infants. One study [51] reported minor side effects in 6 out of 192 infants included in the study. One neonate who received water with pacifier choked when given the water but stabilized within 10 s. Three infants randomized to the sucrose group and two infants randomized to the water with pacifier group showed oxygen desaturation when the study intervention was administered. Each neonate recovered spontaneously with no medical intervention required [51]. In a study on the analgesic efficacy of glucose and pacifier in very preterm neonates during subcutaneous injections, slight (85–88%) and transient oxygen desaturations were observed in 7 out of 54 neonates during administration of interventions [43]. In five neonates it happened during administration of 30% glucose alone and in the other two during administration of 30% glucose plus the pacifier. None of the 24 placebo administrations elicited oxygen desaturations. Regarding blood glucose levels, a study found no significant differences between infants receiving sucrose or placebo in blood glucose levels monitored during the study as well as the incidence of spitting up the sucrose solution [52]. Stevens et al. found no significant differences in incidence rates for necrotizing enterocolitis between

infants who received repeated doses of sucrose over 28 days of life compared to control groups [53].

For repeated administrations of sucrose in infants younger than 31 weeks' postconceptional age (PCA), Johnston et al. reported that higher numbers of doses of sucrose predicted lower scores for motor development and vigor, and for alertness and orientation at 36 weeks' PCA and lower motor development and vigor at 40 weeks' PCA [54]. These results need to be replicated because of their importance and also because the sample size was inadequate to show the same association in the placebo group, which could potentially be a methodological explanation for the observed results. However, Stevens et al. reported no statistically significant differences between sucrose plus pacifier, water plus pacifier, or the standard care group on neurobiological risk status outcomes [53].

12.7.1 Environmental Interventions

So-called environmental interventions aim to decrease the environmental stress of the NICU, where neonates are exposed to numerous repeated noxious stimuli including bright light, loud noise, frequent handling, and repeated painful procedures [12]. The reduction of lighting levels and alternating day and night conditions can reduce stress and promote increased sleep, weight gain, and the development of circadian rhythms [55, 56]. These findings suggest that physical environment has an effect (either direct or indirect) on the subsequent behavior of preterm infants and that exposure to night and day is beneficial. Another study has also shown a reduction of illness severity when light, noise, and handling were reduced [57].

12.7.2 Swaddling, "Facilitated Tucking," Touch, and Positioning

Swaddling is when an infant is securely wrapped in a blanket to prevent the child's limbs from moving around excessively [58]. Facilitated tucking involves firmly containing the infant using a caregiver's hands on both head and lower limbs to maintain a "folded-in" position. Infant may or may not be wearing clothes [58]. Swaddling has been shown to reduce pain-elicited distress during and after heel stick in neonates [59]. This effect was, however, very modest. Fearon et al. studied the responses of 15 preterm neonates to swaddling after a heel stick [60]. They found that in neonates of 31 weeks' postconceptional age or older, the use of swaddling significantly reduced protracted behavioral disturbance. A Cochrane revue published in 2015 on nonpharmacological interventions to reduce procedural pain in infants reported that in preterm infants, there was low to very low-quality evidence to support the use of swaddling/tucking as an effective intervention for reducing pain reactivity (within 30 s of nociception) and immediate pain regulation (after 30 s of nociception) [58]. For term neonates, very low-quality evidence supports the effectiveness of swaddling/tucking-related interventions for pain reactivity [58].

In touch/massage-related intervention, an infant's body is "stroked" to provide some type of counter-stimulation to the nociceptive input [58]. Regarding touch, the above Cochrane revue found in preterm infants that touch and massage-related interventions are efficacious in improving pain reactivity (low-quality evidence), but not efficacious for immediate pain regulation (very low-quality evidence) [58]. In term neonates, low to very low-quality evidence suggests touch and massage-related interventions are not an efficacious intervention to reduce pain reactivity or immediate regulation in neonates.

Grunau et al. have studied the influence of position (prone or supine) on pain responses to heel lance in preterm infants at 32 weeks' gestational age [61]. Thirty-eight neonates were assigned to one of two positions during baseline and heel lance. The authors concluded that placement in the prone position is not a sufficient environmental comfort intervention for painful invasive procedures such as heel lance for blood sampling [61].

12.7.3 Nonnutritive Sucking

The pacifying effect of nonnutritive sucking (NNS) has been clearly shown in humans. Field and Goldson reported decreased crying with NNS in both term and preterm neonates during heel stick [62]. Shiao et al. reported in 1997 a meta-analysis of the effects of NNS on heart rate and peripheral oxygenation [63]. They identified four studies of the effect of NNS on heart rate without stimulations, three studies on heart rate during painful stimulations, and three studies on transcutaneous oxygen tension ($tcPO_2$). NNS significantly decreased heart rate without stimulations ($p = 0.002$) and during painful stimulations ($p = 0.0001$) and significantly increased $tcPO_2$ ($p = 0.0001$). As in all meta-analyses, the authors used the effect size as an index of how much difference exists between the groups. When effect size is based on means, it corresponds to the ratio of the difference between groups to the standard deviation. The total weighted effect size for heart rate without stimulations was small (0.17); however, it was large for heart rate during painful stimulations (1.05) and for $tcPO_2$ (0.69). Larger effects were noticed for preterm infants than for term infants and for longer NNS.

In infants of very low birth weight, Stevens et al. demonstrated that NNS is effective for relieving pain induced by routine heel lance procedures [64]. Corbo et al. investigated the effects of NNS during heel stick procedures in neonates of gestational ages ranging from 26 to 39 weeks [65]. NNS reduced the time of crying, and the heart rate increase during the procedure but had no effect on respiratory rate or $tcPO_2$. In term neonates, other studies have also reported the analgesic effects of sucking a pacifier during heel lance [49, 66] and venepunctures [42]. Blass and Watt found that sucking an unflavored pacifier was analgesic only when suck rate exceeded 30 sucks/min [49]. Bellieni et al. have also shown in term neonates that NNS is effective to reduce heel stick-induced pain [66]. In their study, glucose plus sucking (elicited with the tip of a 1 ml syringe without needle placed in the baby's mouth) was more effective than sucking alone.

Recently, a Cochrane review has summarized the current evidence for NNS sucking interventions [58]. It concludes that for preterm infants, the pooled results suggest that sucking is not efficacious in reducing pain reactivity but is effective for immediate pain regulation. An analysis of significant studies suggests that pain relief will be maximized if sucking begins at least 3 min prior to the painful stimuli. For term neonates, the results suggest that sucking is effective for pain reactivity and immediate pain regulation [58].

Pinelli et al. reviewed in 2002 the available literature to look for negative effects of NNS in high-risk full-term and preterm infants in neonatal nurseries [67]. From this review, it appears that, although harmful effects have not been specifically studied, NNS in preterm and high-risk full-term infants does not seem to have any short-term negative effects. No long-term data on the effects of NNS in high-risk full term and preterm infants are presently available.

12.7.4 Multisensory Stimulation

Multisensory stimulation (massage, voice, eye contact, and perfume smelling) has been shown to be an effective analgesic technique that potentiates the analgesic effect of oral glucose during minor procedures [66]. This interesting method, also termed "sensory saturation," was developed by Bellieni et al. [66, 68]. This intervention consisted of:

1. Laying the infant on its side with legs and arms flexed but free to move
2. Looking the infant in the face, close up, to attract its attention
3. Simultaneously massaging the infant's face and back
4. Speaking to the infant gently but firmly
5. Letting the infant smell the fragrance of a baby perfume on the therapist's hands

A 33% glucose solution was also instilled on the infant's tongue with a syringe to stimulate sucking [66]. In a randomized study conducted on 120 term neonates, these authors found that multisensory stimulation plus glucose was more effective in reducing pain from heel lance than glucose, sucking, or sucking plus glucose. They concluded that sensory saturation is an effective analgesic technique that potentiates the analgesic effect of oral glucose.

12.7.5 Skin-to-Skin Contact (Kangaroo Care)

Gray et al. found that 10–15 min skin-to-skin contact between mothers and their newborns reduces crying, grimacing, and heart rate during heel lance procedures in full-term newborns [69]. A total of 30 newborn infants were randomly assigned to either being held by their mothers in whole body, skin-to-skin contact, or to no intervention (swaddled in crib) during a standard heel lance procedure. Crying was reduced by 82% and grimacing by 65% over the control group during the heel lance procedure.

Heart rate also was reduced substantially by contact. Johnston et al. evaluated the efficacy of maternal skin-to-skin contact, or "kangaroo care," on diminishing the pain response of preterm neonates between 32 and 36 weeks' postmenstrual age to heel lancing [70]. They used a crossover design, in which infants served as their own controls. In the kangaroo care group, the neonate was held in skin-to-skin contact for 30 min before the heel lance and remained in contact for the duration of the procedure. In the control condition, the neonate was in the prone position in the incubator. The ordering of conditions was random. All procedures were videotaped. Research assistants who were naïve to the purpose of the study coded video recordings that were taken with the camera positioned on the neonate's face so that an observer could not tell whether the neonate was being held or was in the incubator. Heart rate and oxygen levels were continuously monitored by computer. Pain was assessed with the PIPP score from videotapes. PIPP scores across the first 90 s from the heel lance procedure were significantly lower by two points in the kangaroo care condition.

Given the analgesic effectiveness of skin-to-skin contact shown in the above studies and the fact that parents of neonates in critical care units want to participate more in comforting their infants, kangaroo care is a potentially beneficial strategy for promoting family health. A recent Canadian study showed that although staff nurses had positive preconceived ideas and reduced concerns on kangaroo use, they did not increase its use as an analgesic intervention for procedural pain [71]. Further research addressing ways to overcome barriers to utilizing KC as an intervention for procedural pain is warranted.

12.7.6 Breastfeeding Analgesia

Breastfeeding maintained throughout a procedure has been shown to be a potent analgesic to relieve procedural pain in term neonates [72–76]. In one study, neonates who were held and breastfed by their mothers during heel lance and blood collection had a reduction in crying of 91% and grimacing of 84%, as compared to infants who had the same blood test while being swaddled in their bassinets [72]. In another study, Carbajal et al. randomized 180 term newborns undergoing venepunctures to receive four different analgesic interventions [73]. Venepunctures were performed in the first group while neonates were breastfeeding and in the second group while neonates were held in their mothers' arms without breastfeeding. In the third group, neonates received 1 ml of placebo (sterile water) 2 min prior to venepuncture, and in the fourth group neonates received 1 ml of 30% glucose 1 min prior to venepuncture and sucked a pacifier before and throughout the procedure. Pain-related behaviors during venepunctures were evaluated using two infant pain scales (DAN scale and PIPP scale). Significant reductions in DAN (Douleur Aiguë Nouveau-né) and PIPP scores were noted for the breastfeeding and glucose plus pacifier groups from the other two groups. Although DAN pain scores were lower in the breastfeeding group as compared to the glucose plus pacifier group, this difference did not reach statistical significance [73]. In 2005, Phillips et al. compared the analgesic effect of breastfeeding and pacifier use with maternal holding in term infants undergoing blood

collection via heel sticks in a randomized controlled study [75]. A total of 96 infants were randomized to three groups for analgesia: (1) breastfeeding, (2) pacifier use while held by mothers, and (3) pacifier use while held by research assistants (non-mothers). The authors found that breastfeeding is more analgesic than pacifier use with nonmaternal holding. They also concluded that maternal holding with either breastfeeding or pacifier use is more analgesic than nonmaternal holding with pacifier use. Shendurnikar and Ghandi randomized 100 neonates so that half of them were heel lanced while being breastfed, whereas the other half were heel lanced after being swaddled and kept in a cradle away from their mothers [74]. Statistically lower pain scores were observed at 1, 5, and 15 min after lancing in the breastfed group. Two other studies have evaluated the analgesic efficacy of breastfeeding prior to a painful procedure but discontinued during the procedure [77, 78]. These studies have shown that if breastfeeding is not maintained during the procedure, there is no analgesic effect. A recent Cochrane revue, evaluating ten studies, has confirmed the analgesic effects of breastfeeding for procedural pain [76].

12.7.7 Breast Milk

Studies on the analgesic effects of supplemental breast milk to reduce procedural pain in neonates have yielded conflicting results [41, 79–83]. In these studies 1–2 ml of breast milk, which contains 7% lactose, was placed in the infant's mouth via a syringe [41, 79–81, 83] or a special cup [82]. Term neonates were given 2 ml of breast milk, and in the only study that included preterm and term neonates [41], infants were given 1 ml of breast milk. Shah et al. reported in a Cochrane review that breast milk was not effective in reducing validated and nonvalidated pain scores such as NIPS, NFCS, and DAN when compared to placebo during painful procedures [76]. Thus, the available evidence does not support the use of milk as the sole intervention to alleviate procedural pain.

12.7.8 Music

Music has been used since ancient times to enhance well-being and reduce pain and suffering [84]. Music is defined as an intentional auditory stimulus with organized elements including melody, rhythm, harmony, timbre, form, and style. By contrast, environmental sounds that exist without controls for volume or cause/effect relations are perceived as noise [84]. Music is ubiquitous in all human cultures and is listened to by persons of all ages, races, and ethnic backgrounds. Music and music therapy may benefit patients both directly and indirectly. Music has physiological, psychological, and socioemotional direct effects. It may also affect patients indirectly through its effects on caregivers' attitudes and behaviors [84].

Bo and Callaghan have tested the effect of NNS, music therapy (MT), and combined NNS and MT (NNS + MT), versus no intervention, on heart rate, $tcPO_2$ levels, and pain behavior of neonates in NICUs having blood taken by a heel-stick

procedure [85]. The researchers used a within-subjects, repeated-measures, counterbalancing design. Each trial consisted of three periods of data collection: a baseline 1 min before the heel-stick procedure, each minute during 5 min of intervention, and each minute for 8 min after the heel stick. During the MT intervention, the researcher played intrauterine maternal pulse sounds with soothing music through a cassette recorder placed near the neonate's head using the same volume each time. In the combined MT + NNS intervention, the neonates had the intrauterine sounds and a latex nipple. The authors found in 27 neonates of 30–41 weeks' gestational age that the three comfort interventions significantly reduced neonates' heart rate, improved their $tcPO_2$ levels, and reduced their pain behavior. NNS + MT had the strongest effect on neonates' $tcPO_2$ levels and pain behavior; MT alone had the strongest effect on neonates' heart rate. Butt and Kisilevsky examined the physiological and behavioral effects of music during recovery from heel lance in 14 preterm infants at 29–36 weeks' PCA in a crossover study [86]. Infants were tested on two occasions: during a music condition and during a no-music control condition. Each condition was videotaped during three periods: baseline, heel lance, and recovery. Heel lance elicited a stress response (i.e., increased heart rate, decreased oxygen saturation, increased state of arousal, and increased facial actions indicative of pain) in both age groups. The stress response was greater in infants of PCA greater than 31 weeks. During recovery, these infants had a more rapid return of heart rate, behavioral state, and facial expressions of pain to baseline levels in the presence of music compared to the absence of music. The authors concluded that music is an effective intervention following a stress-provoking stimulus in infants older than 31 weeks PCA [86]. The limitations of this study include the small sample size and the absence of order effect testing (i.e., if the order of music and no-music conditions affected the outcome). Bergomi et al. reported in 2014 a randomized controlled trial on the management and reduction of heel lance pain using the music of Wolfgang Amadeus Mozart ("Sonata K. 448") in premature infants hospitalized in the NICU [87]. Each of 35 premature infants randomly received three interventions (glucose, music, standard care) during three heel lances; they were their own controls. Compared to baseline, pain PIPP scores change was +3 in the control arm, +1 in the glucose arm, and +2 in the music arm ($p = 0.008$). They concluded that both glucose and music were safe and effective in limiting pain increase when compared to standard care during heel lances in preterm infants [87].

Although methodological limitations exist, results of published studies suggest that music may be useful in reducing procedural pain in neonates. If music is used, it should probably not be provided for longer than 15 min per intervention due to the risk of sensory overload [88].

Conclusions

As stated in the introduction, the alleviation of pain is a basic need and human right regardless of age. Thus, the prevention and treatment of neonatal pain is essential. Procedural pain is the principal source of pain in sick or preterm neonates. These neonates experience numerous heel sticks, tracheal aspirations,

venous and arterial punctures, gastric tube placements, and tracheal intubations. Epidemiological studies still show the need to improve procedural pain management in neonates. Several nonpharmacological interventions are effective in reducing procedural pain in neonates. These interventions are simple, feasible, and accessible and can be easily given by those caring for neonates. Using these interventions may also be cost-effective because they involve minimal effort and time and may reduce or, in some instances, obviate the need for analgesics. Nonpharmacological interventions are also effective adjuncts to pharmacological pain management, and they should be combined as frequently as possible. Nonpharmacological interventions alone should be used for only minor invasive procedures. For more invasive procedures, potent pharmacological analgesics must be used.

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Sensorial Saturation and the 3Ts Rule

13

C.V. Bellieni and G. Buonocore

A heel prick cannot be an automatic procedure. Patients should always be protagonists of their cure even when they are small and nonverbally autonomous, and their parents should never be discouraged from participating in these procedures. Some exceptions are obvious, but the main rule in procedural treatments is "involving the patient" [1–5].

Guidelines for the management of neonatal pain have been suggested [6–9], especially in connection with blood sampling, which is often performed by heel prick. To avoid the drawbacks of general and topical analgesics [10–15], nonpharmacologic methods of analgesia have been proposed. These include nonnutritional sucking [15] and instillation of glucose or other sweet liquids on the tongue [16, 17]; glucose is supposed to provide analgesia by stimulating incretion of β-endorphins [16, 18–20] through a preabsorption mechanism [21]. However, although the methods used have reduced the signs of pain perception, they have not eliminated them [22–27].

All these procedures are indeed far from being completely analgesic and are far from involving the patient and their parents, as far as their state allows it.

To this aim, we developed a nonpharmacologic system to produce analgesia in newborns during minor invasive procedures [28, 29]. It consists in giving stimuli (tactile, auditory, olfactory, and visual) during a painful minor procedure. These stimuli compete with the pain transmission to the central nervous system, and for this reason, we call it "sensorial saturation" (aka "sensory saturation" or "multisensory stimulation"). We have shown that these stimuli are ineffective without the analgesic effect of oral sugar, but, when added to it, they greatly increase the analgesic effect of an oral sweet solution. These three types of stimuli can be resumed in the 3Ts rule: using taste (oral sugar), touch (massage), and talk (speaking to the

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baby to obtain distraction). The "perfect moment" to perform the acute painful procedure is achieved when we see that using the 3Ts the baby fixes his/her sight and sucks rhythmically (Fig. 13.1).

The main explanation of this effect is the so-called gate control theory [30], according to which the brain is not a passive receiver of nociceptive input but can influence the information received, deciding whether or not it is important enough to record. Stimulation of sensory channels prevents nociceptive nerve impulses from getting through [31–33]. We studied this technique in 17 premature babies for whom it was clinically necessary to perform a heel prick five times. To determine which analgesic method was the most effective, we used a different one each of the five times. The order in which the different methods were used was randomized. Either no analgesia was attempted (control sample) or sucking, oral glucose with or without sucking, and sensory saturation were used for analgesic effect. The babies were filmed during the procedure. The Premature Infant Pain Profile (PIPP) was used to score pain as it is precise and takes into account gestational age, wakefulness, oxygen saturation, heart rate,

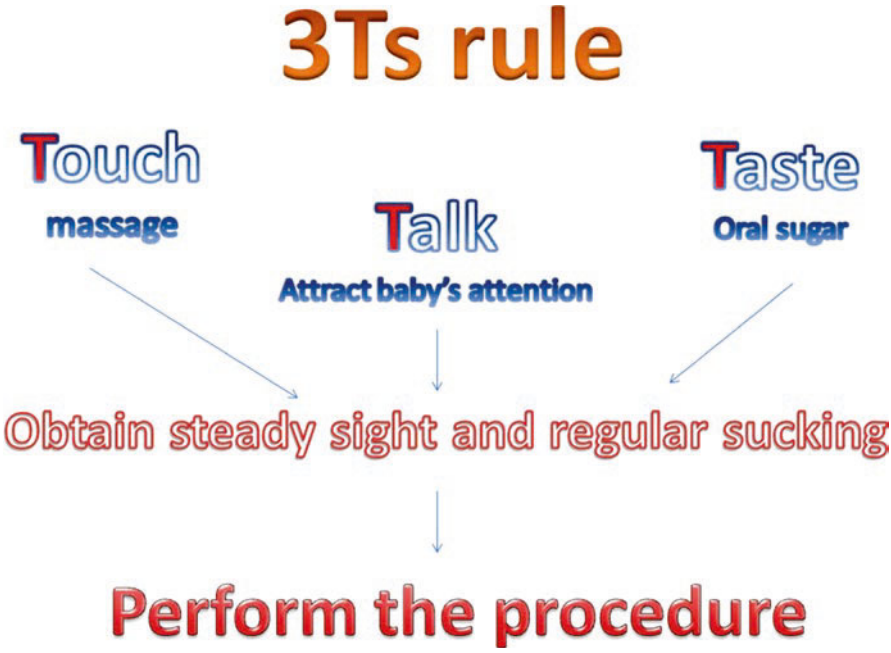

Fig. 13.1 Sensorial saturation: the procedure

and facial expressions. Without glucose, pain scores were high, but glucose alone had little analgesic effect. Glucose plus sucking was associated with a significantly lower pain score. However, with sensory saturation the babies did not feel pain. Glucose plus sucking reduced pain with respect to the control but did not eliminate it. We knew this from the literature: babies sucking sugar solution cry less, but they still cry a lot. In 1996, Abad showed that after oral administration of glucose during blood sampling, babies cried for 20 s during the 3-min observation period [22]. We repeated our study in 120 term babies and saw that with sensory saturation, term babies cried for an average of 2.8 s throughout the heel prick procedure [28].

We also investigated the increase in intracranial pressure during acute pain and whether it was modified by sensory saturation. The instrument we used to measure intracranial pressure was the tonometer that oculists use to measure eye pressure. Measuring intracranial pressure by applying the tonometer to the anterior fontanel was validated in 1982 to assess intracranial pressure in babies with cranial drainage [34]. We studied 51 premature babies: one group was studied during blood sampling from the external jugular vein, a second during heel prick, and a third during heel prick with the aid of sensory saturation [35]. We measured intracranial pressure before and during the various samplings to see how much it increased: sensory saturation almost completely canceled out this increase. Sensory saturation is effective also in the case of intramuscular shots [36].

Sensory saturation is not complex: when correctly explained, the 3Ts rule is easily learnt. Some examples can be seen at URL: http://www.euraibi.com. We recently showed that it can be easily performed by mothers with 5-min training as effectively as by experienced nurses [37]. It is worth remembering that the sign that the baby is ready to receive the prick without pain is his/her having rhythmic sucking, a sign that relaxation and distraction have been achieved.

Nowadays, sensory saturation entered international guidelines in many countries [38, 39].

The main message of using sensory saturation is that the baby should be cared for, even during a routine procedure, because the word “routine” is a misnomer, when dealing with babies. Talking, administering sugar, and massaging should not be optional: they suppress pain and also are a human and holistic way to treat the baby.

As sensory saturation is more effective than oral sugar solution or sucking, it should be implemented as well as other methods which have shown their analgesic effectiveness (e.g., breastfeeding): newborns need not merely a drug or a “good technical procedure” during a painful event but a human presence that accompanies, distracts, and comforts them (Fig. 13.2).

Fig. 13.2 Performing sensorial saturation

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Surgical Use of Analgesic Drugs

14

A.M. Guadagni and L. Manganozzi

14.1 Introduction

Pain is the most common complaint when a patient presents to a physician. Pain management in neonates warrants special consideration because the present knowledge of developmental neurophysiology is improving every day. Neonates are a special group of the population where a fine balance between optimal pain relief and adverse drug effects is greatly important. With the advancement of various surgical techniques and improved perioperative care, an increasing number of sick neonates undergo surgery and optimal perioperative pain management may improve clinical outcomes in these neonates. The neurophysiology of neonatal pain perception, long-term effects of suboptimal pain relief, and role of various drugs and techniques used in acute perioperative pain management in neonates are crucial for the care of surgical neonates, for clinical decisions, and for analgesic selection/dosing processes in order to maximize analgesia and minimize adverse effects.

14.2 Development Neurobiology of Pain

Until the 1980s, it was considered that the newborn was not able to feel pain, a belief mainly attributed to the incomplete myelination of the nervous system [1]. It is now known that the process of myelination in some nerve fibers occurs in the womb and in others occurs only after birth. Moreover, we know that there are also unmyelinated fibers between the fibers that transmit the painful stimulus at any age. It is also known that the incomplete myelination of nerve fibers reduces, but does not eliminate, the speed of conduction of the painful stimulus compared to

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the situation at later ages, and the decrease in speed of conduction is offset by the short length of such fibers. It is now established that, during embryonic and fetal development, the cutaneous sensory receptors appear between the 7th week (perioral region) and the 15th week (abdominal region) of gestational age [2, 3]; the spinal reflex arc, in response to a harmful stimulus, appears during the 8th week of gestational age [4]; and the nociceptive neurons appear in dorsal root ganglia from the 18th week of gestational age [5]. It is also well known that the connections between the thalamus and cerebral cortex are formed between the 20th and 22nd week of gestational age [6], although the real extension of the fibers up to the cerebral cortex begins in the fetus at 24 weeks [7]. From this gestational age onward, both the number of these thalamocortical fiber connections and their function progressively increase, through myelination and mutual synaptic interconnection [8]. This development is led by both genetic factors and sensory stimulation [9]. So, the early perception of pain, especially intense and repetitive pain, causes real anatomical changes with obvious functional consequences, such as the phenomenon of hyperalgesia, which in any case can aggravate the condition or compromise the development of the central nervous system and, to be more precise, compromise the future perception of pain, the associated behavior, and the responses to painful stimuli. Preterm babies undergoing patent ductus arteriosus ligation mount a substantial stress response to surgery under anesthesia, and prevention of this response by fentanyl is associated with an improved postoperative outcome [10]. In full-term infants, the pain experienced during circumcision as newborns influences the pain responses to vaccination as infants. Uncircumcised infants had lower pain responses compared with infants who were circumcised with topical anesthetic, whose pain responses were lower than those who were circumcised without analgesia, suggesting long-lasting effects of pain experienced in early life [11].

14.3 Preoperative Issues

An appropriate pain management plan should be formulated in the preoperative visit that should include detailed history (gestational age, significant events at birth, e.g., asphyxia, meconium aspiration, Apgar score, ventilatory support) and physical examination. Patient's present clinical conditions (hydration status, feeding tolerance), presence of other coexisting medical illness, nature of the surgical procedure to be done, and the area where the neonate will be managed in the postoperative period should be taken into consideration. The information is to be communicated to the parents to minimize their anxiety. Unnecessary laboratory investigations should be avoided to minimize pain associated with invasive procedures. Fasting period beyond the stipulated guidelines should not be extended to avoid unnecessary discomfort. For blood sampling, the heel is preferable, as it is less painful, and mother should be encouraged to

breastfeed the baby whenever feasible or sucrose solution should be used. Topical anesthesia or morphine alone is insufficient for lancinating pain. However, a topical local anesthetic cream may be used during venous/arterial puncture and insertion of peripherally inserted central catheter in neonates aged more than 26 weeks and it is safe in single dose [12].

14.4 Assessment of Pain in Neonates

Because preverbal age children are not able to vocalize, the anesthesiologist has to rely on behavioral and physiological markers of acute pain. Various reliable pain measures exist to assess pain in full-term and preterm neonates. Behavioral indicators of pain (e.g., crying, facial activity, body language, complex behavioral responses) and physiological indicators of pain (e.g., changes in heart rate, respiratory rate, blood pressure, oxygen saturation, vagal tone, palmar sweating, and plasma cortisol or catecholamine levels) can be used to assess pain in neonates as already discussed in Chap. 10.

14.5 Postoperative Pain Management

Neonates undergo a variety of surgeries ranging from simple herniotomy to major thoracoabdominal surgery. The analgesic regimen should also vary according to the severity of surgical trauma and depends on where the baby is being managed in the postoperative period.

The options of postoperative pain management range from simple analgesics such as paracetamol to central neuraxial block such as caudal or epidural blocks. However, an anesthesiologist should remember that a neonate is not a "small child." There is immense anatomical and physiological uniqueness in a neonate that affects the pharmacodynamics and pharmacokinetic characteristics of drugs to a considerable extent.

14.6 Systemic Analgesia in Neonates

14.6.1 Paracetamol

Paracetamol is long being known as an effective analgesic in pediatric populations [13]. Its efficacy in mild to moderate pain in neonates is now well documented. For mild to moderate pain, paracetamol may be used via the oral or rectal route; however, in cases with severe pain, it may be used for its opioid-sparing effects and its opioid-sparing effects in neonates are recently documented [14]. Although rectal paracetamol has a bioavailability almost similar to the oral formulation in

neonates, many factors regulate ultimate absorption and at times it may be erratic. Paracetamol can be administered intravenously as its prodrug propacetamol, which is hydrolyzed very rapidly by plasma esterase to paracetamol even in neonates. Paracetamol is an inhibitor of prostaglandin (PG) synthesis in the central nervous system and also acts peripherally by blocking impulse generation within the bradykinin-sensitive chemoreceptors responsible for the generation of afferent nociceptive impulses. Paracetamol may also inhibit substance P-mediated hyperalgesia and reduce nitric oxide generation involved in spinal hyperalgesia [15]. Absorption of paracetamol is slower in neonates, probably due to a sluggish and prolonged gastric emptying [16].

The hepatic enzyme systems responsible for the metabolism of paracetamol are incompletely developed in neonates. Preterm neonates have lower plasma albumin concentration that may give rise to a higher plasma concentration of free paracetamol. Total body water is higher in lower gestational age and more water is distributed in the extracellular space. The volume of distribution (Vd) of paracetamol may be greater with lower gestational age [17]. However, the higher Vd in preterm infants is of minor significance and is unlikely to influence the loading dose. The clearance of paracetamol is lower in neonates, particularly in preterm babies, and in addition, multiple doses of paracetamol should be given with a longer time interval (8–12 h), or the total daily doses should be lowered to prevent progressive increasing of plasma concentrations. The plasma concentration of paracetamol should be 10–20 mg/L to achieve antipyretic and analgesic effects [13]. The dosing regimen suggested by Allegaert et al. is the most widely used and is also recommended in a recent review [18].

The reserves of glutathione needed for detoxification of the toxic metabolic intermediate from paracetamol (N-acetyl-p-benzoquinone) may be depleted after repeated therapeutic doses. The metabolic activation of paracetamol is a prerequisite for hepatotoxicity. Neonates can produce these potentially hepatotoxic metabolites, but there are suggestions of a lower activity of cytochrome P450 in neonates [19]. This may explain the resistance to paracetamol-induced hepatotoxicity seen in neonates. However, at present, the use of intravenous paracetamol in preterm neonates with a PCA of less than 32 weeks may not be justified before further pharmacokinetic/pharmacodynamics studies are conducted [20].

14.6.2 Nonsteroidal Anti-Inflammatory Drugs

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a heterogeneous group of drugs having antipyretic, analgesic, and anti-inflammatory effects. They act by reducing PG biosynthesis through inhibition of cyclooxygenase (COX), which exists as two major isoforms (COX-1 and COX-2). The PGs produced by the

COX-1 isoenzyme protect the gastric mucosa, regulate renal blood flow, and induce platelet aggregation. The anti-inflammatory effects of NSAIDs are thought to occur primarily through inhibition of the inducible isoform, COX-2. The NSAIDs are well established as a part of multimodal analgesia in older children. Use of perioperative NSAIDs is associated with less opioid consumption and postoperative nausea and vomiting [21]. However, similar robust data in neonates are lacking until today.

Ibuprofen is used in neonates for closure of patent ductus arteriosus, and it is more effective with a lower incidence of adverse effects compared to other NSAIDs. Ibuprofen clearance is reduced in neonates with a prolonged elimination half-life of around 30 h in both preterm and term neonates. The NSAIDs, as a group, are weakly acidic, lipophilic, and highly protein bound (e.g., ibuprofen 98.7%). Ibuprofen use may alter bilirubin binding to albumen and should be avoided in jaundiced premature neonates. Caution must be applied to dosing regime and intervals in neonates (e.g., 5 mg/kg at intervals of 12 or 24 h) with increased vigilance for renal dysfunction and gastric bleeding.

Recently, ketorolac is successfully used in neonates [22]. It has an effective analgesic at a dose of 1 mg/kg without any clinical and biochemical adverse effects on the renal, hepatic, or hematological system [23]. Intravenous ketorolac appears to be safe when used in infants less than 6 months of age with biventricular circulations following cardiothoracic surgery, but it does not decrease the use of standard analgesic therapy [24]. However, infants younger than 21 days and less than 37 weeks' completed gestational age are at significantly increased risk for bleeding events and should not be candidates for ketorolac therapy [25]. In the absence of prospective RCTs, routine use of NSAIDs in neonates cannot be recommended at this time.

14.6.3 Opioids

Opioids are the mainstay of pain management following a major surgery even in neonates. Morphine is the most commonly used opioid in the postoperative period; however, fentanyl is also being increasingly used. Opioids exhibit narrow therapeutic window between analgesic doses and the dose that may cause respiratory depression. Analgesia from opioid is mediated by spinal or supraspinal activation of opioid receptors, leading to decreased release of neurotransmitters from nociceptive neurons inhibiting the ascending neuronal pain pathways and altering the perception and response to pain [26]. Opioid receptors also exist outside the central nervous system in the dorsal root ganglia and on the peripheral terminals of primary afferent neurons [27].

Neonates receiving opioids should have continuous pulse oximetry monitoring and should be managed in a setting in which rapid intervention for airway

management is possible, because respiratory rate monitoring alone may be an inadequate predictor of impending apnea [28].

In contrast to the benefits of opioids in neonatal surgery, recent studies suggest the potential neurotoxicity of anesthetics and analgesics on the developing brain. Studies in neonatal rats (days 1–7 after birth) found adverse long-term effects of morphine in the adult rats including retarded motor development and decreased brain metabolism [29]. Opioid signaling modulates cell cycle progression of neuronal and glial progenitor cells in the developing cerebral cortex in vivo [30]. In vitro studies show apoptosis of human neurons and microglial cells after morphine exposure, which was blocked by naloxone, indicating an opiate receptor mechanism [31]. Morphine dosing also displays a specific effect on hypothalamic nuclei with detrimental effects on pituitary hormone release and thyroid function [32]. These results suggest harmful effects of morphine on neurogenesis in newborn babies, but evidence from human trials is nonexistent. A cohort of preterm neonates (less than 34 week of gestational age) was assigned randomly to three groups—morphine infusion, pancuronium only, and morphine + pancuronium to facilitate mechanical ventilation. All groups were evaluated for intelligence, motor abilities, and behavior at 5–6 years of age. There were no differences between groups, revealing no detrimental effects of morphine on neurocognitive behavior [33]. Another recent study investigated the effects of morphine versus placebo infusions administered in the first 3 days of life in neonates of less than 32 weeks of gestational age on intelligence, visual motor integration, behavior, chronic pain, and health-related quality of life at the age of 5 years. Although a trend toward a more negative outcome was associated with more morphine in the first 28 days of life, a statistically significant association existed between worse performance on the "visual analysis" subtest of the IQ test and neonatal morphine consumption [34]. Of note, the centrally acting a-2-adrenergic agonist, dexmedetomidine, used as an analgesic prevented neurodegenerative apoptosis in immature brains of rodents [35]. This suggests that reduced opioid use and the use of analgesic adjuvants like dexmedetomidine may provide a neuroprotective effect in neonates. Safety and efficacy were assessed in the general NICU population; available studies describe dexmedetomidine use after CV surgery and for procedural sedation.

Hypotension and bradycardia, two of the most clinically significant adverse effects associated with dexmedetomidine, may limit its use in hemodynamically unstable patients in the ICU [36]. These adverse effects are not as well described in neonatal patients. Further studies are necessary for routinary use in neonate postsurgery pain.

14.6.4 Fentanyl

Fentanyl is almost 100 times more potent than morphine and is considered as a selective m-receptor agonist. Fentanyl has a rapid, predictable onset of action with

a short duration of action mostly due to its high lipid solubility. It is associated with greater hemodynamic stability [37].

Fentanyl may be the preferred analgesic agent for critically ill patients with hemodynamic instability and patients with symptoms related to histamine release during morphine infusion [38].

However, fentanyl may be associated with rapid development of tolerance [39, 40] and chest wall rigidity [41]. All metabolites of fentanyl are inactive and a small amount of fentanyl is eliminated by the renal route without metabolism. Fentanyl clearance can be impaired by decreased hepatic blood flow (e.g., from increased intra-abdominal pressure) in neonates after major abdominal surgery [42]. The clearance of fentanyl is immature at birth but increases dramatically thereafter. Fentanyl clearance is 70–80% of adult values in term neonates and, standardized to a 70-kg person, appears to reach adult levels within the first 2 weeks of life [43]. Fentanyl has been shown to effectively prevent preterm neonates from surgical stress responses and to improve postoperative outcomes [44]. Fentanyl may be superior to morphine for short-term postnatal analgesia in newborn infants [45]. Fentanyl may be used as bolus and/or as an intermittent dosing of 0.5–2.0 μg/kg or as a 0.5–2.0 μg/kg/h [46].

14.6.5 Morphine

Morphine is the gold standard opioid with which all other opioids are compared and it is the most thoroughly investigated opioid in neonates. Morphine is water soluble and its solubility in lipids is poor compared with other opioids. Although morphine can also act on k-opioid receptor subtypes [47], its analgesic effect is caused mainly by an activation of m-receptors. Morphine alleviates postoperative pain [48], reduces behavioral and hormonal responses [49], improves ventilator synchrony [50], and may also reduce acute procedural pain [51]. Morphine is metabolized in the liver by the enzyme uridine diphosphate-glucuronosyltransferase 2B7 (UGT2B7) into morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) [52]. M3G has been shown to have higher analgesic potency than morphine and also has respiratory depressive effects. M3G has been suggested to antagonize the antinociceptive and respiratory depressive effects of morphine and M6G and contributes to the development of tolerance. Although UGT2B7 is mainly found in the liver, it is also present in the intestines and kidneys. Clinical trials studying morphine for postoperative analgesia have shown large interindividual variability in morphine plasma levels and a wide range of morphine requirements [53]. However, neonates, particularly those younger than 7 days [49], require significantly less morphine as they have significantly higher plasma concentrations of morphine, M3G, and M6G and significantly lower M6G-to-morphine ratio than the older children [54]. Moreover, morphine metabolism may be delayed during mechanical ventilation [49].

In the postoperative period, morphine can be used as either continuous infusion or intermittent bolus [55, 56]. However, the relative safety and efficacy of either method is controversial. The use of morphine in the NICU for postoperative pain is not free from adverse outcomes. In a spontaneously breathing neonate, obviously the most important adverse effect is respiratory depression [57], but most neonates after major surgical procedures are mechanically ventilated. Respiratory depression may occur at plasma morphine concentrations of 15 ng/mL, and when measured by carbon dioxide response curves or by arterial oxygen tension, similar results are obtained in children from 2 to 570 days of age at the same serum morphine concentration [58].

In mechanically ventilated neonates, the important adverse effects are hypotension [59], prolonged requirement of ventilation, urinary retention, decreased gastrointestinal (GI) motility, and risk of necrotizing enterocolitis [60] and may be long-term neurobehavioral abnormalities as some animal data indicate. At times, morphine may not provide adequate analgesia for short painful procedures [61]. However, continuous infusions of morphine do not increase the vulnerability of ventilated preterm neonates to early adverse neurological events, except in neonates who are hypotensive before morphine therapy or those receiving doses higher than 10 mcg/kg/h [62]. Intravenous morphine boluses should be used with caution in preterm neonates. The routine use of morphine infusions as a standard of care in preterm newborns who have received ventilatory support is not recommended and is not associated with better neurologic outcomes [63]. Also routine morphine infusion in the ventilated newborns is not recommended [64]. However, there is not any difference in mortality, duration of mechanical ventilation, and short-term and long-term neurobehavioral abnormalities but a delayed oral feeding. The main argument against the treatment of neonatal pain with opioids is the uncertainty about their side effects [65].

Animal studies on long-term effects of neonatal opioid use do not provide enough insight and data from the human studies are even sparser. A study [66] in 2009 evaluated the effects of cumulative procedural pain and morphine exposure with subsequent growth and development and found that greater overall exposure to intravenous morphine was associated with poorer motor development at 8 months, but not at 18 months' corrected chronological age. A recent pilot study [67] also concluded that morphine analgesia for procedural pain in preterm neonates may be associated with delayed growth and development. By contrast, in 2005, Grunau et al. [68] found that repeated neonatal procedural pain exposure among preterm infants was associated with downregulation of the hypothalamic and pituitary and adrenal axis, which was not counteracted with morphine. In another recent prospective observational study [69], it was found that repetitive procedural pain in preterm infants during a period of physiological immaturity appears to impact postnatal growth and development.

14.6.6 Tramadol

Systemic tramadol use in neonates and infants is limited because disposition data in young infants are not available. It is primarily metabolized into O-desmethyltramadol (M1) by CYP2D6. The active M1 metabolite has a mu-opioid affinity approximately 200 times greater than tramadol. Tramadol clearance is reduced in premature neonates but rapidly matures to reach 84% of the mature value by 44 weeks postmenstrual age. A target concentration of 300 mcg/liter is achieved after a bolus of tramadol hydrochloride 1 mg/kg and can be maintained by infusion of tramadol hydrochloride 0.09 mg kg/h at 25 weeks, 0.14 mg kg/h at 30 weeks, and 0.18 mg/kg/h at 40 weeks postmenstrual age [70]. The impact of CYP2D6 polymorphism on the variability in pharmacokinetics, metabolism, and pharmacodynamics of tramadol remains to be established. It should be noted that the current license for the use of tramadol is 12 years, and it is not advocated for use in children <1 year unless discussed with the pain service.

14.6.7 Codeine

Codeine is an oral prodrug of morphine and has been commonly used in neonates (at doses of 0.5 mg/kg 6 h), but a significant proportion of neonates cannot metabolize codeine to its active metabolite, morphine [71]. Conversely there may be the occasional neonates that are an ultra-rapid metabolizer (CYP2D6), and this can result in increased morphine production and adverse effects.

Concerns of respiratory depression and death in older children given codeine after tonsillectomy have resulted in reduced use [72] of this drug and license change to 12 years and 18 years for tonsillectomy. Therefore, is not advocated for use in neonates.

The most common analgesic used for pain management in non-ventilated neonates is summarized in Table 14.1.

Table 14.1 Common analgesic used for pain management in non-ventilated neonates

Drug	Route	Dose by post conceptual age			Interval/max dose
Paracetamol	PO/PR	28–32 wks	Loading	15 mg/kg	12 h (30 mg/kg/day)
			Maintenance	15 mg/kg	
		32–53 wks	Loading	oral 20 mg/kg rectal 30 mg/kg	8 h (60 mg/kg/day)
			Maintenance	20 mg/kg	
	IV	<10 kg or 1y		7.5 mg/kg	8 h (30 mg/kg/day)
Ibuprofen	PO	40–44 wks		5 mg/kg	12–24 h
Morphine	IV	Term-44 wks		0.025–0.05 mg/kg/h	Continuous 24–48 h

14.7 Local Anesthetic (LA) Techniques

14.7.1 Topical Local Anesthetic Creams

Topical local anesthetic creams reduce acute pain from venipuncture, venous cannulation, and attenuate physiological response to circumcision. EMLA (eutectic mixture of prilocaine and lidocaine) can cause methemoglobinemia (increased skin absorption due to thin epidermis and increased fetal hemoglobin that has a greater sensitivity to prilocaine) and vasoconstriction, whereas AMETOP (amethocaine gel) does not cause vasoconstriction and has a longer duration of effect.

14.7.2 Local Anesthetic Infiltration, Peripheral Nerve Blockade, and Central Neuraxial Blocks

Local anesthetic infiltration, peripheral nerve blockade, and central neuraxial block have an important role in the treatment of acute postoperative pain or procedures in neonates. The aims are to provide intraoperative nociceptive blockade that reduces anesthetic requirements and residual effects of anesthesia in the postoperative period. Postoperative analgesic requirement for opioids can be avoided or reduced lessening risks of respiratory depression and oversedation. There may be some increased risks to neonates given epidural infusion analgesia, particularly in institutions where fewer than 200 epidurals per year are performed. However, large-scale audits of both central blockade and peripheral local anesthetic techniques have demonstrated an impressive safety profile [73]. Commonly performed nerve blocks are penile block for circumcision, ilioinguinal/iliohypogastric nerve blocks for herniotomy, and intercostal blocks for chest drain insertion. Single injection caudal epidural block is very effective for sub-umbilical surgery and can provide pain relief for 6–8 h. Additives to caudal local anesthetic solutions such as clonidine, ketamine, or opioids are not recommended in neonates. For continuous epidural infusions, there is an increased risk of local anesthetic toxicity in neonates due to reduced hepatic clearance of amide local anesthetic. Infusion rates should be half that of older children. Bupivacaine has been superseded by levobupivacaine or ropivacaine in many centers because these have a lower propensity to produce cardiovascular depression and seizure activity in overdose or after intravascular injection. The most important prerequisite of an epidural block is that the tip of the epidural catheter should be situated at an intraspinal level that corresponds to the dermatome center of the surgical procedure. Caudal bolus injection of 3 mg/kg ropivacaine or a continuous epidural infusion of 0.2 and 0.4 mg/kg/h of the same drug was clinically effective and did not result in excessive plasma levels of the drug [74]. It was found that all the children who had systemic toxicity had infusion rates in excess of 0.5 mg/kg/h of racemic bupivacaine [75]. In a recent review, a maximum bolus

dosage of 1.5–2.0 mg/kg followed by an infusion of 0.2 mg/kg/h was recommended; in addition, this should only be continued beyond 48 h when considerable benefits exist [74].

14.7.3 Regional Anesthesia/Analgesia

Although single injection subarachnoid block (SAB) is the commonly performed regional technique in adults and usually provides immediate postoperative analgesia, SAB is of little use in neonates for postoperative analgesia due to its limited duration of action in this age group.

14.8 Epidural Anesthesia in Neonates: Risk Versus Benefits

Epidural analgesia has been investigated as a modality of pain relief after major surgeries. There is only one RCT [76] that has directly compared the safety and efficacy of epidural analgesia with systemic opioid administration after a major surgery in neonates. The authors reported a faster return of intestinal function and less incidence of pneumonia in neonates who received epidural analgesia. In a small RCT [77] compared the benefits of combined spinal and epidural anesthesia with general anesthesia in neonates undergoing GI surgery. The authors found a significantly less pulmonary complication and more cardiovascular stability in the regional anesthesia group in the postoperative period. Somri et al. [78] reported that combined spinal and epidural anesthesia could be considered as an effective alternative to general anesthesia in high-risk neonates and infants undergoing upper GI surgery when cautiously used by a pediatric anesthesiologist. The use of lumbar/thoracic epidural analgesia in major abdominal surgeries in neonates was associated with a low risk of complication and advantages of reduced need for intraoperative muscle relaxants and opioid analgesics and postoperative ventilatory support [79]. Furthermore the use of continuous epidural analgesia in small infants (1400–4300 g) undergoing major surgery is safe [80]. Neuraxial blockade was not found to be associated with hypotension or hemodynamic instability even in neonates with congenital heart disease [81].

Regional analgesia may also have respiratory stimulant action [82] and has been associated with reduced need for mechanical ventilation. Surgical stress response is more effectively mitigated by regional anesthesia in comparison with systemic opioid, and it is also free of immunosuppressive effects of opioids [83, 84]. The most important consideration in central neuraxial block in neonates is the safety and possibility of inadvertent injury to the developing spinal cord. Serious complications including neurologic injury have been reported in neonates [85], and only experienced pediatric anesthesiologists should perform central neuraxial block in neonates.

14.8.1 Epidural Catheter Insertion: Thoracic Catheter Position Through Caudal Route

Although thoracic epidural catheters have been described in neonates [86], its routine use at this moment cannot be advocated. However, relative fluidity of the epidural fat in neonates and young infants allows advancement of thoracic catheter inserted through the caudal or lumbar route (L3-L4 interspace). It was described [87] the successful insertion of an 18-G epidural catheter up to the thoracic level. A thicker gauge catheter is easier to advance but has a higher possibility of neural damage [88, 89]. There are numerous ways of confirming epidural catheter position, including X-ray [90], electrocardiography [91], ultrasonography [92], and even transesophageal echocardiography [93]. The lumbar epidural route is less preferred in neonates [94], and there are reports of paraplegia due to intraspinal hematoma during attempted lumbar epidural block [95].

The advent of ultrasound may be especially useful in neonates as the ossification of the vertebral column is reduced and the cord structures may be better visualized [96].

14.9 Most Common Surgical Procedures in Neonates

14.9.1 Tracheoesophageal Fistula/Esophageal Atresia

Tracheoesophageal fistula/esophageal atresia (TEF/EA) is a relatively common congenital malformation occurring in 1:3000–4500 live births [97]. Commonly TEF is of five types (A–E), with type C being the most common. It is commonly diagnosed in the delivery room when suction catheter cannot pass from mouth to stomach. Infants with TEF are premature (20–30%) and they have a high incidence of congenital heart disease and other anomalies. Anesthesia for bronchoscopy, intubation, and TEF repair can be induced by IV agents, inhalational agents, or combination of both the techniques with additional use of local anesthetics and opioids. Fentanyl may be used intraoperatively and as continuous infusion for postoperative analgesia. Paracetamol can also be given rectally or IV for postoperative analgesia. A caudal catheter can be advanced to T6–T7 to supplement the general anesthesia (isoflurane/sevoflurane/desflurane/air/oxygen) and provide excellent postoperative analgesia without use of opioid and to facilitate extubation. Local anesthetic clearance is reduced in neonates [98]. Maximum dose of local anesthetic is to be reduced and the duration of infusion should be maximum up to 48 h postoperatively [99]. Local infiltration, intercostal block, paravertebral blockade, or intrapleural infusion of local anesthetics can be considered [97].

14.9.2 Omphalocele/Gastroschisis

Both omphalocele and gastroschisis look similar but are different due to defects of abdominal wall. The incidence of omphalocele is 1:6000 live births, whereas the incidence of gastroschisis is 1:15,000 live births [100]. In omphalocele, there is a midline defect and is associated with other anomalies, whereas gastroschisis is not. In both the cases, large fluid resuscitation is required before, during, and after surgery. Anesthetic maintenance can include fentanyl in addition to inhalational agent as the increased intra-abdominal pressure and diaphragmatic elevation reduces respiratory compliance and makes extubation inadvisable [101]. Nitrous oxide should be avoided. Maintenance of body temperature is essential. Continuous epidural injection provides analgesia and motor blockade without respiratory depression and may reduce the postoperative ventilation [102].

14.9.3 Pyloric Stenosis

The incidence of pyloric stenosis (PS) is 3:1000 live births [102]. Symptoms are apparent from 2nd to 6th week of life. Neonates have severe non-bilious vomiting with resultant hypochloremic dehydration. Before surgery, measurement of electrolyte and correction of hypovolemia and alkalosis can be done. Nitrous oxide is avoided. Maintenance of anesthesia is by inhalational agent with remifentanil [101]. Local infiltration technique can be used for the operation also [102]. Postoperatively wound infiltration, rectal acetaminophen, and ketorolac are very useful [101]. Awake intubation is safer.

14.9.4 Necrotizing Enterocolitis/Intestinal Obstruction

Necrotizing enterocolitis (NEC) is primarily seen in premature [preterm (less than 32 weeks) and low birth weight (less than 2 kg)] infants, whereas intestinal obstruction manifests in 2nd to 6th week of life with incidence 1:2000 [102]. NEC occurs as a result of bowel ischemia and hypotension due to poor cardiac output state, infection, and others, whereas intestinal obstruction is due to congenital malformations, such as duodenal/jejuna atresia, Ladd band, rotations, and others. These emergencies share the same clinical picture with abdominal distention, hypotension, coagulopathy, sepsis, dehydration, and electrolyte imbalances [101]. Ketamine 4 mg/kg/h combined with fentanyl 10–30 μgr/kg and a muscle relaxant can be administered [102]. Inhalational agent can be used with caution; nitrous oxide is contraindicated. Light general anesthesia and epidural analgesia are contraindicated due to sepsis and coagulopathy in NEC. However, this may be considered in intestinal obstruction to avoid the need for postoperative ventilation.

Postoperative management may require meticulous fluid management, inotropic and ventilator support, and antibiotics. Sometimes neonates are managed with placement of an abdominal drain percutaneously in the neonatal ICU with IV analgesia/sedation [101].

14.9.5 Congenital Diaphragmatic Hernia

The incidence of congenital diaphragmatic hernia (CDH) is 1:2500 live births [100]. Herniation of abdominal viscera into thoracic cavity leads to pulmonary hypoplasia due to compression by the viscera on developing lungs. To improve ventilation high-frequency ventilation, extracorporeal membrane oxygenation (ECMO), nitric oxide, and pulmonary vasodilators are used, but nitric oxide use is controversial [103]. Neonates who are not intubated before arrival in the OR are generally intubated awake or after rapid sequence induction. Analgesia may be administered. High inflation pressures for mask ventilation are avoided. Pentothal and fentanyl can be used. Hypoxia, acidosis, and hypothermia are avoided. Isoflurane can be used by administering it through ECMO circuit. Drug also can be given directly to patient or ECMO circuit. Patient on ECMO is heparinized [104].

14.9.6 Neonatal Circumcision

Many circumcisions are done in the awake neonate in the first few hours or days of life; this is reflected in the literature as studies have generally evaluated pain during the procedure. However, for neonatal circumcision, no single technique has been shown to reliably alleviate pain in the awake patient, which therefore presents a clinical challenge. General anesthesia should be considered for neonatal circumcision. A multimodal analgesic approach should include a local anesthetic technique at the time of the procedure in combination with sucrose and paracetamol. Postoperative pain after circumcision in the neonate has not been well investigated, and available studies have all examined pain during the procedure in awake neonates. It has been suggested that the procedure be performed in awake infants only during the first week of life as pain scores during the procedure have been shown to increase to unacceptable levels with increasing neonatal age [105]. For all techniques studied, there was a significant failure rate [106, 107]. The use of LA was superior to either placebo or simple analgesics and sucrose [106]. Dorsal nerve block appears to be superior to subcutaneous ring block or topical local anesthesia (caudal epidural analgesia has not been studied) and was associated with lower cortisol levels, but was operator dependent and not totally reliable. Efficacy of topical local anesthetic agents was very dependent on the technique of application and time allowed [12, 108]. No increased incidence of complications was seen in one technique compared with another. The duration of surgery (and therefore duration

of intraoperative pain) was dependent on the surgical technique with the "Mogen clamp" associated with faster procedures [106].

14.10 Neonatal Abstinence Syndrome (NAS)

Neonatal abstinence syndrome (NAS) is a clinical diagnosis and a consequence of the abrupt discontinuation of chronic use of opioids. Opioid withdrawal is a complex biological phenomenon. The cellular and molecular mechanisms of this process are poorly understood even in adults. The pathophysiology of opioid withdrawal is more complex in neonates as a result of immature neurologic development and impaired neurologic processing. Opioids mostly act through opioid receptors (G protein-coupled receptors μ, κ, and δ), which are extensively distributed across the central nervous system and are also located within the peripheral nervous system, gastrointestinal system, and various other systems [109]. The density and affinity of μ-receptors in neonates are as good as those in adults; however, evidence failed to show similar development of κ and δ receptors, as well as other receptors, in the neonatal brain [110]. A lack of opioids in a chronically stimulated state increases activity in the opioid receptors, leading to increased adenyl cyclase activity and cellular ionic imbalance. Ultimately, this results in the increased production of various neurotransmitters through a cascade of enzymatic activities [111]. The most important center of activity in opioid withdrawal is the locus coeruleus of the pons. This is the principal noradrenergic nucleus of the brain and is extremely sensitive to opioid status [112]. A lack of opioids causes increased production of norepinephrine [113], which is responsible for most of the signs of NAS. The ventral tegmental area of the midbrain, the storage center of dopamine, releases decreased dopamine during opioid withdrawal [114, 115]. Opioid withdrawal also causes decreased serotonin expression in the dorsal raphe nucleus [116, 117], causing sleep disturbances in neonates undergoing opioid withdrawal. Opioid deficits also affect the functioning of the autonomic and peripheral nervous systems, as well as the gastrointestinal system. Opioid deficits cause increased production of multiple neurotransmitters, such as acetylcholine, during withdrawal phase [118]. Opioid withdrawal may activate the hypothalamic-pituitary-adrenocortical axis, leading to increased corticotrophin release [119]. Further, opioid withdrawal may be associated with hyperalgesia [120].

At presentation, signs of NAS usually include tremors, irritability, excessive crying, and diarrhea. Occasionally, seizures also are present. Central nervous system signs, including irritability, jitteriness, tremors, and excessive crying, usually appear first. Hyperirritability, which is a hallmark of this syndrome, can lead to agitation, difficulty sleeping, and inconsolable crying. Tremors, exaggerated Moro reflex, hypertonia, and myoclonic jerks are more common. These can mimic seizures and an EEG may be required for confirmation. Seizures, observed in 2–11% of neonates with NAS, are a serious manifestation of withdrawal and should be treated

immediately [121]. Heart rate, respiratory rate, muscle tone, and other physiological responses to stimuli are impaired in these neonates with NAS as a result of the dysregulation and instability of the autonomic nervous system [122]. Other autonomic nervous system signs include temperature instability, sweating, sneezing, and mottling. Tachypnea, nasal flaring, and nasal stuffiness may be misinterpreted as respiratory distress in newborns. Hyperthermia, although rarely higher than 39 °C, can result in misdiagnosis as sepsis. Poor feeding, excessive motor activity, regurgitation, vomiting, and diarrhea may lead to poor weight gain in these infants. Severe diarrhea can lead to dehydration and electrolyte imbalance. Perianal skin excoriation secondary to excessive loose stools further increases irritability and agitation. Similarly, irritability and agitation may be increased by unattended skin excoriation over the face and body, which are secondary to excessive motor movements. Hyperphagia is widely recognized in infants with NAS, who may require intake of more than 150 calories per kilogram per day [123].

14.10.1 Management

Many scoring systems allow clinicians to assess the severity of NAS, but no scoring system is perfect and all the systems are subject to a strong observer variability. At present, the modified Finnegan scores remains the most common tool that is used [124]. Quantifying the severity of NAS assists in determining if and when pharmacological intervention will be needed. Scoring also assists in monitoring, titrating, and terminating therapy [125]. Scoring should be performed after feeds, at 3–4-h intervals, when the infant is awake. The score should represent the status of the infant both at the time of assessment and during the preceding time period. These scoring systems are generally useful for term neonates, but not for preterm infants. If Finnegan score is $\geq$8 after a too fast discontinuation of opioid therapeutic use, clinicians have to restore last dosage of opioid and proceed more gradually. After a 48-h period of stabilization, the infant may then be gradually weaned from medication. An algorithmic approach for the management of NAS is shown in Fig. 14.1.

Morphine is the most commonly preferred medication [124]. Morphine decreases the incidence of seizures, improves feeding, eliminates diarrhea, decreases agitation, and can control severe symptoms [126]. However, morphine treatment also prolongs the length of hospital stay. Because morphine has short half-life, it must be provided every 3–4 h. When an optimal response is not attained with the maximal dose, additional medications may be considered.

Phenobarbital is another drug of choice for NAS [127]. Although it is occasionally used as a single therapeutic agent for opioid NAS, phenobarbital is more often used as an adjunct to morphine or methadone [125, 128]. Phenobarbital does not prevent seizures at the dosage administered for withdrawal, nor does it improve gastrointestinal symptoms. Methadone is also used for the treatment of

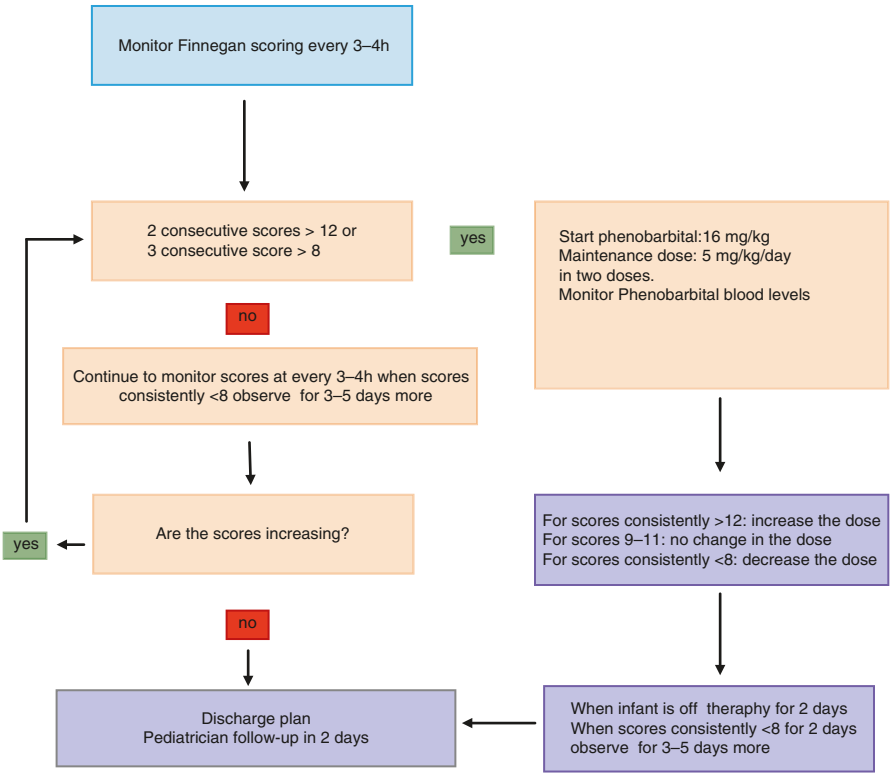

Fig. 14.1 Management plan for NAS in neonates

NAS. Methadone can be administered only twice per day; however, because of the long half-life of methadone, it may be difficult to titrate the methadone dose. The methadone dose also can be increased or decreased depending on the severity score. Caution must be exercised when methadone is used along with other medications, such as phenobarbital [129].

Buprenorphine is a new option for the treatment of NAS and must be given sublingually; however, no large-scale studies are available to support the use of this medication [130].

Clonidine, a centrally acting α-adrenergic receptor agonist, has been studied as a single replacement therapy or adjunct therapy, although the theoretical risk of hypotension and bradycardia may always prohibit increasing its dose. No large-scale studies have proven the efficacy of clonidine for NAS [131]. Clonidine and phenobarbital levels can be monitored, and both are beneficial for decreasing the duration of treatment as well as for curtailing the use of higher doses of morphine or methadone [132].

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Physical Risk Agents in Incubators

15

Renata Sisto

15.1 Noise Exposure

Noise exposure may be quantified according to the objective physical characteristics of the associated acoustic signal or according to the level of subjective loudness and annoyance reported by the listener. Both aspects are relevant to understand the effects of noise exposure on humans; therefore, a short introduction to these two complementary viewpoints seems to be useful.

15.1.1 Physical and Psychological Nature of Acoustic Noise

15.1.1.1 Physics

Any medium characterized by inertia and elasticity may propagate oscillatory waves. An acoustic wave is a perturbation of pressure and velocity field associated to spatially coherent oscillatory motion of the medium (a fluid, like air or water, a solid body, a plasma) propagating through it at the speed of sound.

In air, acoustic waves propagate pressure perturbations that are small fluctuations of the equilibrium atmospheric pressure value (e.g., the pressure fluctuation associated with the rather intense sound level of a crowded restaurant amounts to approximately one millionth of the atmospheric pressure).

Acoustic waves of frequency in the audible range (20 Hz–20 kHz) give rise to sound perception in humans. Infrasonic and ultrasonic waves do not cause auditory perception. Infrasound may be perceived as an annoying vibration, while ultrasounds may be harmful for humans.

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Acoustic noise is a stochastic signal, whose time evolution is characterized by absence of phase coherence, while its spectrum may also show a resonant shape, according to the geometry of the environment. Mathematically, due to complete lack of phase coherence, a stochastic signal is completely characterized by its autocorrelation function or, equivalently, by its power spectrum, which is the Fourier transform of the autocorrelation function.

The acoustic pressure level is expressed in dB SPL and defined as

$$L\left(dB\right) = 10\log_{10}\left(\frac{p^2}{p_0^2}\right), \tag{1}$$

where p is the acoustic pressure, expressed in Pa, and p_0 is the standard reference pressure $p_0 = 20$ μPa. The equivalent sound level is the sound level referred to the square mean value of acoustic pressure calculated over a time interval T:

$$L_{eq}\left(dB\right) = 10\log_{10}\frac{1}{T}\int_0^T \frac{p\left(t\right)^2}{p_0^2}dt. \tag{2}$$

The equivalent level L_{eq} is the level of a hypothetical constant noise that would produce, over the time interval T, the same acoustic energy as the phenomenon under consideration.

15.1.1.2 Psychoacoustics

In psychoacoustics, "noise" is a sound that is characterized by absence of semantic content and that produces annoyance in the listener.

Both the physical intensity and the semantic content of the acoustic signal contribute to the subjective sensation level of annoyance.

The subjective evaluation of the sound intensity is defined in psychoacoustics as loudness. Isophonic curves provide a representation of the psychoacoustical hearing sensitivity as a function of frequency. These curves, for each frequency, connect sound levels corresponding to the same loudness sensation of a 1 kHz tone of a given level.

The parameter commonly used to evaluate the risk associated with exposure to noise is the A-weighted equivalent sound level: $L_{A,eq}$.

The A-weighting curve $A(f)$ approaches the ear sensitivity at the sound level of 40 dB, i.e., equalizes the sound spectrum according to the different sensitivity of the ear at different frequencies at a level of 40 dB. For higher sound levels, which are relevant to the noise exposure issue, other weighting curves have been computed, yet A-weighting is commonly used independently of the noise level, as a standard practice.

The A-weighted sound pressure level is given by

$$L_{A,eq}\left(dBA\right) = 10\log_{10}\left(\sum_k 10^{0.1L_{f(k)}+A_{f(k)}}\right). \tag{3}$$

15.1.2 Effects of Exposure to Noise

15.1.2.1 Auditory Effects

High levels of noise are responsible for hearing loss. A correlation has been well assessed between noise level, exposure duration, and hearing loss [1]. International standard curves take into account also physiological hearing loss associated with aging, which is obviously not present in babies.

The auditory system damage risk starts at sound levels of the order of 78–80 dBA. The inner ear is the most sensitive part of the auditory system. A sophisticated active feedback system involving the outer hair cell (OHC) electromotility, modulated by the peripheral nervous system (at the Brain Stem level), provides active nonlinear amplification of the basilar membrane (BM) transverse vibration, which is responsible for the astounding hearing capability in terms of perception threshold and frequency discrimination. The OHC active filter amplifies the cochlear response by narrowing the bandwidth of the detector. As a consequence, low threshold and good frequency resolution are strictly related to each other.

Unfortunately, OHCs are particularly sensitive to noise exposure. Above noise levels of 100–110 dBA, acute OHC damage effects can cause permanent hearing impairment, but OHCs show generally a good capability of recovering their functionality after acute exposure, if they have enough time to do it. A more subtle risk is associated to chronic exposure to much lower levels of noise (as low as 80 dBA), which can cause permanent damage of the OHCs if recovery cannot be fully reached before the next noise exposure.

15.1.2.2 Nonauditory Effects

The effects of noise on the human health show that noise, also at low levels, interacts with the organism in a complex way giving also rise to nonauditory effects [2]. The nonauditory effects of noise such as annoyance effect can induce psychological and somatic disturbances that can interfere with personal feeling and health, interpersonal relations, and so on. The sound levels that may induce annoyance can be very low. The most important nonauditory effects are relative to cardiovascular diseases, sleep disturbances, and performances at work, while direct effects on psychopathology are still controversial.

15.1.3 Noise Sources in Incubators

15.1.3.1 Typical Noise Levels in Incubators

Noise levels in incubators from various sources under different conditions were evaluated [3] by Bellieni et al. (2003). In the following, we summarize some of the main results of this study:

Main noise sources are:

- Incubator engine (continuous)
- Opening and closing portholes (transient)
- Temperature alarm (occasional, short duration)
- Baby crying (may be frequent, unpredictable duration)

A typical background noise of 50 dBA can be found when the incubator is on. These levels are very high, compared to the background noise measured switching off the incubator: 34–36 dBA. The noise criteria adopted by the ISO to prevent annoyance recommend noise levels for bedrooms or hospitals in the range 25–35 dBA. Noise levels in the range 45–50 dBA exceed tolerability criteria established to prevent sleeping annoyance.

15.1.3.2 Reverberating and Resonant Characteristics of Incubators

As shown in Table 15.1, the highest source of noise in the incubator is the cry of the neonates themselves (noise levels above 80 dBA). The incubator is a highly reverberating acoustic environment, which amplifies the sounds produced inside the incubator itself. As a consequence, sounds originated inside the incubators produce noise levels at neonate ear which typically exceed by about 3 dB the levels which would be produced by the same source in free field.

Moreover, the geometry and size of the incubator are such that the incubator behaves as a resonating cavity for acoustic waves for several frequencies in the audible range (see Fig. 15.1). This causes development of persistent standing waves that can cause both hearing damage and annoyance.

15.1.3.3 Crying Distortion

The reverberating and resonating characteristic of the incubators is responsible for acoustic distortion phenomena. In particular, when the baby is crying, their voice

Table 15.1 Noise levels from various sources under different conditions (from [3] Bellieni et al. Biol. Neonate (2003))

	Noise level L_{eq} (dBA)		
Measurement conditions	Open hood	Closed hood without sound absorber	Closed hood with sound absorber
Background noise incubator OFF	36–37	34–36	33–35
Background noise incubator ON	46–47	48–50	48–50
Opening and closing portholes	70–71	73–74	70–71
Temperature alarm	58–59	56–57	50–51
Baby crying	81–83	84–87	82–85

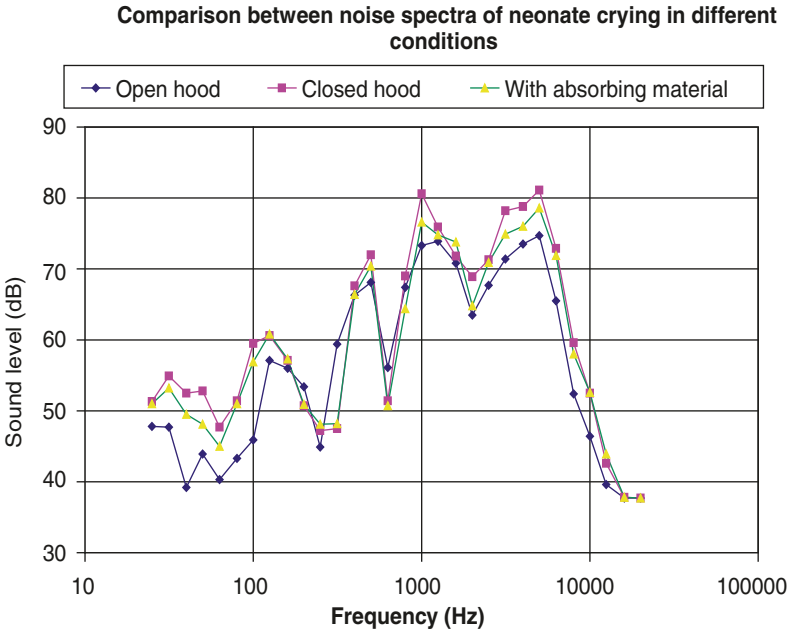

Fig. 15.1 Spectra of neonate cry inside the incubator in three different conditions. Peaks at the cavity resonant frequencies are visible (from [3] Bellieni et al. Biol. Neonate (2003))

results to be amplified and distorted at the neonate ear position. These phenomena were analyzed [3] by Bellieni et al. (2003). In their study an acoustic distortion index was calculated in each frequency band (thirds of octave) using the following expression:

$$Df = L_c - L_a, \tag{4}$$

where L_c is the sound level at the frequency f, measured within closed plexiglass walls, and L_a is the corresponding level measured without plexiglass walls.

15.1.4 Testing the Neonate Auditory System

15.1.4.1 Objective Diagnostic Tools

The neonate acoustic spectral sensitivity curve is not well established, because psychoacoustic techniques are not suitable for non-collaborating subjects such as neonates. However, objective techniques exist, from which it is possible to get information about the auditory function in neonates. Objective techniques include electrocochleography, acoustic brainstem response (ABR), steady-state ABR, and otoacoustic emissions (OAEs) measurements. Electrocochleography is a sensitive but rather invasive technique, requiring direct access to the cochlea by needle

electrodes. ABR techniques require rather long averaging times, due to the low signal level, to reach a good signal-to-noise ratio, and are perturbed by the movements of the subject. Steady-state ABR techniques have been significantly improved in the last years, but are still affected by rather large uncertainties.

OAEs are acoustic signals which can be measured in the ear canal, in presence (evoked OAEs) or in absence (spontaneous OAEs or SOAEs) of an external acoustic stimulus, as a consequence of the OHCs' feedback system activation. Neonates SOAE spectra often show several strong emission lines, with intensity up to 20 dB SPL, as shown in Fig. 15.2.

Evoked OAEs are classified according to the stimulus used to elicit them. Several different techniques are currently available. A main distinction is made between transient evoked OAEs (TEAOEs) and OAEs evoked by pure tones (stimulus-frequency OAEs, SFOAEs) or by two tones (Distortion Product OAEs, DPOAEs) [4].

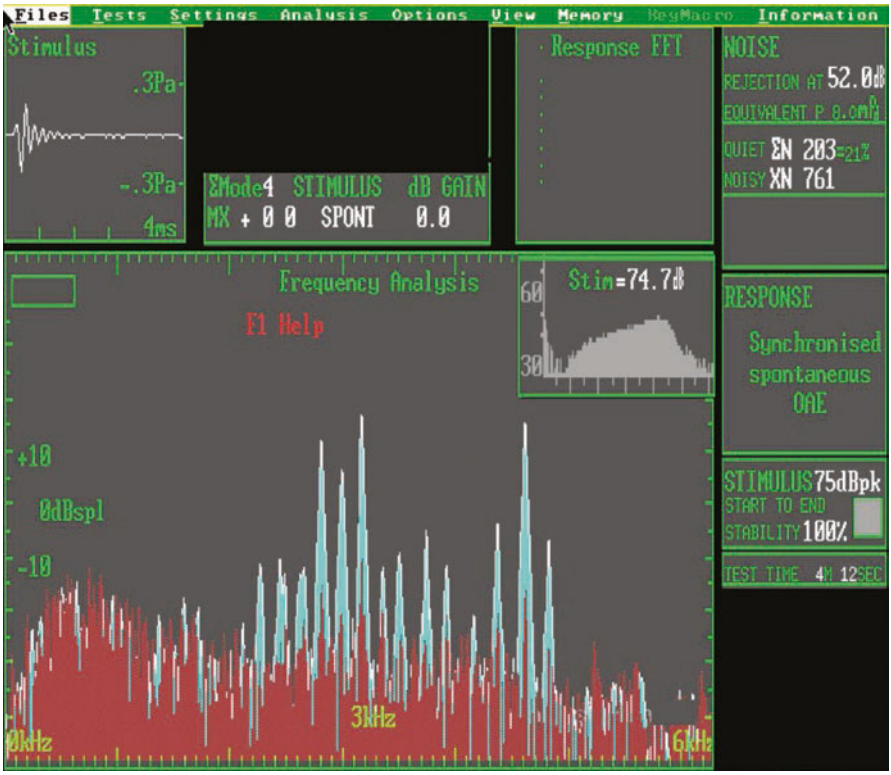

Fig. 15.2 Spectrum of the spontaneous otoacoustic emissions of a neonate

15.1.4.2 Hearing Functionality and OAEs

As mentioned above, the nonlinear amplification active feedback mechanism providing the high sensitivity and sharp frequency resolution of the auditory system is localized in the cochlea and, particularly, in the outer hair cells (OHCs), and OHCs are the first part of the auditory system to be damaged by high levels of noise exposure. The functionality of the cochlear active filter is also a necessary condition for the production of otoacoustic emissions, whose characteristics are therefore very sensitive to small variations of the cochlear filter parameters. In fact, according to cochlear models of the OAE generation, the excitation level of the BM, which is strongly amplified by the active feedback mechanism, is directly related to the level of the OAEs. Therefore the sensitivity of hearing is correlated to the OAE levels.

15.1.4.3 Cochlear Tuning and OAEs

Another psychoacoustical characteristic of hearing, the frequency discrimination capability, is related to measurable OAE parameters, namely, the OAE characteristic delay times, which are often defined as latencies.

In psychoacoustics, cochlear tuning is the capability of discriminating sounds of different frequency, and it is measured using masking techniques. These techniques measure the variation of the perception threshold for a given frequency tone, as the frequency band of a masking noise signal approaches that frequency, identifying a critical band, which defines the frequency resolution of hearing.

In physics, cochlear tuning is related to the sharpness of the tonotopic cochlear filter, which is reflected in the activation pattern of the BM. In animal experiments [5], direct measurements of the BM frequency response have shown that the excitation pattern of the basilar membrane has a typical resonant shape. For each cochlear place x, the maximum BM displacement is given by its characteristic frequency CF(x), with CF(x) being an exponential function [6]. The width of the frequency response curve around CF(x), or, equivalently the width of the excitation pattern due to a single frequency CF(x) around its tonotopic place x, is an increasing function of the stimulus level, and a direct measure of the frequency resolution of hearing.

Moleti and Sisto (2003) demonstrated [7] that it is possible to estimate cochlear tuning also for non-collaborating subjects, such as neonates, using a new technique, based on the time-frequency analysis of OAEs. In fact, the slowing down of each frequency component of the traveling wave approaching its tonotopic place is a function of the sharpness of the corresponding cochlear filter, and, therefore, cochlear tuning may be estimated from time-frequency measurements of the TEOAE latency or from measurements of the phase-gradient delay of SFOAEs [8]. The method has been recently refined [9] by Moleti and Sisto (2016), exploiting recent advances in nonlinear cochlear modeling.

OAE measurements show that average cochlear tuning is sharper in neonates than in adults, as shown in Fig. 15.3, suggesting higher noise vulnerability for

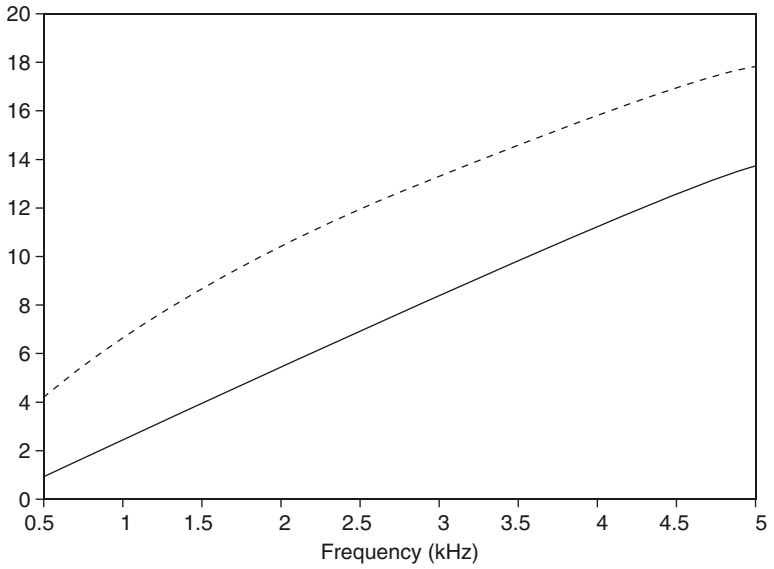

Fig. 15.3 Comparison between the average cochlear tuning curve of adults (*solid line*) and neonates (*dotted line*) derived from otoacoustic emissions latency measures

neonates. In fact, high effectiveness of the cochlear filter for intense signals could be associated with lower effectiveness of the self-defense efferent feedback mechanism protecting the ear from intense noise [7]. Other authors have used DPOAE tuning curves to reach the conclusion that immaturity of the efferent system reduces protection of the hearing system from intense noise in babies [10]. Other studies on premature neonates show that the OAE latency is higher in preterm neonates than in full-term neonates, which would be coherent with the above interpretations.

For the above reasons, neonates could be particularly vulnerable to noise, and among them, preterm neonates, which are more commonly exposed for a rather long time to the incubator noisy environment, could be the most vulnerable.

15.2 Exposure to Electromagnetic Fields

15.2.1 Physical Nature of e.m. Waves

An electromagnetic (e.m.) field consists of variable electric and magnetic fields that excite each other through their time variations. Such variable fields are generated by accelerated charges and variable currents. For oscillating sources, the period of oscillation $T = 1/f$ is related to the wavelength λ by the relation $\lambda = cT$ (or $\lambda = c/f$).

Electromagnetic radiation from a dipole source consists of several contributions, with different dependence on the distance from the source, with terms going as λ/r and $(\lambda/r)^2$, $(\lambda/r)^3$. Only the term with the λ/r dependence propagates energy

irradiating from the source; as for the others the time averaged flux of the Poynting vector over a closed surface is null. The λ/r term is associated to e.m. radiation propagation, and it is dominant at distances much larger than the wavelength (far field). At long distance from the source ($r >> \lambda$), the e.m. fields propagate at the speed of light as e.m. waves.

For visible radiation at macroscopic distances, the far-field approximation is obviously correct, but for microwave and radio waves, the other terms cannot be neglected and become dominant.

At short distance from the source ($r << \lambda$), the near-field terms are dominant, which are time-modulated versions of the static dipole fields, which do not transmit any energy flux across a closed surface. For low-frequency e.m. fields, the near-field condition typically holds; for example, for f = 1000 Hz, λ = 300 km.

15.2.2 Interaction of e.m. Fields with Biological Systems

15.2.2.1 Non-ionizing Radiation and the Role of Frequency

Non-ionizing radiation (NIR) is characterized by the fact that the single photon energy, which is related to frequency by the relation E = hf, is not sufficient for ionizing a single atom or molecule. The quantum nature of the interaction between radiation and matter implies indeed that the single photon energy must exceed a well-defined threshold to excite or ionize an atom.

Frequency is the main physical parameter that influences the interaction between electromagnetic field and biological systems. A distinction is usually made between extremely low-frequency (ELF, between 0 and 300 Hz), low-frequency (VLF-LF, between 300 Hz and 300 kHz), radiofrequency (RF, between 300 kHz and 300 MHz), and microwave (MW, between 300 MHz and 300 GHz) radiation.

At low frequency, the main effect of interaction is associated with the electrical current density (A/m^2) induced in the tissues. The induced current density is the physical quantity used to describe thresholds for acute effects and exposure limits.

At frequencies above 100 kHz, the main interaction effect is the local heating of tissues. In this case, the physical parameter used to describe the effects thresholds is the SAR (specific absorption rate) which is the electromagnetic power absorbed per unit mass (W/Kg).

The development of the human being, from conception to adulthood, is continuous, but the prenatal period and the early stages of newborn development are the ones in which important changes take place in a short time. Therefore, as for other risk agents, non-ionizing radiation exposure may be especially harmful for children and particularly for neonates. Special attention should be given to the central nervous system and the hematopoietic and immune system, which are still under development, and particularly vulnerable, in neonates.

15.2.2.2 Acute Effects of Electromagnetic Fields

Acute effects are deterministic effects, with a typical induction threshold. The safety standards specify exposure limits both in terms of dosimetric quantities, such as

induced current density or SAR, and in terms of maximum permissible amplitude of magnetic and electric fields (reference limits).

The exposure limits given by the ICNIRP (International Commission on Non-Ionizing Radiation Protection) to prevent acute effects at 50 Hz for general population are

$$E_{th} = 5000 \frac{V}{m}, \quad B_{th} = 100 \mu T. \tag{5}$$

15.2.2.3 Electromagnetic Fields: Long-Term Effects

The problem of possible long-term effects due to chronic exposure to low-intensity electromagnetic fields is quite a complex one [11]. The epidemiologic and experimental research carried on in the last years do not generally support the hypothesis of long-term effects due to chronic exposure to low-intensity fields. Nevertheless, an association has been evidenced by some epidemiologic studies [12] between residential exposure to ELF field and risk increase for childhood leukemia, and a possible effect has been hypothesized for brain cancer. On the basis of these studies, the International Agency for Research on Cancer (IARC), in June 2001, decided to classify the ELF fields as possibly carcinogenetic (2B group) for humans.

IARC classification:

1 The agent is carcinogenetic for humans.
2A The agent is probably carcinogenetic for humans.
2B The agent is possibly carcinogenetic for humans.

15.2.3 Electromagnetic Fields in Incubators

Some authors [13] reported the level of exposure to ELF electromagnetic fields in transport incubators and compared them to those typical of hospital incubators. Due to their smaller size, the portable units are characterized by a shorter distance between the electric components and the neonate bed. As a consequence, the level of exposure is significantly higher in transport incubators (Table 15.2):

These values are quite high, if compared with the exposure to domestic ELF sources, and they are also close to the ICNIRP reference levels for population exposure to prevent acute effects (100 μT at 50 Hz). The authors showed that a significant reduction of the exposure may easily be obtained by simply increasing by 10 cm the height of the mattress level.

Table 15.2 ELF exposure levels in transport and standard incubators (from [13] Bellieni et al. Ital. J. Pediatr. (2003))

	ELF e.m. exposure Max B (μT)
Transport incubator	35.7
Standard incubator	8.8

15.3 Summary

Neonates in incubators are subject to health risk due to physical agents such as noise and ELF electromagnetic fields. The levels of exposure are near to thresholds for acute effects both in the case of noise and in the case of EMF. Special care should be given to this problem, because neonates are immature organisms in development, thus they could be more sensitive than general population to risk agents. Otoacoustic emission studies suggest that this may actually be true for noise. Simple, low-cost precautions could be used to significantly reduce the exposure levels.

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Part IV

Pain: A Risk Factor for Brain Damage

Neonatal Stressors

16

M. Delivoria-Papadopoulos and P. Kratimenos

About 2.2 billion years ago, as the oxygen level of the planet was rising, a new sort of life form emerged, forged from a shaky alliance of what were to become the mitochondria and the remainder of the cell. The protomitochondria brought respiration to the partnership, and with it the power to kill every new cell by production of reactive oxygen species—a mechanism of cell death that still exists throughout the eukaryotes.

However, it was about 1.5 billion years later, as multicellular organisms emerged, that our story properly begins. Apoptosis has now evolved as a mechanism of physiological cell death in response to environmental and developmental signals. Apoptotic cell death is observed throughout the animal kingdom. Several recent advances have contributed to an emerging view of how apoptosis proceeds once initiated, to the point that we can speculate on a pathway for this remarkable process.

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In the newborn, we are trying to find out the mechanisms by which the cell dies and brain damage occurs. But the most important part for clinicians is to understand the mechanisms of cell death not before hypoxia but after the hypoxia has occurred. Therefore, the aim of my discussion is to share with you experimental data to show how we have thought of asking and resolving some of the pertinent questions regarding posthypoxic brain damage and ways to learn to understand the mechanism.

16.1 Temporal Profile of Cerebral High-Energy Phosphates, Cell Membrane Na^+-K^+-ATPase, and Nuclear DNA Fragmentation Following Hypoxia

One of our main objectives was to evaluate biochemical changes over time and associated DNA fragmentation in the cerebral cortex of the newborn guinea pig following hypoxia. The experimental protocol was approved by the Institutional Animal Care and Use Committee. We worked with pregnant guinea pigs so that we could control the timing of the birth of pups.

We divided the newborn guinea pigs into the following groups: the normoxic control and the hypoxic group, where the guinea pigs were allowed to breathe 5–7% oxygen for 1 h. Following 1 h of hypoxia, the animals were studied either immediately or after 1 day, 3 days, or 7 days to answer the most crucial questions about delayed cell death in the brain. We measured the level of high-energy phosphates to assess the level of tissue hypoxia. We actually abandoned partial oxygen pressures because it is impossible to compare partial oxygen pressures when the hypoxic response of the animals is different in terms of adaptation. Accurate measurement of the levels of ATP and phosphocreatine in the tissues allows us to compare tissue hypoxia among several groups of animals and across several studies.

We started by measuring the cell membrane function of the neurons and Na^+-K^+-ATPase activity was one of the basic measurements we did. Our aim was to determine the development of DNA fragmentation. The following presentation examines first the results of our three groups of animals, particularly those at 7 days after hypoxia. After 1 h of hypoxia, the ATP levels are significantly decreased, and 7 days later, although there is a tendency for ATP to come up, the level is still decreased and statistically different from corresponding normoxic controls.

Phosphocreatine responded in a similar way. There is a significant decrease in phosphocreatine immediately after 1 h of hypoxia that persists throughout the 7 days. At 7 days, despite a slight increase, it is still significantly lower than in normal controls.

Na^+-K^+-ATPase activity decreased during hypoxia and 7 days later was still decreased, although not statistically different for the number of animals studied.

The DNA fragmentation pattern was very clear. In the normoxic samples, there is a presence of large fragments of DNA along with an absence of small DNA fragments. In the hypoxic samples, the low-molecular-weight fragments are present at 7 days, indicating that there was significant and dramatic fragmentation of the DNA 7 days after hypoxia.

This particular study can be summarized as follows: hypoxia resulted in a decrease in the cerebral level of high-energy phosphates, decreased cellular

membrane Na^+-K^+-ATPase activity, and increased cerebral cortical nuclear DNA fragmentation, and 24–72 h following hypoxia the levels of high-energy phosphates and DNA fragmentation were slightly improved, but at 7 days the level of high-energy phosphates decreased, Na^+-K^+-ATPase activity was lower, and DNA fragmentation had increased significantly. We conclude from these studies that despite an apparent initial recovery following hypoxia, perturbations in cerebral energy metabolism, brain cell membrane function, and cerebral cortical nuclear DNA structure persist in the newborn guinea pig brain. We speculate that a biphasic temporal pattern of the brain dysfunction following hypoxia may represent an initial cellular injury followed by a failure of cellular repair mechanisms, leading to further and delayed brain injury.

16.2 Temporal Profile of Neuronal Nuclear Ca^{2+} Influx and Expression of Proapoptotic and Antiapoptotic Proteins Following Hypoxia

A number of events occur inside the nucleus, including signal transductions that lead to apoptotic cell death. In the next several studies, our objective was to evaluate changes over time in Ca^{2+} influx and Bax and Bcl-2 protein expression in neuronal nuclei of the newborn guinea pig following hypoxia.

Proteins of the Bcl-2 family are known to be critical regulators of programmed cell death and are expressed in neurons of the central and peripheral nervous system [1, 2]. The Bcl-2 protein, which is antiapoptotic in nature and was discovered in association with follicular lymphoma, regulates apoptosis and enhances cell survival in response to diverse apoptotic stimuli [3–6]. Bcl-2 family proteins have been shown by electron microscopy to reside in the nuclear envelope, parts of the endoplasmic reticulum, cytosol, and outer mitochondrial membranes [7–9]. Conversely, overexpression of the proapoptotic proteins such as Bax has been shown to promote cell death by activating caspases [10–12]. The active form of Bcl-2 heterodimerizes with Bax, and their relative ratio appears to determine cellular susceptibility to apoptotic stimuli [12–15]. Bax and Bcl-2 are thought to play a role in cell death following hypoxia and ischemia. Studies have shown that cerebral hypoxia and ischemia alter the expression of these proteins [1, 3, 7, 14, 16, 17]. Studies have also suggested that Bcl-2 may suppress apoptosis by regulating cytosolic and intranuclear Ca^{2+} concentrations [18]. Nuclear Ca^{2+} signals control a number of critical nuclear functions, including transcription, DNA replication, and nuclear envelope breakdown [19–22].

The Ca^{2+} influx into the neuronal nuclei and the ATP-dependent Ca^{2+} influx will be the most important signal for molecular dysfunction. In addition, we focused on the apoptotic proteins, Bax and Bcl-2. The ATP and phosphocreatine decreased in the hypoxic group. However, we focused on the neuronal nuclear Ca^{2+} influx. We have shown before that during hypoxia, Ca^{2+} influx into the nucleus increases and the mechanisms are in place that facilitate that influx within the nucleus. One day after hypoxia, there is a further increase in Ca^{2+} influx. Three days later it is still high, but 7 days later it is very impressive to note that the Ca^{2+} influx is at its

maximum. The proapoptotic protein Bax increased after 24 h following hypoxia. At 72 h it was further increased, and 7 days later it was increased by 66% compared to controls. In contrast, Bcl-2, which is an antiapoptotic protein that maintains a cell life, did not change throughout normoxic states and hypoxic states longitudinally.

In summary, these studies showed that the neuronal nuclear Ca^{2+} influx increases in the postnatal period up to 7 days of age; that hypoxia results in an increased Ca^{2+} influx that remains elevated; and that the nuclear Bax protein expression increases immediately after hypoxia and remains elevated for 7 days, while Bcl-2 protein expression remains unchanged.

We concluded that alterations in neuronal nuclear Ca^{2+} influx after hypoxia and Bax protein expression persist in the newborn guinea pig brain. We speculate that alterations in the nuclear Ca^{2+} influx and the increased ratio of Bax to Bcl-2 proteins during the posthypoxic period promote delayed programmed neuronal death.

16.3 Effect of Allopurinol Administration on the Expression of Apoptotic Proteins and the Activation of Caspases

We can never deduce a concept unless we can eliminate, block, or intercept early steps. To achieve that, we utilized allopurinol, a xanthine oxidase inhibitor. Studies have shown the therapeutic interventions of allopurinol, magnesium, and other substances that inhibit various steps of the apoptotic pathway. We used this inhibitor to prove our point, because if allopurinol inhibits the cascade of events, that would confirm the sequence of cell death mechanism that we have proposed.

We administered allopurinol to newborn guinea pigs to examine the effect of allopurinol administration after hypoxic changes in Bax and Bcl-2 and determined caspase-3 protein expression in the newborn.

Caspases are essential enzymes used for the normal development of the central nervous system. Caspases are cysteine proteases which contain a cysteine residue within their active site and cleave the peptide bond C-terminal to an aspartic acid of the substrate [23–26]. Like many other proteins, caspases are produced as procaspases (the inactive zymogen form) which are then converted to active enzymes following a proteolytic cleavage. Structurally, all procaspases contain a highly homologous protease domain, the signature motif of this family of proteases. The protease domain is divided into two subunits, a large subunit of approximately 20 kDa (p20) and a small subunit of approximately 10 kDa (p10). In addition, there is often a small linker region (about 10 amino acids long) between the two subunits. In addition to the large and small subunits, each procaspase contains a prodomain or NH_2-terminal peptide of variable length. It is this prodomain which contains the caspase recruitment domain (CARD). A charge–charge interaction controls the CARD–CARD interaction. Studies have shown that active caspases such as caspase-1 and caspase-3 contain the large and small subunits which are released from their respective procaspases through proteolytic cleavage, and both subunits are required for caspase activity [27, 28]. Studies have also shown that an active caspase

is a tetramer (homodimer of the p20 and p10 heterodimers arranged in twofold rotational symmetry), with the two adjacent small subunits surrounded by two large subunits [29]. These two heterodimers associate with each other through the interaction between the p10 subunits.

Caspase tetramers have two cavity-shaped active sites which function independently. In the active site, a cysteine (Cys-285 in the p20) is positioned close to the imidazole of histidine (His-237, p20), which attracts the proton from the cysteine and enhances its nucleophilic property. Caspase-9 is synthesized as a 46 kDa precursor protein. Like other caspases, it consists of three domains: an N-terminal prodomain, a large subunit (20 kDa/p20), and a small subunit (10 kDa/p10). Caspase-9 shares 31% sequence identity with the *Caenorhabditis elegans* cell death protein Ced-3 and 29% sequence identity with caspase-3. It contains an active site QACGG instead of QACRG pentapeptide which is conserved in other caspase members.

Caspases are a group of cysteine proteases that are essential for initiating and executing programmed cell death [30–35]. The activity of cysteine proteases is detected in cells undergoing programmed cell death, irrespective of their origin or the death stimuli. Studies conducted in *C. elegans* have demonstrated that an aspartate-specific cysteine protease is essential for programmed cell death of all somatic cells during development [36–38]. Mice lacking caspase-3 or caspase-9 have increased numbers of neurons in the brain, and their lymphocytes are resistant to apoptotic stimuli [39–42]. Furthermore, the expression of baculovirus protein, a potent inhibitor of all known caspases, prevented developmental programmed cell death in *C. elegans* and in a number of cell lines [43–47]. Thus, it has been well established that caspases are critical regulators of cell death initiation as well as executioners of programmed cell death.

Once again in our experimental studies, we used high-energy phosphates to assess hypoxia. We determined expression of Bax, Bcl-2, and the activity of caspase. We documented again the level of hypoxia by ATP and phosphocreatine and the activity of caspase-3, the executioner caspase. The activity of caspase-3 increased significantly during hypoxia and remained high during the posthypoxic period.

Bax is hardly visible in normoxia; it increases in hypoxia and 24 h after hypoxia as shown before. However, following administration of allopurinol after hypoxia, Bax is lower than the posthypoxic level and definitely lower after 24 h. Bcl-2 did not change. The active caspase-3 did not change after an increase following hypoxia and remained the same throughout. Bax increased during the 24 h posthypoxia, and it is decreased in the allopurinol-treated hypoxic group.

To sum up, the findings of these studies were:

- That nuclear Bax protein expression increased immediately following hypoxia and remained elevated 24 h after hypoxia
- That allopurinol attenuated the increase in the nuclear Bax protein expression that was observed immediately after hypoxia and at 24 h after hypoxia
- That the Bcl-2 protein remained unchanged and that caspase-3 expression remained unchanged after it was increased after hypoxia
- That allopurinol did not affect the increase in caspase-3 expression

The speculation was that allopurinol pretreatment prevents alterations of the Bax to Bcl-2 protein ratio following hypoxia and may attenuate delayed neuronal death by a mechanism independent of caspase-3.

16.4 Effect of Allopurinol Administration on Neuronal Nuclear Ca^{2+}/Calmodulin-Dependent Protein Kinase IV and Nuclear DNA Fragmentation

We examined neuronal nuclear protein kinase IV, which is a Ca^{2+}/calmodulin-dependent (CaM kinase IV) enzyme. This is a key enzyme in the transcription of apoptotic proteins. We determined whether pretreatment with allopurinol would attenuate posthypoxic alteration in the neuronal Ca^{2+} and CaM kinase IV activity. After hypoxia, the nuclear enzyme CaM kinase IV increased significantly. Twenty-four hours later, it appeared low, but hypoxia plus allopurinol showed a significant difference.

Posthypoxic treatment with a low dose of allopurinol (20 mg/kg body weight) did not show an effect. Posthypoxic treatment with a high dose of allopurinol (100 mg/kg body weight) significantly decreased Bax without affecting Bcl-2. Twenty-four hours later, there is a smear, 24 h of hypoxia and allopurinol show that there is still presence of fragments, but 72 h later there are fewer fragments of DNA even after post-allopurinol treatment. This was shown at the seventh day, when the fragments are much less and the last two panels, which are 7 days after hypoxia post-allopurinol treatment, look almost like the first two samples, which are normoxic. The DNA fragmentation shows very clearly; the squares are the hypoxic and with allopurinol treatment in which fragmentation decreases while in hypoxia it goes up.

In summary: in the cerebral cortex of newborn guinea pigs, nuclear Bax protein expression increases immediately after hypoxia and remains elevated at 24 h. A further increase is observed at 7 days. Allopurinol treatment following hypoxia prevented the increase in nuclear Bax protein expression observed at 7 days after hypoxia. Bcl-2 protein expression does not change. DNA fragmentation was observed following hypoxia and was decreased at 72 h, but increased fragmentation was again observed at 7 days after hypoxia. Allopurinol treatment following hypoxia attenuated the increase in DNA fragmentation observed at 7 days after hypoxia.

We speculate that treatment with allopurinol following hypoxia prevents alteration in the Bax to Bcl-2 ratio and may attenuate delayed posthypoxic neuronal apoptosis. The proposed sequence of events is this: with the influx of Ca^{2+} inside the cell, there is transformation within the intracellular space of xanthine dehydrogenase to xanthine oxidase, which is obliterated or inhibited by allopurinol. Thus, free radicals that otherwise would promote lipid peroxidation of nuclear membranes enhance intranuclear Ca^{2+} flux and activate CaM kinase IV, which would phosphorylate the cyclic AMP response element binding protein molecule, which subsequently transcribes Bax and Bcl-2 genes.

16.5 Leakage of the Mitochondrial Apoptotic Proteins into the Cytosol Following Hypoxia in the Cerebral Cortex of Guinea Piglet

Smac/DIABLO is a small-sized protein (27 kDa) localized in mitochondria and is subsequently released into the cytosol as a larger mature protein along with cytochrome c, maintaining a dynamic protein–protein interaction [48, 49]. Smac is released concurrently with cytochrome c from mitochondria into the cytosol during apoptosis and reactivates the initiator and effector caspases by deactivating the inhibitor of apoptosis protein (IAP)-mediated inhibition [49–51]. It has been demonstrated that this inhibition of IAPs allows for increased caspase activity, leading to cell death. Under physiological conditions, the IAP is bound to the caspases and deactivates them [48–50].

It is known that perturbations to mitochondria result in the release of cytochrome c, Smac, and other apoptogenic factors [50]. We have reported previously that similar to Smac, fetal hypoxia results in cytochrome c release from mitochondria to cytosol, thus identifying that mitochondrion is a key regulator of programmed cell death. There is a diffuse network of enzymes under the cell membrane in the area of focal adhesions that regulate the cell migration and apoptosis including the mitochondria, probably by increasing the mitochondrial membrane permeabilization or by regulating the mitochondrial permeability transition pore (mPTP) [51–54].

We aimed to study the hypotheses that hypoxia results in increased expression and translocation of Smac from mitochondria to cytosolic compartment in the cerebral cortex of guinea pig fetus at term.

Pregnant guinea pigs at 60 days gestation were divided into normoxic (Nx, $n = 6$) and hypoxic (Hx, $n = 6$) groups. Fetal hypoxia was induced by exposing the pregnant guinea pig mothers to a FiO_2 of 0.07 for 60 min. Fetal hypoxia was documented by ATP and PCr levels. Mitochondrial and cytosolic fractions were isolated from the cerebral cortical tissue. Smac expression in the mitochondrial and cytosolic fractions was determined by western blot using specific anti-Smac antibody and expressed as absorbance (ODxmm2).

Smac expression in the mitochondria was 62.35 ± 4.56 in Nx and 76.65 ± 8.3 (P = NS) in the Hx group. However the Smac expression in the cytosolic fraction was 81.23 ± 7.3 in Nx and increased to 172.34 ± 6.3 in Hx ($p < 0.05$ vs. Nx). The data show that hypoxia results in increased Smac protein in the cytosolic fraction.

Since Smac predominantly resides in mitochondria, we conclude that hypoxia results in increased release of mitochondrial Smac protein into the cytosol. We speculate that Smac translocation is a novel mechanism of hypoxia-induced neuronal cell death in the fetal brain. The increased Smac protein in the cytosolic compartment during hypoxia indicates that hypoxic neuronal death in the fetal brain is mediated by a caspase-dependent mechanism.

In ending, we would like to point out that this is the tip of the iceberg and that a lot of work is still needed in order to see the exact sequence of the apoptotic pathway before we can dream and think of being able to inhibit it therapeutically. These inhibitions only prove the scientific value of the sequence of apoptotic pathways.

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New Insights into Neonatal Hypersensitivity

17

C.J. Woodbury

17.1 Introduction

The skin, the largest sensory epithelium of the body, plays a pivotal role in the homeostasis of organisms and is densely innervated by a wide variety of sensory neurons that have evolved to safeguard its integrity. Nowhere is this more important than in newborns, whose immune systems are not yet fully mature and for whom skin damage can therefore have serious immediate and long-term impacts on the viability of the organism as well as overall function of the skin sensory system. Attesting to this early vulnerability, newborn mammals of all species, including man, display a pronounced hypersensitivity to tactile stimuli over a protracted postnatal period (reviewed in [1]). Throughout this period of cutaneous hypersensitivity, protective withdrawal reflexes exhibit inordinately low activation thresholds and thus can be triggered by relatively innocuous stimuli. Rather than representing a paradoxical, maladaptive behavioural response, this hypersensitivity is undoubtedly highly adaptive in view of the pivotal importance yet extreme vulnerability of the integument and its associated protective/immune functions during early ontogeny.

The mechanisms underlying this early hypersensitivity are poorly understood. It has long been held that such hypersensitivity was triggered by activation of tactile afferents, due to the low activation thresholds of withdrawal reflexes and the widespread belief that the pain system was not yet functional due to delayed development of nociceptors [1]. Indeed, tactile afferents have been thought to invade nociceptive-specific regions of the central nervous system early on and commandeer nociceptive circuitry, in effect serving in the capacity of nociceptors during early postnatal timepoints, while nociceptors were still immature. However, recent findings contradict this widespread belief, providing new insight into the identity of the sensory neurons that underlie neonatal hypersensitivity.

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17.2 Skin Sensory Neuron Diversity

Skin sensory neurons, whose cell bodies lie in the trigeminal and dorsal root ganglia (DRGs), represent the skin's first line of defence against potentially harmful environmental insults. These primary afferents constitute an exceedingly diverse constellation of functional phenotypes, the majority of which are tuned to respond selectively to discrete intensities of mechanical, thermal or chemical stimuli ranging from innocuous to noxious and relay this information to central circuitry [2]. This functional diversity is paralleled by a similarly striking diversity in anatomical, physiological and molecular phenotypic properties of skin sensory neurons. A major challenge in sensory biology is to understand the relationships between these properties and how and when this diversity comes about during development.

From adult studies, while many diverse properties have been shown to be correlated to varying degrees with the discrete functional attributes of sensory neurons, the sheer complexity of these multifaceted interrelationships has strained the utility of broad generalizations. For example, the peripheral axons of cutaneous afferents range in size from large-diameter, thickly myelinated (Aβ) fibres to small, thinly myelinated (Aδ) and unmyelinated (C) fibres. Large fibres have historically been equated with low-threshold mechanoreceptors (LTMRs, or tactile afferents), whereas Aδ and C fibres have historically been equated with nociceptive afferents [2]. This classical account represents an oversimplification, however, as many nociceptors conduct in the Aβ range, while the most exquisitely sensitive tactile afferents often conduct in the Aδ and C ranges. As will become evident below, failure to recognize these important exceptions has helped promulgate a number of major misconceptions surrounding the development and plasticity of the pain system.

Another area where generalizations may be of limited usefulness is in studies of the biophysical properties of sensory neurons. Intracellular recordings have revealed a diversity of somal action potential shapes among sensory neurons, from broad (i.e. long-duration) spikes with a characteristic hump or inflection on the falling limb of the spike to narrow (i.e. short-duration) spikes lacking this inflection. Historically, these quantitative and qualitative attributes of spike shape have been widely viewed as strong predictors of afferent functional identity, with narrow uninflected spikes associated with tactile afferents (LTMRs) and broad inflected spikes associated with nociceptive afferents [3]. Recent work from our laboratory that has examined nociceptor properties at normal physiological temperatures, however, has raised caution concerning attempts to infer afferent identity solely on the basis of biophysical properties [4].

A strong functional correlate is evident in the morphology and laminar termination patterns of sensory neurons where information is relayed by these afferents to central circuits in the spinal dorsal horn. While there are again exceptions, in general the central arborizations of different functional subclasses of skin sensory neurons are highly stereotypical and correlated with afferent modality [5]. In particular, nociceptive afferents have been found to terminate predominantly in superficial dorsal horn laminae I and II, whereas tactile afferents exhibit non-overlapping terminations that remain deep to nociceptive afferents in laminae III–V.

How and when these highly stereotypical laminar termination patterns of skin sensory neurons come about during development is unclear. Neuroanatomical tract-tracing studies, which depend upon bulk application of tracers to label large populations of unidentified sensory neurons, suggest that large-diameter afferents are the first to penetrate into the spinal grey matter of embryos, followed a few days later by small-diameter afferents [6]. It has also been suggested on the basis of such studies that these early-arriving, large-diameter afferents bypass their normal appropriate targets to invade superficial "pain-specific" or nocireceptive laminae during early life [7]. These fibres have been widely assumed to be LTMRs on the basis of axonal diameter and central morphology. This invasion and occupation of pain-specific regions by LTMRs has been thought to extend over a protracted period that in rodents encompasses the first 3 weeks of postnatal life [1, 7]. The adult bulk-labelling pattern is not seen until the third week of life, when these exuberant central arbours retract from superficial pain-specific regions (but see [8]). This has led to a widespread perception that tactile afferents are extremely plastic entities, capable of invading and activating pain circuits directly at different times throughout ontogeny.

During pre- and postnatal development, this invasion of pain circuits by tactile afferents is therefore thought to underlie the hypersensitivity to tactile stimuli seen among neonates. Interestingly, the low activation thresholds of cutaneous withdrawal reflexes extend to about the third week of life, mirroring the time course of these exuberant central projections from tactile afferents. This plasticity of tactile afferents is thought to continue throughout life, with exuberant growth into superficial pain centres again occurring in adults following nerve injury and/or peripheral inflammation [9, 10]. The activation of pain circuits by LTMRs is thought to underlie various chronic pain syndromes, most notably tactile hypersensitivity and touch-evoked pain (mechanical allodynia), that are common sequelae of nerve injury and inflammation.

17.3 Studies of Individual, Physiologically Identified Afferents

An important consideration is that all of these previous conclusions have been based upon morphological studies of unidentified afferents, i.e. the identity or identities of the sensory neurons upon which these hypotheses are based is unknown. To gain a better understanding of the anatomical and physiological development of skin sensory neurons in early neonatal life, we developed an ex vivo somatosensory system preparation wherein the activity of individual sensory neurons could be recorded and functionally identified using natural stimulation of intact terminals in the skin prior to intracellular labelling of the same afferents for anatomical analyses of central termination patterns [11, 12]. In this novel preparation from mice, the spinal cord, DRGs, and cutaneous nerves are isolated in continuity with their innervation territories in the skin. The somata of individual skin sensory neurons are then impaled in the DRG with micropipettes containing Neurobiotin. Somal action

potentials are recorded for analysis of biophysical properties. The latency of action potentials to electrical nerve stimulation provides information on conduction velocity and fibre size. A variety of natural stimuli (e.g. mechanical, thermal and chemical) are applied to the neuron's terminals in the skin to characterize the neuron's peripheral response properties and overall functional identity. Following characterization, Neurobiotin is then introduced iontophoretically into the cell and allowed to diffuse throughout the cytoplasm. The tissue is then fixed, sectioned and processed using standard ABC/DAB techniques, enabling visualization of the entire neuron, including cell body, arborizations in the spinal cord and frequently peripheral endings in the skin. In addition to anatomical and physiological phenotype, the molecular phenotype of the cell can also be examined using fluorescence immunocytochemical techniques to determine whether physiological properties (e.g. heat sensitivity) are correlated with molecular expression patterns [13, 14]. This preparation therefore provides unprecedented power to address issues surrounding afferent identity and plasticity stemming from a variety of manipulations in neonates as well as adults.

17.3.1 Neonatal Tactile Afferents

In studies of newborn mice, the results obtained with this preparation have been strikingly consistent. Low-threshold mechanoreceptors (i.e. tactile afferents) exhibit adultlike central projection patterns in neonates as early as 1 day after birth (the earliest timepoint examined); that is, they do not invade superficial pain-specific laminae during early postnatal life [11]. This result was found for multiple distinct classes of LTMRs, ranging from those that will develop thickly (Aβ) to those that will develop thinly myelinated axons (Aδ) and those that exhibit distinctly different responses to mechanical stimuli (i.e. phasic or tonic). In all cases, the central arbours of tactile afferents remained deep to the superficial dorsal horn, separated from the outermost marginal layer (lamina I) by a distinct gap; this gap was found to be occupied by the central terminals of unmyelinated (C) nociceptive afferents. Along with adultlike laminar termination patterns, LTMRs in neonates exhibit adultlike physiological properties as well, including narrow, uninflected somal spikes as in adults, and remarkably adultlike response properties to a variety of natural skin stimuli. These findings of appropriate, adult central projections in neonatal animals therefore contradict the widespread belief that neonatal hypersensitivity is the result of inappropriate central terminations of LTMRs.

If LTMRs are physically incapable of activating superficial pain circuits directly, what populations of sensory neurons might be responsible for the hypersensitivity of protective nociceptive withdrawal responses in neonates? Another important point to note is that myelinated nociceptors have been ignored in all previous studies. These fibres bind the same cholera toxin labels that have been used previously to argue for a "transient invasion" of myelinated fibre inputs into superficial pain-specific regions of the dorsal horn, yet the contribution of myelinated nociceptors to

bulk-labelling patterns has been overlooked in previous studies. Here again, the use of this semi-intact ex vivo preparation to study the early postnatal development of individual myelinated nociceptors has provided a number of important insights into neonatal pain [15].

17.3.2 Neonatal Myelinated Nociceptors

With this detailed approach, we found two basic types of myelinated nociceptors in neonates as exemplified in Fig. 17.1 One class, with the most slowly conducting axons (i.e. future Aδ fibres), exhibited the classical central morphology associated with myelinated nociceptors, with terminations primarily restricted to the most superficial layer (marginal layer, or lamina I) of the dorsal horn. In addition to lamina I terminations, however, these afferents also projected into the substantia gelatinosa (lamina II; Fig. 17.1a), the region widely perceived to contain only unmyelinated C-nociceptor terminations. The other basic morphology (Fig. 17.1b) was seen

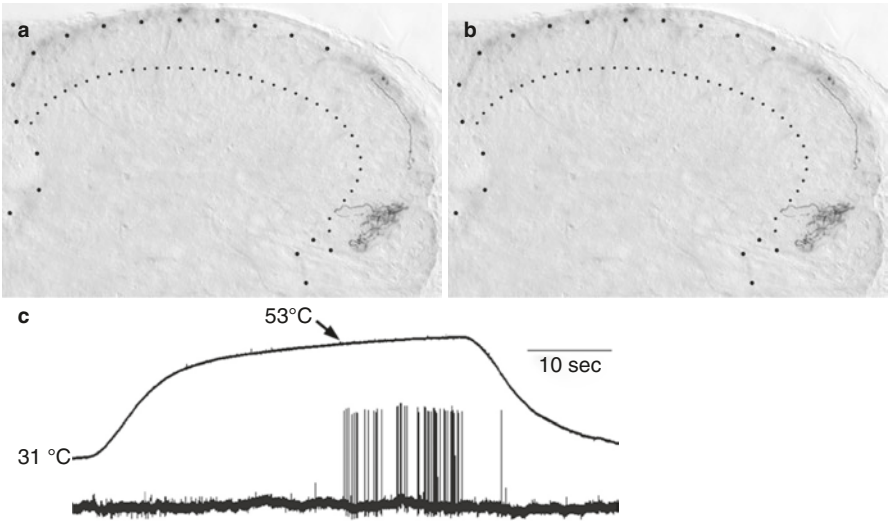

Fig. 17.1 (**a–c**) Examples of central arborizations from two different types of myelinated nociceptors innervating hairy skin in neonatal mice. (**a**) Slowly conducting (future Aδ) lamina I-/II-specific myelinated nociceptor from a P2 neonate. (**b**) Fast-conducting (future Aβ) lamina I/V myelinated nociceptor. *Large, widely spaced dotted lines* delineate the grey/white border of the dorsal horn; *small, narrowly spaced dotted lines* delineate the ventral border of the substantia gelatinosa (i.e. lamina II). (**c**) Example of response to noxious heating of the receptive field of a myelinated nociceptor from a P2 neonate. Note that the activation threshold was 53 °C, essentially identical to the average heat threshold (52 °C) for myelinated nociceptors in adults. Responses to mechanical stimuli of this afferent are not shown; for examples, see [11]. Scale bar (photomicrographs) = 100 μm

among a different subclass of myelinated nociceptors with relatively fast conducting axons, a population that has been largely ignored to date. Many of these exhibited conduction velocities that were as fast as future Aβ LTMRs. In neonates, this population of future Aβ nociceptors gave rise to dorsally recurving arbours that were similar in many respects to the classical "flame-shaped" arbours described for LTMRs that innervate hair follicles. Similar to the latter, the central arbours from this population of nociceptors arborized throughout deeper dorsal horn laminae (III–V). However, in contrast to LTMRs, the distinctive arbours of these nociceptors did not stop abruptly at the ventral limit of the substantia gelatinosa but instead continued uninterrupted into more superficial pain-specific regions, with extensive projections throughout superficial pain-specific laminae (I and II). Interestingly, this distinctive morphology has been found to persist throughout adult life in both in vitro [15] and in vivo studies (unpublished observations). Therefore, rather than a transient developmental phenotype, this population would be expected to contribute to cutaneous sensation throughout life.

As with other nociceptors, these afferents responded tonically throughout the duration of maintained stimuli, and their response became increasingly vigorous to higher forces in a manner capable of encoding the intensity of stimuli [15]. Interestingly, many of these afferents exhibited surprisingly low activation thresholds to mechanical stimuli, and tonic discharges were seen to innocuous stimuli. While these earlier studies were restricted to mechanical characterization, more recent studies of these afferents in neonates have found that many are also sensitive to noxious heat as exemplified in Fig. 17.1c. Indeed, these afferents responded to heat in a manner indistinguishable, in terms of threshold and evoked discharge, from heat responses seen across the same population in adults. Thus, similar to findings with tactile afferents, these studies reveal that myelinated nociceptors are also adultlike in all major respects in early life. Furthermore, in view of their relatively low mechanical activation thresholds and projections to superficial pain-specific laminae in the dorsal horn, this neglected and poorly understood population probably represents the afferent limb underlying hypersensitivity seen among neonates.

The existence of this novel central morphology in normal adults is particularly noteworthy. That these inputs to the substantia gelatinosa are not revealed with bulk-labelling techniques indicates that the latter are not sufficiently sensitive for these purposes. Nevertheless, these dorsally recurving flame-shaped myelinated nociceptor arbours are indistinguishable from those of the unidentified fibres interpreted as "sprouted LTMRs" in earlier nerve injury studies [9, 10]. As with hypotheses that invasion of tactile afferents into pain-specific regions of the dorsal horn could explain hypersensitivity in newborns, the latter studies in adults suggested that the same regions were invaded by these afferents again after injury, thereby explaining similar touch-evoked pain states. We now know this to be incorrect.

17.3.3 Inflammation in Newborns

While our recent findings from identified afferents have failed to support the major tactile afferent plasticity invoked in these earlier scenarios, the possibility that such

plasticity may be present in nociceptive afferents remains a distinct possibility in view of recent work involving damage and/or inflammation of the skin in newborns [16]. We are currently examining this important issue in a mouse model of neonatal inflammation, and our results to date suggest that certain nociceptors are indeed highly plastic and may be particularly vulnerable to early tissue damage as exemplified in Fig. 17.2. Inflammation at birth resulted in greatly expanded receptive fields among classical (i.e. lamina I-/II-specific) myelinated nociceptors; in some,

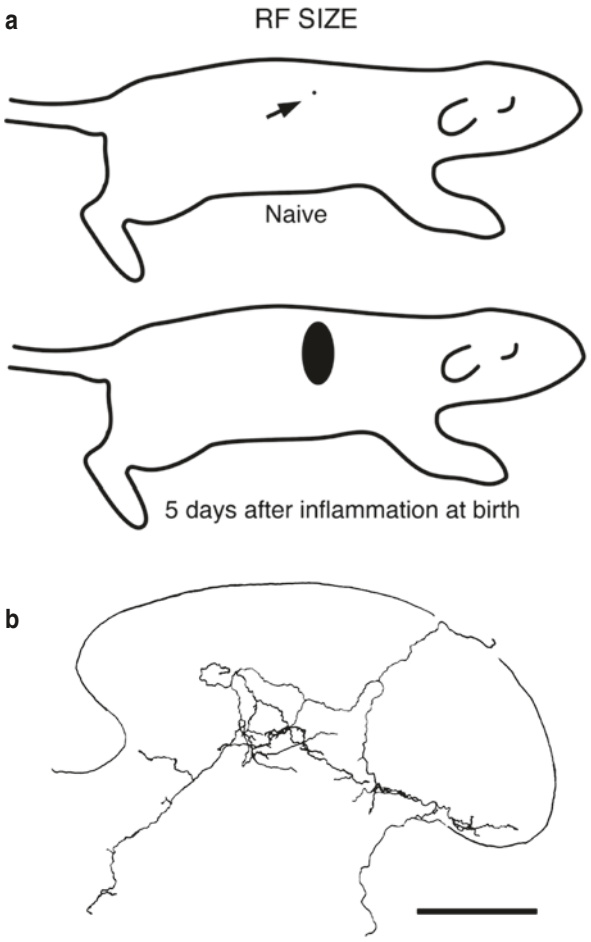

Fig. 17.2 (**a, b**) Changes in myelinated nociceptors following neonatal inflammation. (**a**) Schematic diagrams of neonatal mice (6 days after birth) showing approximate receptive field size of myelinated nociceptors in naïve, untreated animals (*small dot, top*) compared to animals that had experienced adjuvant-induced inflammation at birth (*large oval, bottom*). (**b**) Example of altered central projections from a myelinated nociceptor (5 days after birth) that had been exposed to adjuvant-induced inflammation at birth (compared to normal nociceptor arbours in Fig. 17.1); medial is to the *left*. Note the substantial disorganization and expansion of central arbours into somatotopically inappropriate regions of the dorsal horn; all normal afferents from dorsal cutaneous nerve nerves terminate in the lateral third of the dorsal horn. Scale bar = 100 μm

innervation territories expanded to cover the entire innervation territory of the dorsal cutaneous nerve. By contrast, the receptive fields of these afferents in naïve animals are universally small and spot-like (Fig. 17.2a). Similar plasticity was also seen in the central terminals of these afferents as well, where arbours became highly disorganized and expanded into novel territories (Fig. 17.2b). Our findings so far have been restricted to the first week of life; whether these disruptive effects of neonatal tissue damage lead to permanent changes in the organization of the pain system is of considerable clinical importance and is currently being examined in long-term studies using this model.

Conclusions

These recent studies of the physiology and anatomy of identified skin sensory neurons have revealed that the skin sensory system is adultlike at birth. Tactile afferents in neonates are essentially miniaturized versions of their adult counterparts, and are not in a position to activate pain circuitry as previously believed. By contrast, myelinated nociceptors are also well developed early on, displaying thresholds to mechanical and noxious heat stimuli that are essentially indistinguishable from their adult counterparts. Unlike tactile afferents, however, these nociceptive afferents project extensively throughout superficial pain-specific laminae and can thus account for bulk-labelling patterns that were previously attributed to tactile afferents. The relatively low mechanical thresholds of certain subclasses of myelinated nociceptors can explain the marked hypersensitivity seen among neonates. However, as their mechanical thresholds are essentially identical to those seen in adults, the loss of neonatal hypersensitivity is likely to be due to alterations in central processing, and a potentially fruitful avenue for future research will be the examination of the development of central inhibition. Our recent studies also suggest that the nociceptive system may be particularly vulnerable to early inflammation and/or physical trauma. An important goal of future research will be to assess the long-term impact of this phenomenon and the molecular identity of the factors involved.

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From the Gate-Control Theory to Brain Programs for Neonatal Pain

18

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Large numbers of low-birthweight (LBW) and preterm neonates are born in developed and underdeveloped countries each year [1], and many of them are extremely premature (<1500 g). For their normal, routine care, it may be necessary for these infants to undergo repeated or prolonged exposure to stress, pain, and maternal separation in the neonatal intensive care unit (NICU). At this stage, the brain's architecture and vasculature are very immature, and these neonates can only survive because of improved obstetric and neonatal care [1]. Despite an increasing survival rate, preterm infants develop a high prevalence of cognitive deficits, learning difficulties, and abnormal behaviors during their early childhood and primary school years. Multiple follow-up studies of ex-preterm neonates have reported neurodevelopmental deficits [2–4], with needs for special assistance [5] and increasing burdens for health care and society [6].

We performed a meta-analysis on the cognitive and behavioral outcomes of ex-preterm children (cases) at school age compared to term-born children (controls), which showed that the mean IQ score of ex-preterm children is 11 points lower than that of children born from term pregnancies. The lower their gestational age and birthweight, the greater was the difference in mean IQ scores between the cases and controls [7]. Hack et al. showed a five-point difference between the IQ scores of 8-year-old ex-preterm and term-born children, and this cognitive difference persisted until their follow-up at 20 years of age [8]. Despite their enormous significance for society, the biological mechanisms underlying these neurodevelopmental deficits remain unclear and underinvestigated.

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18.1 Mechanisms Leading to Long-Term Effects of Pain and Stress

Neuronal cell death and altered synaptogenesis in the immature brain may offer plausible explanations for changes in cortical and subcortical brain regions noted from human and rodent studies. We investigated the mechanisms leading to these changes in order to explore the long-term effects of pain, stress, or other adverse experiences of the neonatal period. A theoretical framework [9] suggests that immature neurons and glial elements are vulnerable to apoptosis or excitotoxicity, and that repetitive pain or stress may have a significant impact on neuronal survival.

18.1.1 Enhanced Vulnerability of Immature Neurons

Brain development is critical just before and after human birth, which corresponds to the neurological maturity of 0- to 14-day-old rat pups and is characterized by peak rates of brain growth [10], exuberant synaptogenesis [11], and expression of specific receptor populations. Neuronal receptors include the excitatory N-methyl-D-aspartate (NMDA) receptors, AMPA/kainate receptors, and metabotropic glutamate receptors, with widely distributed sites in the brain and key roles in neuronal proliferation, differentiation [12], migration [13], synaptogenesis [14], and synaptic plasticity in the immature brain [15]. NMDA receptors allow Ca^{2+} entry into the cell, which leads to phosphorylation of second messengers and changes in gene regulation. NMDA receptors reach a peak density at birth [16, 17] and are coupled with an increased magnitude of ligand-gated Ca^{2+} currents [18] in newborn rats. They are abundantly expressed in the human fetal brain as well [19]. Immature neurons appear to have greater vulnerability to excitotoxic damage [20], which may be due to altered molecular mechanisms for Ca^{2+} signaling [21]. This enhanced vulnerability of the immature nervous system, in the setting of increased stimulation by pathological stressors, may lead to an excessive amount of Ca^{2+} entry into the cell, which can initiate excitotoxic cell death. Prolonged blockade of NMDA receptors or the activation of neuronal cytokine receptors (e.g., the TNF-α receptor) may also trigger apoptosis in developing neurons [22], directly or indirectly, through the sequential activation of initiator (e.g., caspase-8, caspase-9) and effector caspases (e.g., caspase-3) [23–25]. This critical period is also characterized by enhanced degrees of naturally occurring neuronal death (or physiological cell death) via apoptotic mechanisms [26, 27]. Such neuronal cell death follows a developmental pattern, affecting particular brain regions during specific developmental phases, such as the brain stem in the perinatal period [28], thalamus, and other subcortical areas soon after birth [29, 30], and cortical areas in the first 2 postnatal weeks [30–32]. The regional expression of Bcl-2 and caspase-3 appears to mediate this susceptibility to neuronal apoptosis, and this phase is terminated by the reduced cellular expression of caspase-3 [33]. In situ hybridization revealed a profound developmental regulation of caspase-3, the main effector enzyme for neuronal apoptosis, with a high abundance of caspase-3 mRNA observed in fetal and neonatal neurons and decreased expression in adult neurons [34]. Rabinowicz

et al. calculated that large numbers of cortical neurons undergo apoptosis after 28 weeks of human gestation, with neuronal numbers decreasing by more than 50% to achieve a stable number of neurons at birth [26]. This vulnerability is not limited to neurons but extends to glial elements of the nervous system as well. Volpe and colleagues demonstrated that oligodendroglial cells present in premature human infants are exquisitely sensitive to free radical injury [35]. The predominant mechanism of oligodendroglial cell death occurs by apoptosis. This sensitivity to free radical injury is maturation-dependent, as mature oligodendroglia survive in much greater numbers when exposed to free radicals [36].

18.1.2 Effects of Early Adverse Experiences

The developmental regulation of excitotoxic and apoptotic mechanisms heightens the susceptibility of the immature nervous system to the adverse experiences in preterm neonates. Accordingly, models of hypoxic-ischemic injury in neonatal rats show increased neuronal necrosis in the cerebral cortex, striatum, thalamus, and hippocampus [37]. Viral infections of the neonatal mouse brain can cause increased cortical and hippocampal apoptosis [38], whereas remote stressors such as neonatal peritonitis also lead to neuronal and astrocytic injury, associated with impaired integrity of the blood-brain barrier in the frontal cortex [39]. Survival experiments further report a hyperresponsiveness of the hypothalamic-pituitary-adrenal (HPA) axis, correlated with permanent changes in the median eminence and hippocampus of adult rats following exposure to neonatal endotoxemia [40]. Neonatal intensive care aggressively treats hypoxia, hypoglycemia, or sepsis, but other factors contributing to neuronal damage, such as repetitive pain or maternal separation, have received little therapeutic attention until recently [41]. When the 8-year-old children studied by Peterson et al. were receiving neonatal intensive care, it was customary to ignore the effects of invasive procedures (e.g., heel lancing, venous catheterization, chest tube placement, etc.) or adverse environmental stimuli (e.g., loud noises, bright lights). Recent clinical and experimental observations suggest that the repetitive pain caused by invasive procedures, and maternal separation leading to a lack of social (tactile, kinesthetic, and verbal) stimulation, may have independent and perhaps interrelated [42] effects on the developmental vulnerability of immature neurons.

That repetitive or prolonged pain can insidiously hinder cognitive development has been largely ignored. For example, a similar pattern of long-term behavioral changes was noted in adult rats that were exposed to repetitive acute pain during the first postnatal week. Rats exposed to neonatal pain had lower pain thresholds during infancy, with increased alcohol preference, defensive withdrawal behavior, and hypervigilance noted during adulthood [43]. Neonatal rats subjected to inflammatory pain (induced by injection of formalin, complete Freund's adjuvant (CFA), or carrageenan) also manifested robust behavioral changes during adulthood. Following CFA injection in the neonatal period, adult rats were hyperresponsive to subsequent painful stimuli (pinch, formalin injection) [44]. Following exposure to repeated formalin injections, adult rats showed longer latencies to the hot plate, decreased alcohol preference, and diminished locomotor activity [45].

It is likely that these long-term behavioral changes may result from developmental alterations of the immature pain system at the peripheral, spinal, and supraspinal levels [41]. At the peripheral and spinal level these changes include increased sprouting of peripheral cutaneous nerves and their primary afferent connections with the dorsal horn of the spinal cord, corresponding to the area of tissue injury [44, 46]. Somatotopically related dorsal horn neurons showed a marked hyperexcitability, both at rest and following noxious stimulation, as well as decreases in their receptive field size [44, 47]. In the cortical areas associated with pain processing, it appears that repetitive inflammatory pain in neonatal rats leads to a significant accentuation of naturally occurring neuronal cell death [48]. Specific regions, particularly in areas of the piriform, temporal, and occipital cortex, show twice as many neurons dying in the 1-day-old and 7-day-old rats subjected to inflammatory pain as compared to age-matched controls, but this vulnerability was not evident in 14-day-old rat pups [48]. Mechanisms leading to these long-term changes may include neuronal excitotoxicity (mediated via activation of NMDA or other excitatory receptors) or apoptosis (mediated via inflammatory cytokine receptors or mitochondrial injury). NMDA-dependent mechanisms not only mediate the spinal transmission of pain but also the long-term effects of pain, such as hyperalgesia, allodynia, windup, and central sensitization [49], involved in the pathogenesis of chronic pain states [50, 51].

Accumulating data suggest that exposure to neonatal pain promotes an increased susceptibility to chronic pain states mediated by NMDA-dependent neuroplasticity [52, 53]. If neonatal pain or localized inflammation truly produces these long-term changes, then analgesia or anti-inflammatory treatment should prevent or ameliorate the expression of the reported cellular and behavioral changes. A paucity of published data, however, does not allow any firm conclusions in this regard. One recent experiment showed that preemptive analgesia with morphine in neonatal rats exposed to inflammatory pain reduced some, but not all the long-term behavioral changes noted in adult rats [45]. Preliminary evidence for the beneficial effects of preemptive morphine analgesia in preterm neonates comes from a blinded and placebo-controlled, randomized clinical trial, which suggests a reduced incidence of early neurological injury in the morphine-treated neonates [54]. The cognitive and neurobehavioral outcomes from a larger clinical trial (currently underway) may answer the question of whether the outcomes reported by Peterson et al. are altered by opioid analgesia, thus supporting the possibility that these changes resulted from pain-induced neuronal or white matter damage [55].

18.2 Summary and Conclusions

The neurodevelopmental outcomes of preterm neonates remain a cause for deep concern. We propose that NMDA-mediated excitotoxicity resulting from repetitive or prolonged pain and enhanced apoptosis due to maternal separation are the two primary mechanisms leading to enhanced neuronal cell death in the immature brain. Thus, neurodevelopmental abnormalities will depend on genetic variability as well

as the timing, intensity, and duration of these adverse environmental experiences. Altered development during infancy may lead to reductions in hippocampal volume, abnormal behavioral and neuroendocrine regulation, and poor cognitive outcomes during subsequent life. Ameliorating the subtle brain damage caused by these mechanisms may have a colossal public health and economic importance. Thus, concerted efforts by neuroscientists and clinicians to investigate the mechanisms underlying early neuronal stress, efforts to minimize the impact of adverse experiences in the neonatal period, and novel strategies for improving neurodevelopmental outcomes are justified.

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Long-Term Consequences of Pain and Stress in Neonates

19

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19.1 Introduction

Historically, researchers believed that human neonates were not fully capable of perceiving localized pain. This was based on the assumption that immaturity of the nervous system, more specifically, incomplete cortical synaptogenesis with thalamocortical fibers and incomplete myelination limit the processing of nociception. Numerous investigations in both animal and human studies conducted over the past 30 years have produced evidence to strongly dispute this view [1–4]. Moreover, studies utilizing near-infrared spectroscopy (NIRS) have demonstrated specific somatosensory cortical hemodynamic changes during painful stimuli (venipuncture) in preterm neonates suggesting the conscious sensory perception of pain exists [5]. Evidence is clear that in even the most prematurely born infants, for example, at a postmenstrual age of 24 completed weeks of development, the neuronal circuitry and neurochemical capacities necessary for nociceptive processing of pain are intact [6].

It is well known that neonates experience frequent and often unnecessary procedures which invoke moderate to severe-level pain experiences, with the most common of these observed exposures being skin-breaking procedures, i.e., heel prick and vein punctures [7]. The frequency of these procedures may be as often as 16 daily to as many as 134 times during the first 2 weeks of life [8–10]. While the physiologic and behavioral responses of pain in neonates have been well described (see previous discussion, Chaps. 9 and 10), the long-term effects of repeated exposures to pain and stress have been less well studied. The purpose of this chapter is

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to first present a review of stress dysregulation within the context of prematurity and stress-related painful events; second, to examine potential linkages among pain/stress exposures, brain structure, and neurobehavioral development; and, third, to address the potential long-term impact of pain/stress exposures on families and health care providers.

19.2 Prematurity and Stress Regulation

Selye described the human stress system as a highly complex system that acts to protect the body by responding to internal and external stressors to achieve stability for the organism. Agents or experiences that cause stress or "stressors" arise from the organism's external, internal, and psychosocial environment [11]. Accordingly, organisms show a systemic response of resistance to a stressor, which in the early phase enhances system functioning. Over time with repeated exposures to the same stressor, unique responses occur related to the adaptive capability or "conditioning factors" required for systemic regulation. Development and neural function establish the extent to which responses to stressors are efficient or dysregulated, creating allostatic load [12]. In situations where environmental demands exceed coping regulatory capabilities, toxic stress and neurobiologic dysfunction may result [13].

In comparison with the protection of the intrauterine environment, the infant was required to leave prematurely; the neonatal intensive care unit (NICU) is harsh. Despite developmental sensitivity to NICU design [14], preterm infants experience bright lights, noise, disruption of sleep with frequent handling episodes and multiple invasive procedures [15]. These experiences of high environmental demand on an immature system lead to repeated episodic periods of autonomic and hypothalamic-pituitary-adrenal (HPA) activation, which are associated with dysregulation and higher morbidities during the neonatal period [16–18]. Also, heightened sensitivity to noise and handling episodes (i.e., diaper change) can be perceived as painful in vulnerable preterm infants [16, 19, 20]. Early life experience and the impact of protracted stress activation during this critical window of postnatal development are known to alter neuroendocrine and neurobehavioral development and function [21–23]; however, the long-term impact of these effects is less well understood.

Several observational studies have demonstrated that children born preterm, but without neurological deficits, that are followed through school age have subtle neurobehavioral changes including lower cognitive scores and higher rates of behavioral problems [24–26]. The incidence of mild to moderate deficiencies occurs with high frequency in this population. In fact, more precisely, reports indicate that for preterm infants born less than 30 weeks gestation as many as 40% will show mild motor deficits [27] and upward from 30 to 60% will experience cognitive impairments and emotional/social problems at school age [28].

In a carefully designed meta-analysis using pooled data from 31 studies conducted from 1980 to 2001, 1556 preterm-born cases were compared with 1720

term-born controls for cognitive and behavioral outcomes at school age. Quality assessment of studies was determined by selecting studies that used only case-control study design (with concurrent evaluation of controls) and an attrition rate of less than 30% [29]. Investigators found that term-born controls had significantly higher cognitive test scores at school age than preterm-born cases, with mean cognitive scores directly proportional to both birth weight and gestational age (GA) ($p < 0.001$ for both). Also, preterm-born children demonstrated higher externalizing and internalizing behaviors as well as more than a twofold increased incidence of attention-deficit hyperactivity disorder (ADHD) [29]. However, the specific impact of repeated stressors, painful procedures, and/or maternal separation on outcomes at school age was not evaluated in these studies.

19.3 Impact of Stress/Pain Exposures on Brain Development and Behavior

Brain behavior studies in animal models have helped to elucidate the role of early postnatal environment on endocrine responsiveness and later social development. Meaney and colleagues (1988) studied the effects of an early postnatal environmental manipulation on behavioral and endocrine responses in newborn male Long-Evans rats. Neonatal rat pups in the experimental group were subjected to the stress of handling and separation from dams for 15 min daily during the first 3 weeks of life. Subsequently, after the handling episode, both dams and rat pups were returned to their cages without further environmental manipulation. The control litters were left undisturbed in their cages. Findings of this research were that the handled rat pups at all ages secreted less glucocorticoids in response to stress and had lower basal glucocorticoid levels than non-handled rats. The physiological mechanism underlying the lower glucocorticoid response was a permanent increase in glucocorticoid receptor (GR) concentration within the hippocampus in the handled rat pups [30].

The hippocampus is a critical region in the brain for glucocorticoid feedback inhibition over hypothalamic corticotropin-releasing hormone (CRH) synthesis. Glucocorticoids damage the neurons indirectly by disrupting energy metabolism, thus compromising the ability of the neuron to survive metabolic changes. Hypersecretion of glucocorticoids causes neuronal cell death within the hippocampus leading to a degenerative cognitive impairment. The non-handled neonatal rat pups progress in adulthood to show higher spatial memory deficits as well as increased neuronal cell loss in comparison with the handled aged rats. The diminished rate of hippocampal neuron loss in the adult rats handled during the neonatal period is secondary to the lower cumulative lifetime exposure to glucocorticoids [30].

Investigations by Francis and associates (1999), Ladd et al. (2000), and Liu and colleagues (2000) provide further elucidation to the physiological mechanisms that impact stress in the newborn rat. In these studies, it was demonstrated that handling

of rat pups resulted in changes in the dam-pup interaction. Dams of handled pups had shorter but more frequent nesting bouts and demonstrated increased licking and grooming of pups with less passive posturing during nursing. Furthermore, offspring of the high licking-grooming mothers showed increased glucocorticoid feedback sensitivity and decreased hypothalamic CRH expression mediated by increased glucocorticoid receptor (GR) expression, suggesting a non-genomic behavioral transmission of individual differences across generations of rats [31–33].

Increased hippocampal GR mRNA expression, increased central benzodiazepine receptor levels in the central and basolateral nuclei of the amygdala, and decreased corticotropin-releasing factor (CRF) mRNA in the paraventricular nucleus of the hypothalamus occurred in the offspring of high licking-grooming mothers [31, 32]. Tactile stimulation through maternal licking and grooming regulates pup physiology and affects central nervous system development. More specifically, the effects of maternal care on the development of the stress system are thought to be mediated through changes in the levels of expression of specific genes in the brain that regulate stress response.

In an experiment by Francis, Diorio, Plotsky, and Meaney (2002), researchers set out to determine the effects of reversibility of behavioral, hormonal, and glucocorticoid receptor (GR) expression of maternal separation on offspring in Long-Evans rats. The rats were exposed to either handling (H) or maternal separation (MS) daily for the first 2 weeks of life. HPA responses to stress were significantly greater in MS control rats compared with H rats. At the time of weaning, peri-pubertal control rats were reared in standard housing, while the environmentally enriched rats were housed in larger cages with interconnecting burrows and novel toys. There were no group differences in HPA responses to stress among animals raised in the environmental enrichment program. In addition, the control MS rats were more fearful than those raised in the environment enrichment program. Thus, environmental enrichment reversed the effects of maternal separation on both HPA and behavior responses to stress. The authors posit a functional reversal of the neural and behavioral effects of early life adversity caused by maternal separation, and HPA activation occurred because of environmental enrichment. However, there were no changes on the permanent effects of increased hypothalamic corticotropin-releasing factor (CRF) gene expression [34].

The theories derived from these early animal investigations have been supported through a growing body of evidence in studies reported since 2004. In a recent systematic review of 40 studies (13 animal studies and 27 human studies), the impact of early adverse life experience, persistent changes in glucocorticoid receptor gene expression through methylation and persistent behavioral changes have been demonstrated [35].

Similar to rat pups, preterm human neonates are especially vulnerable to stress as they have multiple and repeated exposures to vast amounts of noxious environmental stimuli (i.e., handling, light/noise exposure, painful procedures) at a time when they are not yet developmentally capable of successful adaptation ex utero and often are separated from their mothers for extended periods of time. However, studies to examine repetitive stress exposures in human neonates are more challenging due to

both practical and design issues. In part, this is secondary to the difficulty of maintaining large datasets for extended periods (neonatal period through school age). For example, prospective longitudinal studies, best suited for studying the long-term effects, require large sample sizes to control for the many confounding factors, i.e., sociodemographic, genomic, and environmental factors that are known to impact long-term neurobehavioral development. In addition, these types of studies are more difficult because of the often high numbers of subjects lost to follow-up. Despite these challenges, numerous quality investigations have been conducted to evaluate the effects of neonatal stress/pain in the neonatal period, through infancy, and development at school age and adolescence.

In 2011, Smith and colleagues conducted a prospective cohort study of preterm infants (<30 weeks GA) where a cumulative stress index was documented from enrollment within 24 h of birth until discharge or term equivalent using the Neonatal Infant Stressor Scale (NISS) [36]. Magnetic resonance imaging (brain metrics, diffusion, and functional MRI) and neurobehavioral testing at term equivalent postmenstrual age (PMA) were used to evaluate cerebral structure and function. Importantly, covariates of immaturity and severity of illness were controlled for in the analysis of data. Investigators found increased cumulative exposure to stressors was associated with lower frontal and parietal brain width, alteration in diffusion and functional connectivity within the temporal lobes, and motor behavioral abnormalities (movement and reflexes) at term equivalent age [37]. Findings from this study importantly demonstrate that cumulative stress exposure in the NICU environment is associated with regional alterations in brain structure and function during the neonatal period through term equivalent gestation. However, a sufficient number of subjects were lost to follow-up or technical difficulties, as only 26 of 44 subjects had sufficient quality MRIs for diffusion analysis. Although long-term outcomes have not yet been reported, investigators report planning to follow this cohort of former preterm infants at school age for neurocognitive, behavioral, and motor testing.

In a longitudinal study, Grunau and colleagues (2009) studied 211 (137 preterm- mean gestation 29 weeks GA, 74 term- mean gestation 40 weeks GA) infants prospectively from birth to term gestation with both groups of infants born during the same time frame and delivered at the same hospital, the major tertiary center for the province of British Columbia, Canada. Infants underwent follow-up neurodevelopmental testing at 8 and 18 months corrected chronological age (CCA) using Bayley Scales of Infant Development with subscales of Mental Developmental Index (MDI) for cognitive and language development and Psychomotor Developmental Index (PDI) for gross motor development. Chart reviews were conducted to determine infant neonatal and maternal characteristics (i.e., GA, BW, illness severity, and maternal sociodemographics) as well as infants' days of mechanical ventilation, daily dosage of intravenous morphine, and total sum of all skin-breaking procedures (including failed attempts). Parents completed surveys on parenting stress using the Parenting Stress Index and were rated on videotaped interactive parent-child play sessions conducted at 8 and 18 months CCA. Parents were rated by blinded,

inter-rater reliable coders for parenting interaction domains of gratification, affect, sensitivity, and organization. Data were analyzed using RMANOVA, Pearson correlations, and hierarchical regression to examine unique relationships of neonatal and parent predictor factors on each outcome measure at 8 and 18 months CCA. The key finding was an association between a higher frequency of neonatal skin-breaking procedures and poorer cognition and motor function at an adjusted chronological age of 8 and 18 months. This association was independent of early illness severity, overall intravenous morphine, and exposure to postnatal steroids. Also, exposure to morphine was associated with poorer motor development only at 8 months CCA; however, the extent to which morphine alone impacted this negative outcome is inconclusive, it could not be concluded. Investigators also found that lower parenting stress modulated the effects of neonatal pain, but only on cognitive outcome at 18 months [38]. These findings are among the first to demonstrate a direct link between early repeated episodes of pain-related stress with poorer neurodevelopmental outcomes in infancy and toddlerhood.

In a prospective cohort study of 86 very preterm infants (24–32 weeks gestational age [GA]), Brummelte et al. conducted an investigation to study linkages between procedural pain exposure in the NICU and early brain development using magnetic resonance spectroscopic imaging (MRSI) and diffusion tensor imaging (DTI) at median 32 and 40 weeks GA born neonates at the regional tertiary referral center British Columbia, Canada [39]. Careful documentation of every skin-breaking procedure was conducted by the bedside nurse throughout the course of the infant's hospitalization and later quantified by the research team as total number of skin-breaking events from birth to term gestation. Research nurses also collected data via chart review on infant demographic characteristics, illness severity, mechanical ventilation days, daily morphine doses, surgeries, and morbidities, for example, infections and necrotizing enterocolitis. Cumulative exposure to sedatives and narcotics was calculated and averaged as daily dose per day, times number of days the drug was administered. Generalized estimating equation modeling was used to examine relationships among procedural pain and clinical variables with white and subcortical gray matter diffusivity. Investigators found that after adjusting for multiple clinical factors (infection, illness severity, and analgesic medication), greater procedural pain exposure was associated with reduced white matter and reduced subcortical gray matter. Sequential analysis of scans showed that there was a primary and early effect on subcortical structures with secondary white matter changes [39]. These findings support that early and frequent stress-inducing painful procedures alter the developing brain structure.

As part of the ongoing research program of pain-related stress and brain development in very preterm infants conducted at the Child and Family Research Institute, BC, Canada, investigators proposed to investigate the effects of neonatal pain-related stress (adjusted for clinical confounders of prematurity) on cortical thickness in a group of 42 very preterm (24–32 weeks GA) born infants now at 7 years of age. Children with major sensory/cognitive impairment and severe brain injury were excluded from participation [40]. The results showed that after adjusting for

neonatal confounders, greater neonatal pain-related stress exposure was associated with significant thinning of cortical thickness mainly in the frontal and parietal regions at school age [40].

Isaacs and colleagues compared a group of 11 adolescents who were former very low birth weight (VLBW) preterm infants born at less than 30 weeks gestation with age-matched controls, former term infants with normal neonatal courses [41]. The purpose of the study was to explore the relationship between memory deficits and neuropathology using cognitive testing, parent questionnaires, and quantitative magnetic resonance imaging. The results showed that the children who had been VLBW babies had significant deficits in every day memory, by cognitive testing and parental report, and had a striking deficit in mathematics ability, especially numerical operations. In addition, in the former preterm, VLBW group, there was a significantly lower bilateral hippocampal volume ($p = 0.002$), despite normal total intracranial volume and normal head circumference [41]. Certainly, the exact mechanism for this finding is unknown. However, it is reasonable to postulate that the mechanism involves adverse early life experience on an immature stress system with related impact on brain structure and neurobehavioral functional changes.

In a longitudinal study, children between 9 and 14 years of age who were former NICU preterms (GA $\leq$ 31 weeks; $N = 19$), NICU full-terms (GA $\geq$ 37 weeks; $N = 20$), and control full-terms (GA $\geq$ 37 weeks; $N = 20$) were tested for perceived sensitization to tonic heat and repeated mechanical stimulation as well as heat pain and mechanical pain thresholds at thenar and trigeminal nerve regions [42]. Thermal stimulation was done using a small, 16 × 16 Peltier thermode, and subjects were instructed to increase the temperature until pain threshold was reached and then held constant for 30 s with subjects unaware. Subjects were next asked to readjust temperature by lowering, increasing, or leaving it when at pain threshold. Difference between timepoints was used to determine perceptual sensitization. Heat pain threshold was determined by increasing temperature from baseline by 1 °C/s. Subjects were asked to press a button when they felt pain; temperature was then returned to baseline by researchers. Thresholds were determined by averaging five trials.

For mechanical stimulation, a blunt tip adapter was used with seven standardized punctate probes. Mechanical perceptual sensitization was determined using a numeric rating scale (NRS) from 0 = no pain to 100 = worst pain. Change in NRS was averaged over three trials. Children were blind-folded during trials. Mechanical pain threshold was done starting with lowest intensity pinprick and applied in five ascending and descending series. Mechanical pain threshold was calculated as the mean of the five sub- and suprathreshold intensities. Group differences were analyzed by ANOVAs and Tukey's post hoc tests. Results showed that both preterm and full-term-born children with NICU exposure had greater tonic heat perceptual sensitization and elevated heat thresholds at both thenar and trigeminal sites. Mechanical pain threshold and perceptual sensitization were not different between groups [42].

These findings show that NICU experience with repeated stress-pain exposures is associated with enhanced perceptual sensitization to prolonged painful stimuli and hypoalgesia to brief painful stimulation. Thus, repeated pain exposures during the neonatal period, a critical period of development, likely alter functioning of pain pathways that persist to school age. While the exact nature of this process is unknown, studies in rodents demonstrate that pups that undergo separation from dams and/or undergo repeated handling have higher somatic pain thresholds, elevated morphine analgesia, and reduced stress-induced analgesia as adults [43]. Taken together, these studies support that early life environmental pain-stress exposures lead to altered HPA responsiveness and altered pain processing through central sensitization and changes in pain modulation. Additional long-term follow-up studies are needed to disentangle these exact relationships.

19.4 Consequences of Pain/Stress on Families and Health Care Providers

The unexpected birth of a preterm infant, characterized by incomplete prenatal preparation and sudden separation from the newborn [44], and admission to the NICU lead to parental stress, fatigue, financial worries, separation, and isolation from home and family and might adversely affect the parent-infant relationship [45]. Parents often struggle to understand and deal with the special developmental needs of prematurely born infants, and their anticipatory thoughts and related emotions are likely to make effective behavior toward their preterm infant even more difficult for them [46]. Helping parents to understand the "behavioral language" of their preterm infant, including responding to behavioral distress/pain signals, can strengthen parental mastery and self-confidence, leading to a sense of control, competence, and empowerment [47]. However, parents often relay that they have limited knowledge and involvement concerning pain management in the NICU setting. Our group conducted a single-center pilot, observational study on 20 parent couples regarding their stress-related experiences and parental advocacy role during the time of their infant's hospitalization in NICU. Findings were that while both mothers and fathers felt confident in their neonate's care, felt they had adequate access and time with their infant, and felt supported in their role as parent, a major issue for parents was that they expressed concern regarding their lack of involvement in providing pain relief/comfort to their infant. Mothers reported considerably more distress in their lack of involvement in providing pain relief than fathers (Unpublished data, Veneman, Brelsford, Doheny, 2013).

Similar to our findings, results from a multi-site study conducted on parents of hospitalized NICU infants in both the UK and US demonstrate that parents have concerns regarding pain related to the following issues: the potential impact of pain on developing organ systems, lack of consistent information on pain management, barriers to their involvement in staying with and comforting their babies during painful procedures, and health care providers inconsistency in the recognition and management of pain [48]. Parents reported that pain associated with procedures was

the most common cause of their infant's pain [38, 48]. Parents expressed emotional distress over their infant's pain experience and also voiced concern about the potential long-term impact these experiences of their own worry and distress may have on their future relationship with their infant [48]. These concerns have merit, as studies have demonstrated that lower parental stress modulates the impact of neonatal procedural pain on cognitive function at 18 months adjusted chronological age [38]. Further, parents continue to recall and voice concerns many years after discharge from the NICU related to the potential long-term impact that the high technological environment and many procedures their infant endured will have on their child's development [49].

Strategies that include parents in providing care and comfort to their infants like staying with their infant and providing skin-to-skin holding during a procedure or learning sensorial saturation and massage are opportunities to help parents to feel more involved and important to their infant's recovery [50–52]. In addition these strategies provide important opportunities for parents to act as *parents*, the caregivers most capable of providing protection, nurturing, and love to their infant. By promoting parental advocacy within a family-centered developmental model of care, parents are more likely to feel welcomed and involved in the caregiving, and thus more confident in the provision of care and successful in their parenting role.

The consequences of unmitigated pain/stress on neonates and families also place significant burden on health care providers. While this is not limited to nurses, nurses are often the most highly impacted because they are the ones having the most direct and frequent exposures to observing pain and distress in infants and families. The issue of iatrogenic pain caused by life-sustaining technologies is of grave concern for neonatal and pediatric intensive care unit nurses. This concern may rise to the level of "moral distress" whereby one's personal moral judgment is competing with barriers or constraints to action [53]. This may be the case when nurses are caring for ventilator-dependent infants that are not receiving adequate pain control [54], or if they are required to inflict pain in order to provide life-sustaining care to the extremely premature neonate [55]. In a qualitative study of 24 neonatal nurses regarding nurses' experiences of caregiving dilemmas with extremely premature infants, the major theme that emerged was "inflicting pain," whereby nurses often viewed themselves as "torturers" and expressed "it's agony for us as well" [55]. This dilemma is understandable when the relief of pain and suffering is germane to the discipline of nursing, pain assessment documentation is reported as the fifth vital sign [56], and, yet, the complexity of pain assessment and uncertainties in management of pain for the extremely preterm are tangible [57]. Overwhelming conflict arises when professionals are expected to relieve pain and suffering, but, they are unable to do so. Unresolved conflict leads to feelings of powerlessness and despair or in detachment and distancing behaviors to avoid emotional burnout [55]. Possibly the best solution to dealing with moral distress related to unmitigated pain is to support a mutual understanding of all stakeholders perspectives through open dialog with all members of the health care team, including families, in order to determine a consensus for a unified and ethical approach to relieve pain and suffering. In addition, future efforts in neonatal care must be directed at

technological advancements to reduce pain and stress for procedures known to cause pain, critically evaluating that a painful procedure is truly warranted, and eliminating all unnecessary procedures.

19.5 Additional Ethical Considerations

Infant advocacy groups have spoken on the behalf of infants and parents to assure that the humane treatment of newborns includes limiting research studies on pain to those well-designed studies that carefully weigh benefit above risk [58–60]. In fact, ethics committees and institutional regulatory bodies have argued against research studies exposing newborns to additional risk [61, 62], for example, as with a research protocol that would include any additional procedure likely to cause discomfort. In this view, research studies on pain should be carefully designed around necessary procedures scheduled as part of clinical care (not only for the purpose of research) and where standardized approaches to pain treatment are always offered.

Ethical considerations also encompass providing a welcoming space for families to stay with their neonates in the intensive care environment and to facilitate that families provide ongoing nurturance to their infant [63]. This importantly includes the provision of comfort care and assurance that adequate pain relief is provided during painful or stress-evoking procedures. The context of caregiving for the preterm infant should be within an individualized and developmentally supportive environment that places the family central to providing emotional support and comfort to their infant [64]. When procedures are necessary, appropriate pre-procedure analgesia and supportive developmental approaches administered by parents, for example, containment and skin-to-skin holding [65] should be utilized to reduce stress and pain.

19.6 Summary

As presented in this chapter, repeated exposures to pain-induced stress reactivity during the neonatal period have been associated with long-term deleterious effects including alterations in pain sensitivity and cortisol regulation, changes in brain structure and function leading to potential cognitive impairment, and behavioral and emotional regulation issues at school age. Repetitive episodes of pain/stress also have been shown to produce permanent alterations in sensory processing (i.e., ADHD, behavioral problems), with the severity of long-term effects dependent on the maturational stage during which the exposures occurred. The prevention of pain is imperative as unmitigated pain is unethical given the strong evidence demonstrating the neonate's ability to experience pain and suffer long-term consequences.

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Drawbacks of Analgesics in Neonatal Age: How to Ensure Safe and Effective Use in Newborns

20

Karel Allegaert and John N. van den Anker

20.1 Introduction

The myth that immaturity protects neonates from pain perception and its negative effects was shown to be untrue by Anand et al. when they demonstrated that untreated perioperative pain resulted in increased morbidity and mortality. Moreover, these negative effects were also observed in later pediatric life and beyond [1, 2]. In essence, adequate analgesia in neonates should not only be given because of empathy or ethics, but it is a valid, appropriate, and needed part of medical and nursing care. More recently, experimental data in animals have provided evidence that perinatal exposure to analgesics also results in reduced brain growth, decreased neuronal packing density, and less dendritic growth and branching [3, 4]. This is because analgesics affect axonal growth and neuro-apoptosis. There seems to be an

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age-related window of vulnerability for apoptosis or dendritic changes related to human neonatal life and infancy, respectively. These anatomic findings are associated with persistent motor and learning disabilities. Besides the neurodevelopment issues, other compound-specific side effects (e.g., bleeding tendency, hepatic impairment, atopy, renal impairment, blood pressure) should also be considered. Although some of the concepts discussed in this chapter can also be applied to other compounds (e.g., benzodiazepines, propofol, inhalational agents, dexmedetomidine, clonidine, ketamine) or techniques (locoregional or spinal techniques) considered for analgesia, this chapter will focus on the short- and long-term side effects of opioids, nonsteroidal anti-inflammatory drugs (NSAIDs), and paracetamol (acetaminophen) in neonates. At the end, we will provide some guidance on how we think the topics on side effects discussed in this chapter should be integrated into clinical pain management in neonates.

20.2 Opioids: Still the Golden Standard Analgesics?

Morphine is probably the most extensively evaluated analgesic in neonates and can be administered by oral (bioavailability 30%) or intravenous route. Morphine is a narcotic analgesic that stimulates opioid receptors within as well as outside the central nervous system. This explains effects (sedation, analgesia, miosis) and side effects (bladder retention, paralytic ileus, respiratory depression). *Fentanyl* is the first of a sequence of synthetic, fat-soluble opioids (sufentanil, alfentanil). It penetrates faster into the central nervous system because of its fat solubility, resulting in a faster effect as compared to morphine. Besides morphine and fentanyl, there are also observations on shorter-acting opioids in neonates. *Alfentanil*, *sufentanil*, and more recently *remifentanil* have been used mainly for procedures with an anticipated short duration, such as endotracheal intubation, retinal laser surgery, or percutaneous intravenous central catheter placement while there is anecdotal experience during major surgery and to maintain analgo-sedation during mechanical ventilation [5]. The analgo-sedative effects disappear very soon after discontinuation of remifentanil since the drug is cleared very rapidly. When used for major surgery, anticipation and replacement by another (longer) acting opioid or non-opioid analgesic is needed, or the remifentanil infusion should be prolonged. Further continuation will, however, more likely result in potential negative effects such as opioid-induced tolerance or hyperalgesia since these phenomena are more common when opioids with a short elimination half-life are administered as compared to morphine [5].

The benefits of morphine in neonatal pain largely depend on the clinical indication (ventilation and respiratory support, surgery, procedural pain). In the latest meta-analysis on the use of opioids in ventilated preterm neonates ("Neopain studies"), Bellu et al. concluded that there is insufficient evidence to recommend routine use of opioids in mechanically ventilated newborns [6]. It was suggested that opioids should be used selectively, when indicated by clinical evaluation of pain indicators. We hereby should not forget that—if sedation is required—morphine is safer than midazolam. In the setting of postoperative analgesia following "major" surgery, opioids are needed, either as monotherapy or as part of multimodal analgesia.

There is even strong evidence from a randomized controlled trial supporting the benefits of opioids on neonatal outcome [1], either as continuous or intermittent administration, in part driven by the pharmacokinetic characteristics (morphine versus shorter-acting opioids). Finally, the evidence concerning the effective use of morphine for procedural analgesia is much more limited.

20.2.1 Short-Term Side Effects

Short-term side effects of morphine observed in the abovementioned studies include intestinal paralysis, bladder dysfunction, hypotension, and respiratory depression. Rarely, seizures may occur. A similar pattern of side effects can be seen with the use of fentanyl.

Decreased intestinal motility has been observed and was associated with reduced morphine clearance and higher morphine concentrations, while oral naloxone coadministration improved intestinal motility [7, 8]. As a consequence, morphine delayed attainment of full enteral feeds (+3 days, 20 instead of 17 days) in the Neopain trial [9]. We could not find data on the incidence and extent of bladder dysfunction, but based on our clinical practice, this does occur. Hypotension is a more relevant side effect, and the available data reported in different studies likely reflect differences in the morphine doses used. Simons et al. were unable to document differences in arterial blood pressure, the need to coadminister inotropic medications, and blood pressure variability related to the morphine infusion (100 μg/kg loading dose, 10 μg/kg/h maintenance dose) [10]. In contrast, preemptive morphine infusions (100 μg/kg loading dose, 10–30 μg/kg/h maintenance dose), additional morphine administration, and lower gestational age were associated with hypotension among preterm neonates in the Neopain study [2]. Finally, the respiratory depression also results in prolonged duration of ventilation (+1 day) [11].

Based on the available evidence, fentanyl does reduce acute pain, but does not reduce prolonged pain and adds an additional cost caused by an increase in duration of ventilation and paralytic ileus. Chest wall rigidity and/or laryngospasm has been associated with fentanyl administration in neonates, while withdrawal symptoms should be anticipated when continuous infusion goes beyond 5 days. High doses of fentanyl may result in neuro-excitation and, rarely, seizure-like activity. Compared to morphine, tolerance (higher dose/concentration needed for same effect) during continuous fentanyl appears sooner. Its use has also been associated with postoperative hypothermia [12].

20.2.2 Long-Term Side Effects

Neonates requiring intensive care experience a significant and clinical relevant number of stressful and painful procedures. Management of stress and pain is therefore an important issue. To mitigate the effects of repeated painful stimuli, opioid administration for analgeso-sedation is very common in neonates. A growing body of laboratory and animal evidence suggests a link between long-term harm and the

use of opioids in newborn infants [13]. This has also initiated clinical research investigating the relationship between exposure to morphine and neurodevelopmental outcome. For neurocognitive outcome after neonatal exposure, there seems to be an age-dependent trend when we combine the results of the Neopain and Rotterdam studies [2, 10].

At term equivalent age, neurobehavior (Neurobehavioral Assessment of the Preterm Infant, NAPI) in former preterm neonates included in the Neopain study documented subtle differences (motor scores, popliteal angle) in those exposed to morphine analgesia [14]. Poorer cognition was associated with a higher number of skin-breaking procedures, independent of early illness severity, overall intravenous morphine, and exposure to postnatal steroids at the corrected age of 8 and 18 months in a cohort of 137 preterm (<32 gestational age) infants. Higher exposure to intravenous morphine was associated with poorer motor development at 8 months, but no longer at 18 months corrected age [15]. For the Neopain study, there is only a small pilot study looking at the effect of preemptive morphine administration on head circumference (smaller), social behavior (more social problems), and response latencies (slower) at the age of 5–7 years, while IQ tests were similar [16].

The Rotterdam group reported more recently on the outcome of their morphine cohort at the age of 5 years and observed some minor differences in specific subtests (visual analysis) of the IQ tests [17]. At the age of 8–9 years, there was no longer a negative neurocognitive outcome association with morphine exposure in the same cohort [18]. Van den Bosch et al. documented in a small subgroup of 19 former preterm neonates at the age of 10 years of this cohort that the brain volume was significantly associated with prematurity, the number of painful procedures, and the extent of opioid exposure [19]. However, morphine exposure itself had no effect on neurocognitive development. Similar positive observations were reported by the same research group for patients after neonatal ECMO and surgery [20]. Finally, using a structural search of experimental and clinical data on morphine exposure in preterm neonates, Schuurmans et al. recently concluded that experimental animal and human clinical data displayed conflicting results on the effects of neonatal morphine on neurodevelopmental outcome. In contrast to specific short-term neurological outcomes, long-term neurodevelopmental outcome in human neonates seems not to be affected by morphine [21].

20.3 Nonsteroidal Anti-inflammatory Drugs: Rarely Administered as an Analgesic

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a heterogeneous group of medicines that act by cyclooxygenase (COX) inhibition, resulting in antipyretic, analgesic, and anti-inflammatory effects. NSAIDS are considered to be very useful analgesics as part of multimodal analgesia in, e.g., postoperative care or after trauma

in children and adults [22]. A meta-analysis of perioperative NSAID administration in children showed that it reduces opioid consumption (standard mean difference −83%) and PONV (OR 0.75) in the first 24 h after surgery [23]. However, in (pre) term neonates, the clinical use of these compounds has been primarily focused on closing a patent ductus arteriosus, and the clinical experience with its use as an analgesic is very very limited, but not nonexisting.

The available observations are limited to retrospective analyses of effects and side effects in five small cohorts in a total of 157 (pre)term neonates or infants exposed to ketorolac. A retrospective review of ten patient records of infants (<6 months) suggested an opioid-sparing effect of ketorolac administration [24]. In 18 spontaneously breathing former preterm infants with chronic lung disease, ketorolac (intravenous, 1 mg/kg) administration resulted in pain control in 17/18 cases after surgery, and no hematological, renal, or hepatic side effects were observed [25]. In 57 surgical neonates and young infants exposed to ketorolac, bleeding events were observed in 17.2% and were more common in neonates below 37 weeks of correct gestational age [26]. Using a similar study population, 4/53 had minor episodes of bleeding, and all showed a minor increase in creatinine shortly after cardiac surgery [27]. Finally, ketorolac was tolerated well (renal, hepatic, transfusion needs, analgesia) in 19 young infants (<6 months) following cardiac surgery (biventricular circulation) [28].

Common short-time side effects relate to gastrointestinal, renal, and thrombocyte functions. While gastrointestinal- and thrombocyte-related side effects are less pronounced in neonates, renal impairment is more pronounced in neonates with a transient reduction in glomerular filtration rate of 20% during acetylsalicylic acid or ibuprofen up to 40% during indomethacin exposure [29]. A similar pattern can be observed for the incidence of necrotizing enterocolitis or for the duration of mechanical ventilation (ibuprofen to indomethacin, relative risk 0.64 and mean difference −2.4 days, respectively) [30]. Another additional specific issue in preterm neonates may be the appearance of acute pulmonary hypertension during ibuprofen infusion.

For some of the potential long-term side effects (atopy, neurodevelopmental impairment), we refer to the section on paracetamol, since this drug [31] also has some peripheral and more robust central COX inhibitory effects. Besides these issues, there is a concern that exposure to NSAIDs in preterm neonates may also result in long-term drug-induced renal damage through impairment of glomerulogenesis [32]. Bueters et al. recently described the impact of early postnatal NSAIDs (indomethacin, ibuprofen) treatment on nephrogenesis in the Wistar rats and hereby documented that these compounds inhibit kidney development (e.g., nephron numbers −12%) [33]. This confirms earlier findings on the impact of ibuprofen on the developing kidney in a preterm baboon model (30% reduction in nephrogenic width) [34] and of perinatal indomethacin but not ibuprofen exposure on the glomerular number (−12–15%) in the adult rat [35].

20.4 Paracetamol in Neonates: An Old Dog with New Tricks?

Paracetamol, N-acetyl-p-aminophenol (acetaminophen), is a readily available, over-the-counter analgesic and antipyretic drug. It is the most commonly prescribed medicine to treat mild to moderate pain or fever in neonates and can be administered by different (oral, rectal, intravenous) routes. Although intravenous paracetamol is still off-label for specific subpopulations (e.g., limited to term neonates or under the age of 2 years in the United States) within the neonatal setting, these formulations are increasingly used in (pre)term neonates in an attempt to avoid or reduce opioid exposure [36, 37].

Adequate management of pain in neonates is a major issue in contemporary neonatal care. In an attempt to avoid opioids, there is an emerging use of paracetamol. However, we should be aware of the differences in currently available evidence to support the use of paracetamol for procedural versus postoperative pain in neonates. In essence, the available data suggest a poor analgesic effect of paracetamol for *procedural* pain relief [37]. In contrast, there is published data on the morphine-sparing effect of paracetamol in neonates and young infants following noncardiac *surgery* or during stay in the NICU. The currently available evidence on paracetamol as analgesic supports the use of paracetamol for minor to moderate severe pain syndromes in early infancy, and paracetamol has a very relevant opioid-sparing effect (−66%) after major noncardiac surgery in neonates, but also in preterm neonates (−54%) when we focus on cumulative morphine exposure [38, 39]. Following recruitment of 71 neonates and infants undergoing major noncardiac surgery in a randomized placebo-controlled setting, coadministration of intravenous paracetamol resulted in a significant reduction (−66%) in morphine exposure [38]. In a cohort of 108 preterm neonates (<32 weeks gestational age), paracetamol (loading dose 20 mg/kg, followed by 7.5 mg/kg q6h) was administered in early (<72 h) neonatal life and resulted in a reduction of 54% compared to a historical control group from the same unit and using the same pain assessment tool (neonatal infant acute pain assessment scale) [39]. In contrast, there is only a very poor analgesic effect of paracetamol when used for procedural (e.g., heel lancing) pain relief [37].

20.4.1 Short-Term Side Effects

Undesired short-term side effects of paracetamol described in other populations mainly relate to *hepatotoxicity* or *hemodynamic* effects. Prospective data suggest good hepatic tolerance, but individual cases with hepatic toxicity potential related to paracetamol in newborns have been observed, and more advanced tools for pharmacovigilance have been suggested. Similarly, hemodynamic effects of paracetamol in neonates are modest with the suggestion to be more careful in the specific setting of impaired hemodynamics in neonates. There were no signs of hepatic intolerance during and following repeated administration of intravenous paracetamol [40]. The hemodynamic side effects of intravenous paracetamol in neonates are very modest,

similar to the recent quantification (mean arterial blood pressure, 1.85; 95% CI −2.6 to −1.1 mmHg) in healthy adult volunteers, and were explained by a transient reduction in systemic vascular resistance [41, 42].

However, these observations have mainly been made in (pre)term neonates <32 weeks gestational age, while reported observations on tolerance in extreme preterm neonates <28 weeks are very limited. Despite these limitations, case reports, case series, and randomized trials describe the use of paracetamol in neonates who had contraindications or who previously failed nonsteroidal anti-inflammatory drug therapy for a patent ductus arteriosus. An association between paracetamol exposure and closure of the PDA has been reported in a limited number of preterm neonates. High doses of paracetamol are hereby suggested, and a median paracetamol serum concentration of 15 mg/l is likely after a dosing regimen of 15 mg/kg q6h dosing. However, a target paracetamol concentration that induces closure of the ductus arteriosus is still unknown, while the safety of such a high-dose paracetamol in extreme preterm neonates is still uncertain [37, 43].

20.4.2 Long-Term Side Effects

Besides these short-term outcome side effects, recent epidemiological data also show a possible link between the (over)use of paracetamol in pregnancy as well as in infancy and an emergence of different kind of pathologies throughout childhood (immune deviations, neurodevelopmental impairment). Because these studies describe associations, causality remains questionable and certainly not yet proven. At least, further pharmacovigilance is warranted to unveil the complex, potential causal association [37, 43].

There has been an exponential increase in the frequency of immune deviations in young children. Consequently, research investigating environmental causes for this increase became a public health priority. Paracetamol—similar to, e.g., ibuprofen—has a nonselective inhibitory action on peripheral cyclooxygenase 2 activity, besides its central action. This inhibition of acetaminophen on COX2 only relates to low arachidonic acid concentrations and explains the difference between ibuprofen and paracetamol in anti-inflammatory effects. The impact of repeated mucosal PGE2 synthesis inhibition on the development of tolerance to food antigens has been demonstrated in some animal experiments and should be further explored in human infants [44].

A recent meta-analysis of epidemiological datasets suggests a link between paracetamol exposure and subsequent risk (odds ratio 1.2–1.3) to develop asthma [43]. However, exposure to paracetamol throughout pregnancy and/or lactation in a pregnant mice model had no effects on allergic airway diseases in the offspring at weaning and at 6 weeks of age (house dust mite intranasal model). Consequently, these mechanistic observations do not support the hypothesis that perinatal paracetamol exposure increases the risk of childhood asthma [45]. This may at least in part be explained by confounding by indication, i.e., antipyretic intake because of

respiratory tract infections. Sordillo et al. recently tried to control for this confounder. Adjustment for respiratory tract infections in early life substantially diminished, but did not completely abolish the association between infant antipyretic use and early childhood asthma (paracetamol and ibuprofen, unadjusted odds ratio 1.21 and 1.35 to 1.03 and 1.19, respectively) [46].

Similar to atopy, animal experimental findings as well as epidemiological associations suggest a link between paracetamol exposure and adverse effects in the developing brain. In view of a recent report in mice of adverse effects on the developing brain from paracetamol [47] and reports of an association between prenatal paracetamol and the development of autism or autism spectrum disorder in childhood [48–50], long-term follow-up to at least 18–24 months postnatal age must be incorporated in any studies of paracetamol in the newborn population [51].

Brandlistuen et al. explored the impact of prenatal paracetamol exposure using a sibling-control approach and observed an impact on gross motor development, communication, externalizing and internalizing behavior, and higher activity level. Ibuprofen exposure was not associated with these neurodevelopment outcome parameters [48]. Bauer and Kriebel described a synchronous rise in autism spectrum disorder prevalence in paracetamol use-associated prenatal or postnatal paracetamol (circumcision) exposure and hereby provided an ecological link between both [50]. Frisch and Simonsen provided indirect evidence and confirmed the association between neonatal circumcision (3347) and autism spectrum disorder (4986/342,877 cases) with a hazard risk of 1.46 (95% CI 1.11–1.93) but linked this observation to associated paracetamol exposure in Denmark [49].

A similar link has been suggested between perinatal paracetamol exposure and attention deficit hyperactivity disorder (ADHD, 13–37% relative increase) [52]. Similar, Thompson et al. documented an association between paracetamol intake during pregnancy (in 49.8% of pregnancies) and subsequent ADHD symptoms at the age of 7–11 years. Assessment was based on validated questionnaires; the total study population was based on 871 infants, and similar associations were not documented for maternal intake of, e.g., antibiotics or antiacid drugs [53]. Finally and based on a Danish National Birth Cohort ($n = 64{,}322$ children), hyperkinetic disorders (hazard ratio 1.37) and ADHD (risk ratio 1.13) were associated with maternal prenatal paracetamol use [54].

We should be aware that the analgesic effect of paracetamol goes through the central nervous compartment, through inhibition of cyclooxygenase activity. Interestingly, the inducible form of cyclooxygenase 2 (COX2) gene is polymorphic, and the C allele variant (associated with reduced COX2 activity) was independently associated with worse cognitive outcome at 2 and 5 years in a cohort of 207 Caucasian preterm (<32 weeks) neonates [55]. This suggests that the phenotypic cyclooxygenase activity may affect neurocognitive outcome and may hereby provide a pathophysiological link between the long-term neurobehavioral outcome and perinatal paracetamol or ibuprofen exposure [47, 50, 55].

20.5 Discussion

Effective pain management remains an important indicator of the quality of care provided to neonates, but observations on neuro-apoptosis and integration of newer techniques and compounds force caregivers to reconsider the clinical and research aspects of "effective" pain management. There are data on an association between major neonatal surgery (number of interventions, disease severity) and neurodevelopmental impairment. However, exposure to analgo-sedatives is only one of the factors associated with negative outcomes [3, 4]. Obviously, neonates who repeatedly underwent anesthesia during infancy are more likely to have other risk factors for impaired neurodevelopment. At the same time, we know from animal experimental studies and clinical studies of Anand et al. that surgery without analgesia has also major impact of morbidity and mortality [1].

Effective and safe pharmacotherapy can only be achieved if integrated in a structured approach on pain management. Such a structural pain management plan should be based on *prevention*, *assessment*, and *treatment* followed by a *reassessment*. Effective pain control is based on preventive strategies including the decrease in the number of painful procedures and environmental stress, driven by systematic assessment of pain based on a validated assessment tool, and followed by titrated administration of the best fitted analgesic and subsequent reassessment. Systematic evaluation hereby will likely also result in decreases in analgesia exposure, when appropriate. The most recent observations on morphine strongly suggest to use lower doses [56] and to consider multimodal analgesia [38]. Data on paracetamol pharmacokinetics/dynamics in neonates are available and suggest that the same effect compartment concentration (10 mg/l) should be aimed for [57]. However, pain treatment in neonates is not limited to pharmacotherapy. Non-pharmacological interventions stress the fact that not only the kind of procedures matters, but also the way we perform painful procedures matters [58]. The focus needs to be on less invasive techniques, preventive strategies, or complementary techniques.

There are also shifts in our clinical practices and subsequent needs for analgesia. Although both the avoidance of mechanical ventilation and less invasive surfactant application are associated with reduced duration of analgesic or sedative treatment, the percentage of VLBW infants who receive analgesia and/or sedation has remained unchanged in Germany in recent years (German Neonatal network, 2003–2010), but with shifts toward novel drugs like sufentanil, propofol, and intravenous paracetamol [59]. Based on these data, it is clear that there are still important issues on pharmacokinetics, effects, and side effects of analgesics that deserve further evaluation, especially for the newer compounds that are used in neonates off-label and without sufficient validation (e.g., propofol, dexmedetomidine). We encourage all stakeholders to design dose-finding studies that are needed to improve adequate (i.e., effective, but without overexposure) administration of analgesics in neonates.

The feasibility of this approach has been illustrated for morphine studies [60]. As reflected in this chapter, such studies should not only focus on short-term outcome but should also cover different aspects of long-term outcome.

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Part V

Pain and Communication

Disclosure of Pathology to the Newborn's Family

21

P. Arosio

Care of high-risk newborns often involves complex ethical problems, such as quick decisions about questions with a high degree of uncertainty. It is not always possible to define recovery, establish a long-term prognosis or predict future quality of life. This indicates the complexity of factors involved in relating to parents. I have been working for 20 years in the Neonatal and Intensive Care Unit of San Gerardo Hospital, Monza. I am also the president of an association of families with handicapped children (Gli Amici di Giovanni), which is affiliated with the national association "Famiglie per l'Accoglienza". I am not an expert in communicating, but all neonatologists have had to break "bad news", such as neonatal pathology, to parents, and have therefore had occasion to reflect on this experience. I shall touch on some points that seem important in the dynamics of communication between neonatologists and parents. I shall start with some data relating to my background experience.

Communicating a diagnosis means "making it *common*", entering into a relationship with the family and child. Communication of a diagnosis should not be an isolated event, but the first step in a therapeutic journey, a journey that should be planned and accompanied in the best possible manner for the parents.

Once the diagnosis has been communicated, the parents must not be left alone with their doubts, fears and anguish. They must be able to spend as much time as possible near their child and take part in the therapy. Open wards where parents can be present for most of the day are of great importance. However, the initial approach is crucial and can predispose parents to accepting or rejecting the baby (especially if affected by certain pathologies). For example, communication of an unexpected disease, such as Down syndrome to young parents, dashes the image

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of the awaited child. The doctor must try to have awareness and courage in being as little invasive as possible, creating affinity and empathy with the parents to enable the dialogue to continue.

To prepare this paper, I used a letter that Dr. Bellieni wrote to me about 4 years ago, submitting a lesson of his to an updating course on neonatal care. The title of the lesson was "Who is the premature baby?" I remembered two points which are fundamental for entering into a relationship with the parents and for communicating the diagnosis [1]. It is not possible to talk of "what to do" or "what to say" to someone without having some idea of "who" that person is. Before being a clinical case or a set of symptoms, the newborn is a patient and a "you", a fragile you in a situation of great need and dependence [2]. This person or "you" cannot be conceived outside a history, a family, a couple, without risking interrupting the continuity of a life made of sensations, smells, sounds, movements, that the baby is experiencing and experienced throughout fetal life [2]. We therefore have to think of the newborn within the unit it forms with its mother and father. I think it is helpful to bear these two points in mind when continuing the work of forming a relationship with the parents.

Let us now look at two aspects of the problem: the reaction of parents to communication of the diagnosis (Table 21.1) and the reaction of the doctor and how the doctor approaches this problem. The reactions of parents when told about the baby differ but follow a well-defined sequence of emotions which may vary in intensity and duration [3]. It is difficult to predict which reaction will prevail, and sometimes backward steps are made. The state of shock is a condition of confusion and impotence in which even the simplest information is difficult to grasp. It is followed by despair, sadness, disbelief (hope in a diagnostic error), anger and denial (when there may be confused hopes that the baby will die or hasty decisions to abandon the child). They are human reactions, understandable and even necessary, though not all are obligatory for the situation to mature and evolve towards acceptance and hopefully taking in (embracing) of the child. This phase becomes evident when the

Table 21.1 Reaction of parents to news of an ill or malformed baby

Towards themselves	*Within the couple*
Shock	Trust
Blaming	Detachment
Despair	
Sadness	
Disbelief	
Anger	
Denial	
Taking responsibility	
Towards the baby	*Towards the staff*
Hyperprotection	Discharging tension
Medicalisation	Absolute dependence
Alienation	Dissatisfaction

parents begin to see and describe their baby as it is (as it laughs or cries, sucks, sleeps or fusses) [4]. This experience eventually reinforces the couple. Reactions to the child may be characterised by conflicting emotions (overprotectiveness–coldness). Later, reactions towards the ward staff also develop [5].

Much also depends on the reactions of the doctor. Apart from their specific skills, doctors find themselves in a situation of stress, the intensity of which is related to the gravity, chronic nature or untreatable nature of the baby's condition and prognostic uncertainty. Our words and attitude should take account of the different prognostic implications of the pathology in question (Table 21.2). In cases with good prognosis, the medical practitioner should explain the risks and benefits of therapy, reassuring the parents and eliciting their trust. When the prognosis is uncertain, possibly good, the doctor should sustain hope day by day, building trust in the treatment, in a therapeutic process that involves the parents and establishes human relationship. The figure of the doctor in charge of the case is important [6].

In cases where the pathology cannot be treated, it is more difficult to tell the parents, and the emotional impact on them is greater. Much depends on the doctor's experience, including experience of life, and his or her attitude towards disease and handicap. Limitations may be encountered in this area. The parents are initially in a state of shock and confusion that makes them unable to fully grasp the information the doctor gives them; however, they will sense if the doctor is willing to understand and participate in their situation. Much of the work parents must do to receive and care for their child is mediated by us, our glances, our words and our silences, an embracing or a cold and detached attitude. The way we look at the baby, the patient, is the same as the way we look at the people around us, our colleagues, the way we look at ourselves. Knowing this, some aims of communication (what to say, when and where to say it and how to say it) can have a truer content, a less technical and certainly less sentimental form [7].

Table 21.2 Prognoses of some neonatal pathologies (modified from [3])

Good prognosis
Hypothyroidism
Adrenogenital syndrome
Congenital treatable cardiopathy
Uropathy due to malformation
Omphalocele
Uncertain, possibly good prognosis
Severe prematurity with possible sequelae (bronchopulmonary dysplasia, retinopathy of prematurity, brain haemorrhage with hydrocephalus)
Untreatable
Down syndrome
Severe intraparenchymal brain haemorrhage
Severe asphyxia

21.1 What to Say?

The truth is essential, but with how many details? In the case of pathologies with a rapid course, such as extreme prematurity with possible sequelae such as retinopathy and chronic lung disease, the truth should be told as it evolves, with doses and timing appropriate for parents undertaking a difficult and not always linear process, with its ups and downs, like the baby's pathology. The truth is our capacity to accompany the baby and its parents, observing timing that is not ours.

If possible, the diagnosis should be communicated to both parents, showing and giving them the baby. Reality is always less dramatic than imagination. Today the procedures of prenatal diagnosis have reduced the emotional trauma of communicating malformations or pathology at the moment of birth as many are diagnosed in utero and have already been communicated. Collaboration is necessary with obstetricians and gynaecologists so that the problem can be tackled together [8].

21.2 When and Where to Say It?

The diagnosis must be communicated as soon as possible and with every new development. It should be done in a suitable quiet place, to both parents and no one else, giving them the opportunity to freely express their feelings (including weeping) and to ask questions [9].

21.3 How to Say It?

This is the most difficult part and the one that most involves the doctor emotionally. Basically, it should be said with clarity and simplicity. Technical terms should be kept to a minimum, especially initially, on the first occasion. Parents more readily grasp the non-verbal dialogue, expressed through the attention and willingness of the doctor to understand and share what they are going through.

The facts should be presented as they stand, realistically. This seems a play on words, but is not intended as such. It should be done without prejudice and preconceptions that we doctors have sometimes borrowed from the media. It should be done with awareness of that unique "you" mentioned at the beginning.

Some optimism should be maintained, without denying the problems, but underlining the positive aspects and possible therapies.

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Pain and Grief in the Experience of Parents of Children with a Congenital Malformation

22

Luigi Memo and Emanuele Basile

Approximately 3–4% of newborn babies have a congenital malformation. One of the most devastating life-changing events for parents is to find out their baby has a congenital malformation. The disclosure of a postnatal diagnosis of congenital malformations disrupts parental expectations of a healthy infant and changes the quality of life of parents and family functioning, activating a process of long and tiring adaptation [1, 2].

Clinical experience and studies have highlighted the importance of communication of diagnosis, which is one of the most critical moments of parents' experience.

In the last years, several studies have reported clinically significant stress reactions in parents particularly in the immediate period after communication of diagnosis. Some of these important psychological reactions are acute traumatic stress symptoms such as shock, disorientation, emotional instability, anger, loss, and hyperarousal [3, 4].

The presence of these reactions has led some researchers to propose post-traumatic stress disorder as a model to explain emotional and psychological reaction of parents after communication of diagnosis [5].

Communication of a pathological condition is a fundamental moment. The experiences of some parents are complicated and deep: "When she was born they told me that she was a beautiful child; I did not accept this, because it was not the truth."

"They give you the news and then they leave you alone, you don't know anything." "They said very little, very quickly, about everything our baby was showing."

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In a study in which 170 parents of children with a congenital syndrome answered a clinical–psychological questionnaire about how they received the information of their child's condition, the results showed that the news was extremely distressing and stressful in 53% of cases, hastily communicated and difficult to understand in 25%, and satisfactory as regards content and the manner of communication in only 15% (S. Intini, personal communication, National meeting of Italian CDLS Parent Support Group, Pesaro 2000).

In literature, several studies have highlighted wide variability of effects about communication of diagnosis on parents. Some researchers believe that the variability of effects may be on the one hand related with the severity of the child's physical and clinical characteristics and on the other hand with parental factors such as personality, intrapersonal resources, social context, and quality of family relationships [6].

Clinical experience emphasizes the difficulty of parents to understand and remember the contents of the communicated diagnosis. These results may be correlated with the way in which the diagnosis is communicated (use of technical language, lack of empathy, inadequacy of the context, etc.); on the other hand, it emphasizes the role of the emotional component in understanding the diagnostic information [7].

It is important to remember that during the communication of the diagnosis, the effect of the emotions on parents' rational ability reduces complete comprehension of information. Unsatisfactory communication has a negative influence on parents' emotions and psychological feelings, and this has consequences for clinical and diagnostic plans by reducing parent compliance. This fact can cause a break in the medical relationship and increase the time taken for examination and specialist consultations.

For these reasons, it is important to support the family, helping parents to process and overcome their sense of loss, with the aim of restoring family comfort and obtaining complete collaboration for diagnostic and therapeutic indications.

Several studies have highlighted the effects of poor communication on the process of care and on treatment choices. Frequently, parents consider the communicative competence of the physicians a point of weakness of their professionalism. For this reason, in recent years guidelines and communication protocols have been outlined with the aim of improving the competencies for physicians and promoting better communication of diagnosis [8, 9].

Studies regarding parents' preferences about communication of diagnosis emphasize the importance of three components: physician's expertise, emotional support provided, and comfortable setting [10].

Regarding the physician's expertise, there are some important competencies related to health-care communication such as to ascertain and respond to the parents' doubt, concerns, and expectations; to assist parents to reflect on the impact of their alternative decision; to develop a partnership with parents; and to agree on an action plan and define a follow-up program [9].

Moreover, it is important that communication takes place in a comfortable environment. Parents should be able to express their emotions and be supported. Some

aspects promote this aim such as private setting, empathic attitude, maintaining eye contact, speaking slowly and paying attention to the emotional reactions of parents, and spending whatever time is required on this critical moment.

In the immediate aftermath of the communication of diagnosis, parents' distress reduces their coping abilities. For this reason, the psychological support should promote the recovery of the rational skills needed to understand the severity of the child's problems and to make decisions.

In some cases, it may be useful to provide for the intervention of a psychologist with a dual purpose: to contain and support the emotional distress but also to provide the physician information about what the parents' understanding of the diagnosis and the child's problems is.

The severity of the information forwarded and its impact on parents' lives leads to consider the time of diagnosis in terms of communication process. The communication process must provide a number of meetings with parents to verify the correct interpretation of contents, to integrate information, to answer questions, and to support the decision-making process without affecting choices. After the first meetings, it is very important to organize subsequent meetings to gradually explain the various diagnostic aspects and their possible development [11].

The period after hospitalization is a very delicate phase of the experience of parents and should be properly organized. It must be planned in agreement with parents and the external pediatrician. A precise follow-up program should be offered and help in dealing with social services.

It is also important to identify a case manager that coordinates these activities. This figure could be the family pediatrician, the neonatologist, or the pediatrician who first examined the child. If possible, this specialist should be a pediatrician who specializes in genetic problems and is skilled in general pediatrics and clinical genetics.

Parents need to be aware of the social services available in their area in terms of specialized services for children with disabilities. Organizing this for them will reduce the distress of searching through different referrals providing supervision and fulfillment of their needs.

We recommend the development and establishment of an infrastructure within each hospital system that makes routine the provision of up-to-date and accurate information and the referral to parent support groups or other experienced parents of children with congenital malformation.

It is important for the couple to exchange practical information, receive support, and share experiences with other people in a similar situation. Hinkson et al. (2006) [11] feel that disorder-specific support groups are crucial, since they are composed primarily of caregivers who have first had experience of caring for similarly affected children and can provide appropriate information that is most likely to match the changing concerns of other caregivers. The neonatologist and/or the pediatrician should provide the family with information about these types of associations, leaving the parents free to decide themselves how and when to make contact.

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Invest in Prenatal Life: A High-Yield Stock

23

M. Enrichi

In the last 20 years, various associations have been formed, inside and outside academic and health circles, to provide information on prenatal life and the importance of this special period for our physical and emotional life and relationships. Founded in 1992, Associazione Nazionale Educazione Prenatale (ANEP) is the Italian chapter of the Organisation Mondiale des Associations pour l'Education Prénatale (OMAEP), founded in France in 1982, which now contains 18 national associations. Another association, Associazione Nazionale Psicologia Educazione Prenatale (ANPEP), was founded in 1999. An understanding of prenatal life needs to become part of the cultural heritage, especially for couples planning to have a family or expecting a baby and for school children. Thus the fascination of the first 9 months of life will leave a mark in the DNA of the heart, as well as in the personal cultural heritage that school inculcates in us all, and respect for life and its wonders will have a stronger foundation. Knowledge and respect for life, especially the bud of life, when it is so small as to seem insignificant and so defenceless as to seem in our power, can enable us to know our origins, which are the same for all of us—the basic equality—and need to be embraced in common by all of us, reinforcing ancient words of peace. Knowledge of prenatal life is therefore precious.

Prenatal life is a high-yield stock, because an increase in fetal health becomes an increase in adult health and because health is a basic right and social aim, especially at the start of life [1]. Investment in prenatal life pays because 9 months is worth a life. Prenatal life is a fundamental time for life because it is at the beginning. The environment, especially the mother's body, moulds prenatal development through experience. Experience is nourishment: biochemical, metabolic, sensory, cellular, emotional, relational, and cognitive.

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The studies of Barker have shown that physiology and metabolism change permanently when the foetus has to adapt to an unfavourable environment and that these programmed changes may underlie illness in adulthood [2]. The role of the environment is becoming increasingly recognized. It is increasingly evident that almost all illnesses have an environmental component, that the effect is much more harmful in the aetio-pathogenesis of disease if exposure to insult occurs during development, and that the outcome may be not only malformation but also functional deficit which may manifest later in life [3].

Prenatal experience is also a sensory experience. The work of Mauro Mancia, pioneer in the encounter between neuroscience and psychoanalysis, has shown that "sensoriality"—i.e. fetal sensory experience through integration of pons structures—underlies active sleep, stimulates synaptogenesis, determines implicit memory, participates in control of the vegetative system, and takes part in sensory transmodality. Active sleep is the nucleus of the baby's representational mode at birth; synaptogenesis is the process behind learning, memory, and intelligence and hence the cognitive self. Implicit memory is the nucleus of the emotional self and the axis of the self, namely, the emotional centre of personality. Sensory experience takes part in the control of the vegetative system, including the heart and respiratory control, and is linked with postnatal life, as we shall see. Sensory experience is involved in sensory transmodality, which is the fetal and neonatal capacity to pass information from one channel to another [4]. Finally, emotional and sensory experience is the experience of suffering and pain, lived through the troubles and stress of the mother or directly in the fetal body, and its mark remains long after birth as many neuroendocrinological, neuropsychiatric, and neonatological studies show [5–7].

Studies in psychology and psychoanalysis show that maternal representations in pregnancy—that is, the idea the woman has of herself during pregnancy, as a pregnant woman and as a mother, that she has of the baby and the relationship between them (which underlie motherhood and bonding of the newborn and baby with its parent figures)—are the operative model through which adults form relationships [8–11]. These representations form a thread of continuity between pregnancy and the postnatal period. They are present from the start of pregnancy and are quite well formed in the second trimester, a crucial time in which their construction undergoes acceleration [9–11]. These representations can be revised during pregnancy as new information is acquired and can therefore be modified [10].

Sensory experience is therefore a stimulus for sensorineuromotor construction. Sensory stimuli take part in the control of vegetative life before and after birth. It has been demonstrated that auditory input is important for maintaining diencephalic respiratory centre function. Auditory input in particular has a major role in regulation of breathing during neonatal sleep, to the extent that development of environmental acoustic stimuli is a major protective factor against cot death, as acoustic stimulation reduced the risk of central apnoea [12].

The fact that fetal cells pass into the maternal circulation during pregnancy has been known for over a century [13]. Recent studies indicate that cells transfer between the foetus and mother during pregnancy and can persist in both decades later, almost lifelong [14]. The presence within one individual of a small

population of cells from another genetically distinct individual is referred to as microchimerism [15]. A term pregnancy is not required for the development of fetal cell microchimerism and for a woman to become a chimera following pregnancy [14].

Natural microchimerism is maternal (mother's cells in the foetus) and fetal (fetal cells in the mother) [14]. The potential role of such placental transfer is still unknown, but the finding of a high frequency of fetal microchimerism in the maternal liver suggests the possibility that this migration may be important in the induction and subsequent maintenance of tolerance towards the foetus during pregnancy [16]. Persisting maternal and fetal microchimerism could be involved in the induction of some autoimmune diseases [15]. Alternatively, maternal and fetal cells may migrate to areas of tissue damage secondarily and function beneficially in repair [13, 17]. The medical consequences of pregnancy, therefore, appear to extend well beyond delivery [18].

Psychiatrists and psychologists have long since brought to light the importance of the mother–child relationship in utero. Prenatal life sculpts one's personality on the basis of this relationship. The basic principle of the human being is to-be-in-relation, to-be-with, Mitsein, dialogue [19]. Every stimulus is relative. The stimulus and the reaction will become a component of the personality that is to be, becomes "biologic", a true imprinting that is there to stay and yields the "personality's sculpture". Every stimulus reaches the foetus, and the foetus unfailingly reacts against the events reaching him; if they repeatedly trouble him, they produce a "silent trauma", forcing the foetus to raise defensive barriers that require a continuous waste of energy [19]. The mother and the environment send messages that influence the foetus, his relation with his mother and the world around him, and his brain development [6]. Today stress is a subtle and ever-present toxin that when acting on a pregnant woman can cause premature birth and infantile psychopathologies [5]. Clinical studies in the third trimester of pregnancy have proved that an important stress is associated with a statistically significant risk of neurobehavioural dysfunctions [6], that the first modality of "to-be-with" is built with the parents' emotional mood pattern [19], that there is reciprocity between bonding (the parent's attachment toward the child) and attachment (the child's tendency to attachment) [20], and that there is a correlation between prenatal bonding and postnatal attachment [21].

Prenatal education is to let both parents know how important it is that they are parents, because:

- The father is always present, even when he is not, because he is in the mother's mind [22].
- The primary triangle (first relation mother–father–child) is present starting from the prenatal period [20].
- Triadic interactions (mother–father–child) arise early if the parents respond appropriately and if they create an ever-growing state of awareness and if it is possible for there to be continuity in the interactive and affective organization between the prenatal and the postnatal period [20].

At this point, we can draw some conclusions about the principles of prenatal education. First and foremost is the principle that every child is a miracle, and since these days we all talk by slogans, we will conclude with some slogans:

- For the mother: "Take care of yourself."
- For the father, family, and society: Take care of the mother and prepare to welcome the baby. In other words—"Number one: ecology."
- For everyone: Get to know prenatal life—"To know it is to love it."
- For the mother and the father: Trust in your abilities and competence and at the same time trust in the vital strength of the child—"Equal dignity."
- For the mother and the father: Maximize all sensorial communications as much and as soon as possible—"It's never too early, it's never too late."

What can we do to invest in prenatal life? Apart from health policy and medical aspects, it is important that people be informed about prenatal life. Knowledge of prenatal life arouses a sense of wonder and rapture, potentiating the perception of fetal life as something precious and increasing respect for the developing embryo and the woman bearing it. This has many good repercussions, making pregnant women prefer a healthier and more appropriate lifestyle. Couples also weave a richer and more complex relationship by thinking and caring about the baby. Finally, all of us and the society itself begin to wish to create a more protective environment for the unborn baby and its mother.

We believe that all this can contribute to change the experience of prenatal life on which the life of adult humans is built.

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Erratum to: Neonatal Pain—Suffering, Pain, and Risk of Brain Damage in the Fetus and Newborn, Second Edition

Giuseppe Buonocore and Carlo Valerio Bellieni

Erratum to:
Chapters 20, 21 and 22 and Appendix in: Giuseppe Buonocore and Carlo Valerio Bellieni *Neonatal Pain*, DOI 10.1007/978-3-319-53232-5

Owing to an unfortunate oversight the contribution by Karel Allegaert and John N. van den Anker was initially published as an Appendix to the book and incorrect authorship had been provided. It has now been changed into a regular chapter and author names have been corrected. The Appendix has been integrated as chapter 20, "Drawbacks of Analgesics in Neonatal Age: How to Ensure Safe and Effective Use in Newborns"

As a consequence the chapter numbers and the page numbers for the following chapters have changed compared to the originally published versions as follows:

Chapter 20: Disclosure of Pathology to the Newborn's Family by P. Arosio has become chapter 21

Chapter 21 Pain and Grief in the Experience of Parents of Children with a Congenital Malformation by Luigi Memo and Emanuele Basile has become chapter 22

Chapter 22 Invest in Prenatal Life: A High-Yield Stock by M. Enrichi has become chapter 23

In addition, the Preface and the Introduction have been included in the Table of Contents.

The updated online versions of Chapters 20, 21, 22, and 23 can be found at:
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