■ REVIEW

The Neurobiology of Pain: Developmental Aspects

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Invasive procedures that would be painful in children and adults are frequently performed on infants admitted to the neonatal intensive care unit. This article discusses sensory responses to these procedures in the immature nervous system and highlights the fact that, in addition to causing distress and delayed recovery, pain in infancy is also a developmental issue. First, the immaturity of sensory processing within the newborn spinal cord leads to lower thresholds for excitation and sensitization, therefore potentially maximizing the central effects of these tissue-damaging inputs. Second, the plasticity of both peripheral and central sensory connections in the neonatal period means that early damage in infancy can lead to prolonged structural and functional alterations in pain pathways that can last into adult life. NEUROSCIENTIST 7(3):246–257, 2001

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It has been reported that a preterm infant in intensive care can undergo more than 300 invasive procedures in a week, many of which are tissue damaging (Barker and Rutter 1995; Stevens and others 1999). The effect of these procedures on the developing sensory nervous system responses is a subject of considerable recent research and has highlighted the importance of the developmental aspects of pain.

Pain responses can be simply divided into three types: immediate responses lasting seconds to minutes, persistent responses lasting days and weeks, and prolonged responses that outlast the clinical period and may continue for years. Current research suggests that each type is triggered by different neurobiological mechanisms. Here we discuss the postnatal development of these three types of pain response and their underlying developmental neurobiology.

The Immediate Infant Pain Response

The response to invasive procedures and tissue damage such as heel lancing and venipuncture in preterm infants has been measured in a variety of ways, notably in terms of crying, changes in facial expression, heart rate, respiration, sweating, body movement, hormonal responses (see Franck and Miaskowski 1997), and flexion reflex responses (Fitzgerald and others 1988b; Andrews and Fitzgerald 1994). Spinally mediated reflexes to mechanical skin stimulation are exaggerated in young infants compared with the adult, with lower thresholds and more synchronized, longer-lasting reflex muscle contractions. This is paralleled in laboratory animals, where thresholds for withdrawal from mechanical, heat, and chemical stimuli are also lower and responses greater in amplitude in younger animals (Fitzgerald and others 1988b; Guy and Abbott 1992; Falcon and others 1996; Hu and others 1997; Teng and Abbott 1998; Marsh and others 1998a). The exaggerated spinal responses are in contrast to the facial expression response, which is weaker in younger infants and increases with postnatal age (Johnston and others 1993, 1995). This may reflect a slower onset of the affective or emotional response to pain as compared with the sensory-motor response, but because little is known about the maturation of central pain processes in the brainstem, thalamus, and cortex, this remains speculation.

The Persistent Infant Pain Response—Inflammation

The trauma of repeated procedures will result in local inflammation and hypersensitivity, which, in adults, is characterized by an enhanced sensation of pain to a noxious stimulus (hyperalgesia) and an abnormal sensation of pain to previously nonnoxious stimuli (allodynia). In addition, there may be spontaneous or ongoing pain (Cervero and Laird 1996). The hyperalgesia that follows tissue injury can be divided into primary and secondary hyperalgesia. Primary hyperalgesia develops at the site of an injury and appears to arise largely from peripheral nociceptor sensiti-

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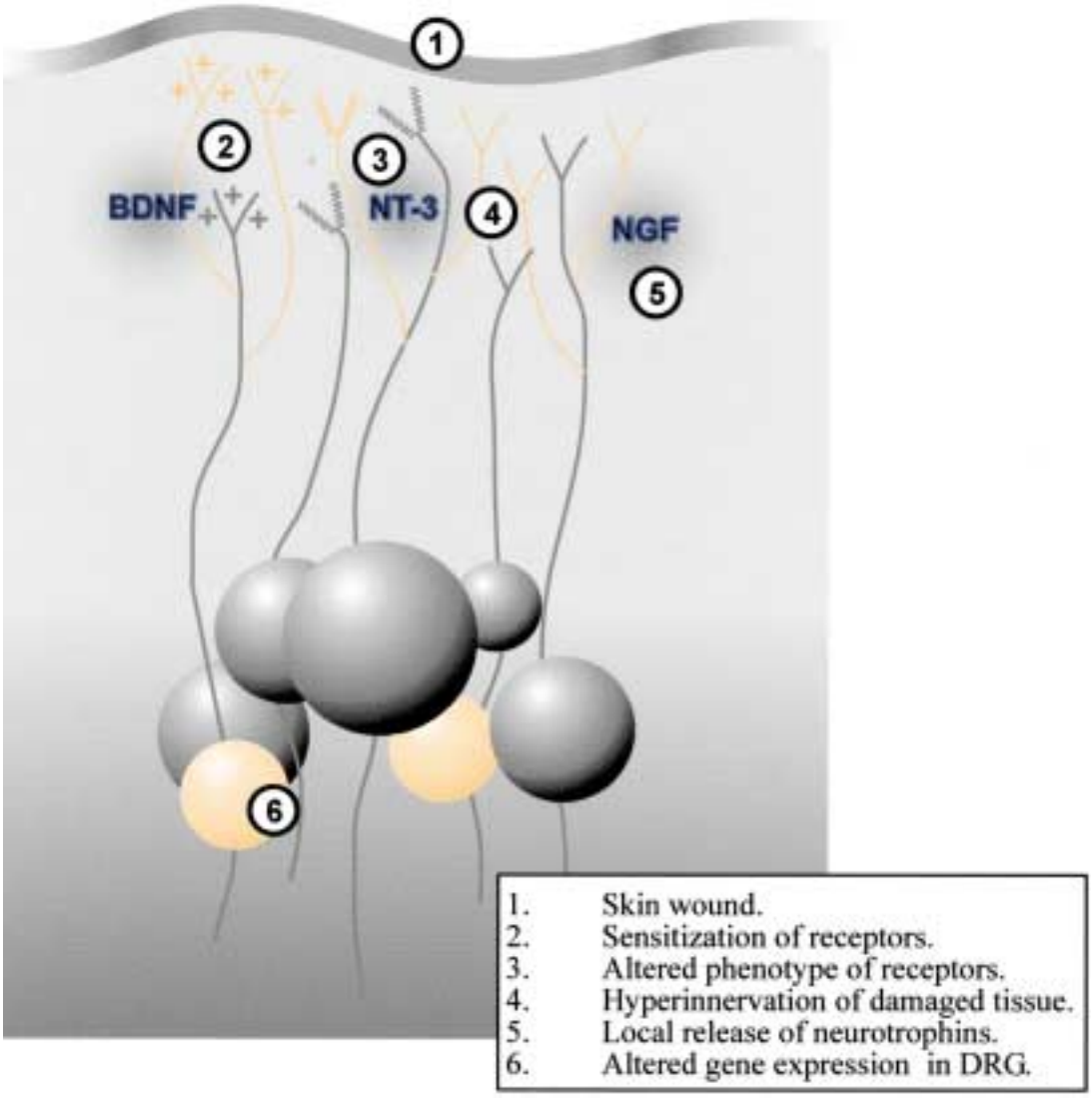

Fig. 1. Different ways in which early tissue damage in infancy can influence peripheral cutaneous sensory terminal structure and function. While many of these changes will subside with the resolution of the inflammation, others may outlast the injury for a prolonged period. DRG = dorsal root ganglion.

zation. Surrounding this is a zone of secondary mechanical hyperalgesia and allodynia, which is proposed to arise from central plastic changes in spinal cord connectivity modifying CNS responsiveness to future stimuli.

In very young infants, cutaneous reflex responses become sensitized upon repeated mechanical stimulation even in the innocuous range. Response magnitude increases and threshold decreases after repeated innocuous mechanical stimulation at 10-sec intervals. This effect is greatest at the 28- to 33-week postconceptional age group and is lost by 42 weeks (Fitzgerald and others 1988a; Andrews and Fitzgerald 1994, 1999). There is also good evidence that even the youngest infants are capable of displaying hypersensitivity following noxious, inflammatory stimuli. The mechanical sensory reflex threshold of preterm infants in an area of local tissue damage created by repeated heel lances is half the value of that on the intact contralateral heel (Fitzgerald and others 1988). The "tenderness" is established for days and weeks in the presence of tissue damage but can be alleviated by repeated application of lignocaine-prilocaine cream (EMLA) (Fitzgerald and others 1989). This response can spread outside the immediate area of injury. Preterm infants that have spent time in intensive care and have done so with established leg injuries from repeated procedures also show significantly lower sensory thresholds even on the intact, contralateral foot (Andrews and Fitzgerald 1994). The low thresholds are similar to those of much younger preterm infants. Although spinal responses are sensitized under these conditions, maturation of facial expressions in response to heel lances are delayed by frequent invasive procedures (Johnston and Stevens 1996). Recently, a striking hypersensitivity has been observed in preliminary studies in postsurgical infants

less than 1 year old, where abdominal surgery leads to a fall in the sensory reflex thresholds in the wound area and surrounding hyperalgesia (Andrews and others 2000).

Behavioral responses to persistent inflammatory pain have not been fully examined in young rat pups. The hyperalgesia or drop in mechanical threshold that follows carageenan injection (Marsh and others 1998b) and mustard oil application (Jiang and Gebhart 1998) is smaller in amplitude in postnatal day 3 (P3) rat neonates than at P21. This may be a reflection of the high level of background sensitivity of young rats, which limits the degree of hypersensitivity that they can display. Nevertheless, it is possible to demonstrate a clear enhancement of amplitude and duration of the flexion reflex (de Lima and others 2000) and of dorsal horn cell responses (Torsney and others 2000) following carageenan injection from P3.

The Prolonged Infant Pain Response—Beyond the Clinical Period

Sensitization to early injury may last even longer than the period of clinical care. The most notable example of this is the observation that circumcised infants display a stronger pain response to subsequent routine vaccination at 4 and 6 months than uncircumcised infants. This effect is attenuated by preoperative treatment with lidocaine-prilocaine cream (EMLA) (Taddio and others 1997). In addition, it has been reported that children with birth weights under 750 g but without overt neurological damage are still at high risk for neurobehavioral dysfunction and poor school performance. Social disadvantage as a major determinant was ruled out, and although many factors are likely to be involved, early sensory experiences in intensive care may be important (Hack 1994). This issue has been addressed in a study in which infants born at less than 1000 g were assessed at 18 months of age and found to have significantly lower pain sensitivity compared with controls (Grunau and others 1994a, 1994b). Unlike control children, the pain sensitivity of the very smallest group was not related to temperament, which suggests that some mediator has interfered with normal pain development. At 4 months of age, these infants' biobehavioral pain responses were similar to normal birth weight infants, but subtle differences were observed in cardiac autonomic responses to heel lancing among ELBW infants (Oberlander and others 2000).

The effects of repeated neonatal noxious stimulation in infancy upon later development have been modeled in rat pups by application of needle prick stimulation four times each day from P0 to P7. As adults, the rats displayed decreased withdrawal latencies to intense heat, increased preference for alcohol, increased latency in exploratory and defensive withdrawal behavior, and a prolonged chemosensory memory in social discrimination tests. The authors suggest that the rats have an altered ability to cope with stress and pain in adulthood (Anand and others 1999).

Advances in the developmental neurobiology of peripheral and spinal mechanisms of somatosensory processing and pain have opened new avenues of research in infant pain. Below, we discuss possible neural mechanisms for immediate, persistent, and prolonged infant pain.

The Neurobiology of Infant Tissue Damage—Peripheral Effects

Repeated tissue damage in newborn infants will naturally activate primary sensory neurons in the skin and underlying tissues. In many ways, these afferents respond in a similar way to those of adults. In the rat pup, C-fiber polymodal nociceptors, responding to mechanical, thermal, and chemical noxious stimuli, have mature thresholds and firing patterns at birth. High-threshold Aδ mechanoreceptors are less mature, and low-threshold, rapidly adapting Aβ mechanoreceptors responding to touch or brush are, relatively, the most immature at birth, with lower frequencies of firing and response amplitudes than those of adults (Fitzgerald 1987; Fitzgerald and Fulton 1992). At 2 weeks of age, mice A fibers still have reduced conduction velocities and immature stimulus-response functions (Koltzenburg and others 1997).

Local consequences of inflammation include the release of algogenic substances from damaged cells, recruitment of inflammatory cells, and release of further mediators from cells in the vicinity. These include H^+ and K^+, serotonin and histamine, bradykinin and prostaglandins, nitric oxide, cytokines, and growth factors (Woolf and Costigan 1999). These may directly activate peripheral nociceptors to cause pain, but more often they act indirectly to sensitize nociceptors and alter their response properties to subsequent stimuli (Yaksh 1999). The recent molecular cloning of membrane receptors for capsaicin, protons, and heat has greatly advanced knowledge of nociceptive signal transduction (Caterina and Julius 1999), but as yet very little is known about the developmental regulation of these receptors (see Alvares and Fitzgerald 1999). A component of the adult inflammatory response is neurogenic, arising from the release of substance P (SP) from peripheral C-fiber terminals. The onset of production of neuropeptides by C fibers appears to be triggered by peripheral innervation (Jackman and Fitzgerald 2000), and levels remain low in the postnatal period (Marti and others 1987; Reynolds and Fitzgerald 1992). SP is apparently not released in sufficient quantities from peripheral C fiber terminals to produce neurogenic extravasation until P10 in rats (Fitzgerald and Gibson 1984), although exogenously applied SP can produce extravasation before this age (Gonzales and others 1991).

The Importance of Neurotrophic Factors

The release of neurotrophic factors upon tissue damage is a major factor in the production of inflammatory

pain. Nerve growth factor (NGF) injections cause hyperalgesia in neonatal rats (Lewin and Mendell 1993), and neutralization of endogenous NGF prevents the hyperalgesia and sensitization of nociceptors supplying inflamed skin (Woolf and others 1994a; Koltzenburg and others 1999a). Tissue damage in infancy causes substantial up-regulation of neurotrophins in the skin, almost fourfold that seen in adults (Constantinidou and others 1994; Alvares and others 1999).

Neurotrophic factors will have more far-reaching effects if administered or up-regulated in the neonatal period. The physiological properties of primary sensory neurons are highly influenced by the levels of neurotrophic factors in the skin during a critical period of development. In addition to the well-known regulation of sensory neuron survival by neurotrophic factors earlier in development (Snider 1994; McMahon and others 1996), they also regulate physiological phenotype. NGF and neurotrophin 3 (NT-3) levels are critical for the differentiation and the mechanical sensitivity of Aδ and C fiber nociceptors, and brain-derived neurotrophic factor (BDNF) also influences A fiber mechanoreceptor properties (Lewin and Mendell 1994; Carroll and others 1998; Koltzenberg and others 1999b). Therefore, while also triggering mechanical hyperalgesia and pain from the injury (Koltzenburg and others 1999a), increased levels of nerve growth factor protein levels in the skin in infancy could produce permanent change in the relative proportions of nociceptors and low threshold mechanoreceptors and their final physiological sensitivity. In addition, in early postnatal life the majority of C nociceptors express trkA and are therefore NGF sensitive (Bennett and others 1996), and this is down-regulated over the postnatal period, making them especially sensitive in this period.

Equally important is the fact that neurotrophin levels determine the innervation density of the skin. Excess NGF and BDNF lead to skin hyperinnervation (Albers and others 1994; LeMaster and others 1999), whereas excess IGFII leads to hypoinnervation (Reynolds and others 1997). The up-regulation of neurotrophic factors provides a likely explanation for the observation that early skin wounds lead to long-term hyperinnervation and hypersensitivity of the injured area. Tissue damage in the early postnatal period in rats causes a profound and lasting sprouting response of the local sensory nerve terminals, leaving an area of hyperinnervation in the wounded area that extends into adulthood (Reynolds and Fitzgerald 1995; Alvares and others 2000). Parallel behavioral studies show long-lasting hypersensitivity and lowered mechanical threshold in the injured region (Reynolds and Fitzgerald 1995; De Lima and others 1999a). The sprouting response is clearly greatest when the wound is performed at birth and declines with age at wounding. The response to adult wounds is weak and transient in comparison, resulting in a temporary hyperinnervation that recovers after a few weeks. Both myelinated A fibers and unmyelinated C fibers, but not sympathetic fibers, contribute to the sprouting response (Reynolds and others 1992), and it is unaffected by local anaesthetic block of the sensory nerve during wounding (De Lima and others 1999a).

It seems likely that the sprouting results from the release of neurotrophic factors from the damaged region. NGF is a candidate but is unlikely to be the main factor in that systemic anti-NGF treatment fails to prevent sprouting (Alvares and others 2000) or the neurite outgrowth toward injured skin in an in vitro coculture model (Reynolds and others 1997). BDNF, NT-3, and NT-4 are also all expressed in the skin during development (Ernfors and others 1992) and may be up-regulated following skin wounds along with other growth factors (Whitby and Ferguson 1991; Alvares and others 1999). Furthermore, the immune cytokines, released after tissue injury from macrophages or neurons and glia, may directly affect neurite outgrowth.

Novel Genes

An important additional mechanism of central sensitization is the induction of novel genes. After inflammation, A fiber neurons begin to express SP and BDNF, and this may contribute to the allodynic response (Neumann and others 1996; Ji and others 1999). This also occurs in the neonate, but the pattern of change is somewhat different. CGRP (which is co-expressed with SP) expression is up-regulated in A cells and is switched on in IB4-positive C neurons following carageenan inflammation and returns to normal only after the inflammation has subsided (Beland and Fitzgerald 2000a). This suggests that the nonpeptidergic C fibers have the ability to express peptides under some circumstances in the postnatal period.

Summary of Peripheral Events

Figure 1 illustrates the different ways in which an early tissue damage in infancy could influence peripheral sensory structure and function. Whereas many of these changes will subside with the resolution of the inflammation, others may outlast the injury for a prolonged period.

The Central Effects of the Tissue Damage in Infancy

The region of secondary hyperalgesia and allodynia that surrounds an area of tissue damage results from central synaptic rather than peripheral receptor alterations. The hyperexcitability of sensory neurons in the dorsal horn of the spinal cord and brainstem that follow inflammation is termed *central sensitization* (Woolf and others 1994b). Activation of these central cells by repetitive Aδ- and C-fiber inputs initiates sensitization such that they respond to normal inputs in an exaggerated and extended manner and allow inputs that were previously ineffective to activate the neurons (Woolf and Mannion 1999). The effect of this enhanced neurotransmission and hyperexcitability includes enlargement of receptive

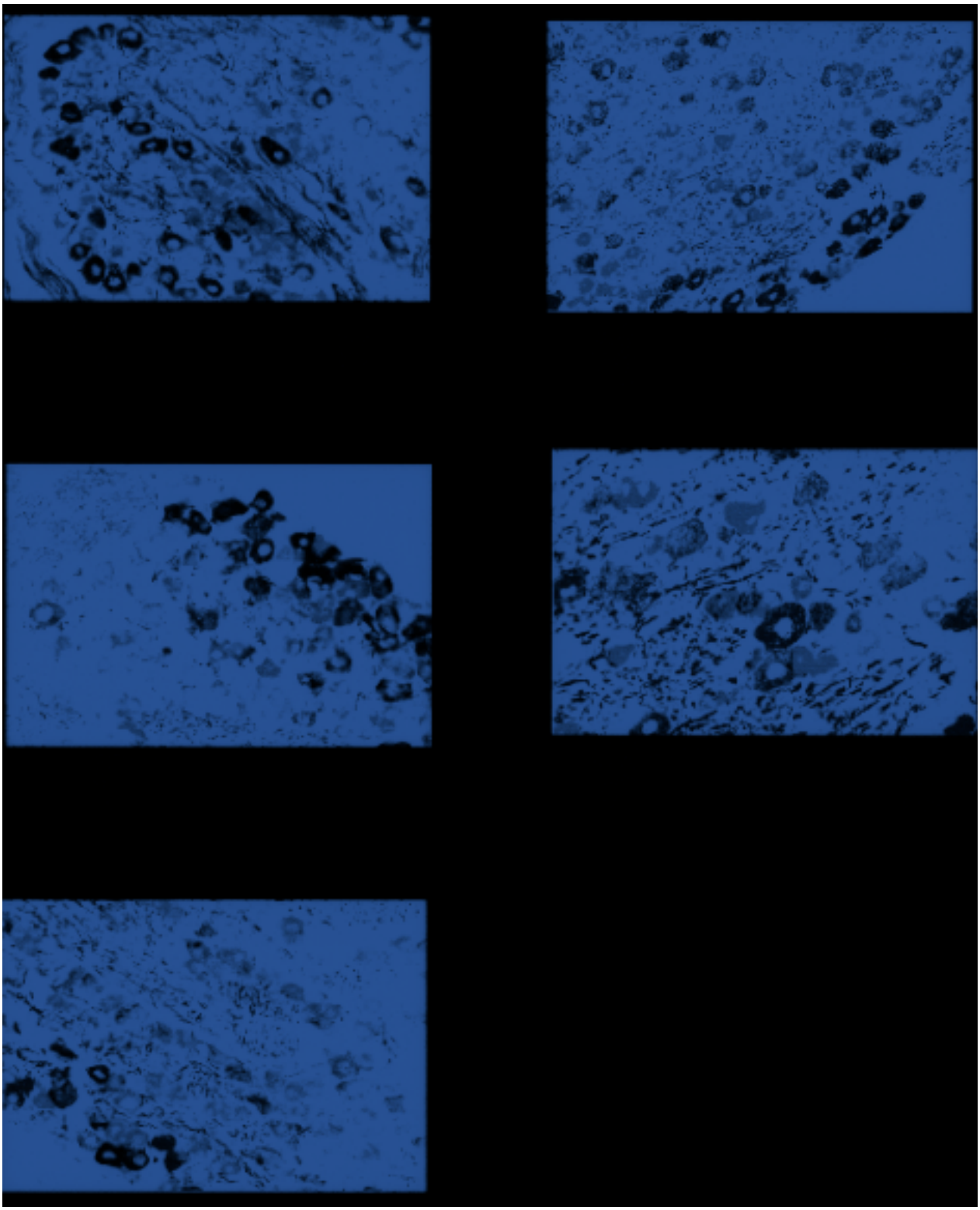

Fig. 2. In the adult spinal cord, low-threshold A-fiber and nociceptive C-fiber afferent sensory terminals are located in separate laminae of the gray matter. In the neonate, however, A-fiber terminals are exuberant and overlap with those of C fibers in the superficial laminae (see Fitzgerald and others 1994).

fields, increased spontaneous activity, greater discharges to mechanical, thermal, and electrical stimulation, and sometimes decreased thresholds (Ren and Dubner 1996). All of these will lead to increased neuronal activity transmitted to supraspinal sites and therefore the onset of persistent pain.

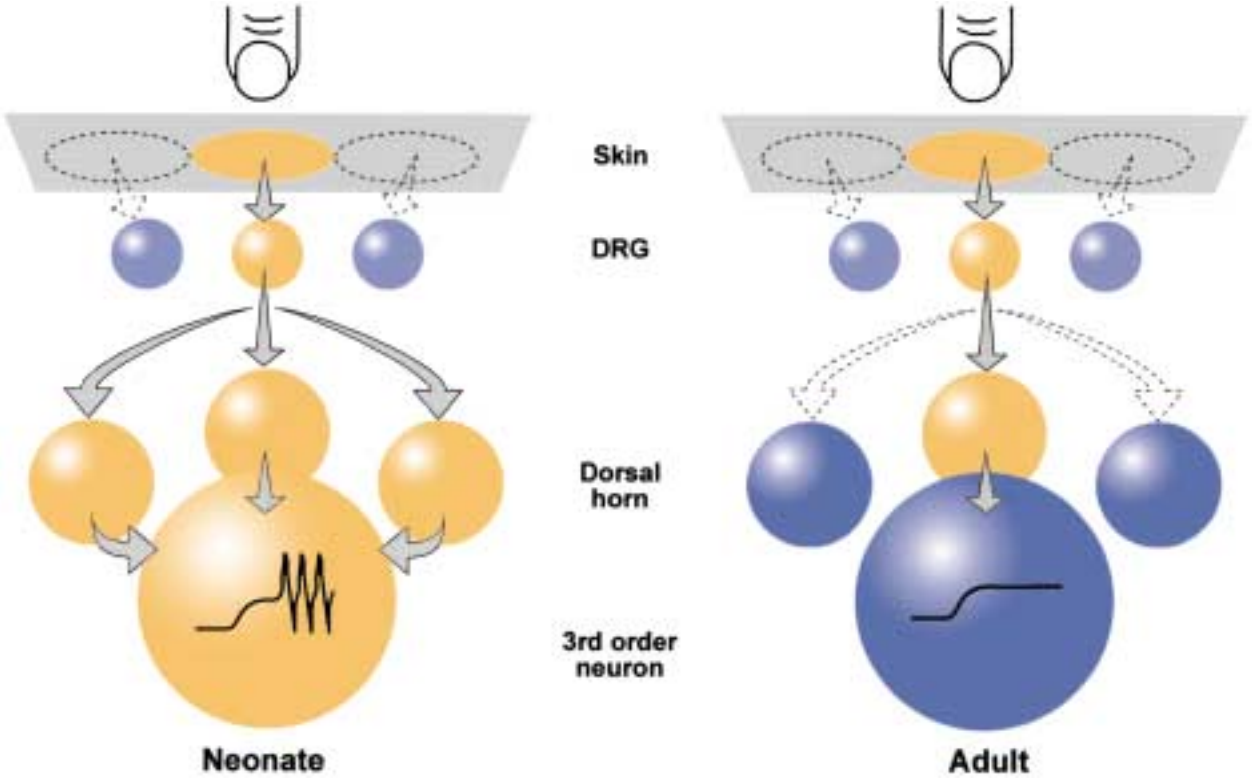

Fig. 3. A diagram to show how larger dorsal horn receptive fields can lead to reduced sensory thresholds in third-order cells. DRG = dorsal root ganglion.

Immediate Central Response

In the rat, both A and C fibers have grown into the spinal cord by birth but C-fiber terminals are very immature and many C-fiber-specific chemical markers are not apparent in the spinal cord until the perinatal period (Jackman and Fitzgerald 2000). Synaptogenesis in the rat dorsal horn is at its maximum in the first postnatal weeks. C-type afferent terminals within synaptic glomeruli are not observed at electron microscope level until P5 (Pignatelli and others 1989). The pattern is similar in the primate but maturation occurs earlier so that by embryonic day 40 (of a 165-day gestation), all types of primary afferent terminal and postsynaptic specialization can be observed in substantia gelatinosa (SG) (Knyihar-Csillik and others 1999). The growth of both A and C fibers into the rat cord is somatotopically precise (Fitzgerald and Swett 1983; Fitzgerald 1987b), such that the pattern of skin innervation by individual peripheral nerves is preserved as an interlocking pattern of terminal fields within the spinal cord. This is not true of the laminar organization, however (Fig. 2). In the adult, Aβ afferents are restricted to laminae III and IV, whereas in the fetus and neonate their terminals extend dorsally right up into laminae II and I (SG) to reach the surface of the gray matter (Fitzgerald and others 1994). This is followed by a gradual withdrawal from the superficial laminae over the first 3 postnatal weeks (Fitzgerald and others 1994). C fibers, on the other hand, grow specifically to laminae I and II, and for a considerable postnatal period these laminae are occupied by both A- and C-fiber terminals (Fitzgerald and others 1994). During their occupation of SG, A-fiber terminals can be seen to form synaptic connections at electron microscope level (Coggeshall and others 1995). C fibers play an important role in the withdrawal of A fibers from laminae I and II, because administration of neonatal capsaicin, which destroys the majority of C-fibers, leaves A-fiber terminals located more superficially than in normal animals (Shortland and others 1990; Torsney and others 2000).

In the newborn rat, the synaptic linkage between afferents and dorsal horn cells is still weak and electrical stimulation often evokes only a few spikes at long and variable latencies (Fitzgerald 1985; Jennings and Fitzgerald 1998). Despite this, noxious skin damage will produce afferent activation of dorsal horn cells that outlasts the stimulus and which lead to the rapid, transient central response. In immature rats, excitatory postsynaptic currents can be elicited by Aβ afferents in the majority of SG neurons, whereas in adults this is only possible with Aδ or C afferents (Park and others 1999). Expression of c-fos in SG neurons, which in adults is evoked by noxious and Aδ and C fiber inputs only, can also be evoked by innocuous inputs and A-fiber activation in the newborn (Jennings and Fitzgerald 1996). Fast excitatory synaptic transmission in adult

spinal sensory pathways is mediated by glutamate acting on AMPA (amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid) and kainate ligand gated ion channels (Li and others 1999). AMPA and kainate receptors are expressed early in the developing spinal cord and are evident in the first trimester of human development (Akesson and others 2000). Expression in the neonatal rat spinal cord shows a wider distribution than in the adult and decreases over the first postnatal 3 weeks (Jacowec and others 1995a, 1995b). The Glu R1, 2, and 4 subunits are generally more abundant in the neonatal compared with the adult cord although the ratio of Glu R2 to Glu R1, 3, and 4 is lower. Different combinations of subunits will affect desensitization, ionic permeability, and current/voltage relationships. There are also changes in the distribution of the flip-flop variants with postnatal age; the flip variants are generally more sensitive to agonists than the flop, resulting in higher levels of depolarization from glutamate release (Watanabe and others 1994). The implications of these findings on immature sensory processing in the spinal cord are not clear.

Another important feature of infant dorsal horn cells is that their receptive fields are larger, that is, occupy a relatively larger area of the body surface, than in adults. The fields gradually diminish over the first 2 postnatal weeks (Fitzgerald 1985; Fitzgerald and Jennings 1999). This is not due to a gradual reduction in the receptive field size of the primary afferents themselves (Fitzgerald 1987) but to a gradual inhibition and reduction of effective afferent inputs to dorsal horn cells. Enhancing these inputs with 4-aminopyridine can cause instantaneous enlargement of receptive fields in the adult (Saade and others 1985). Figure 3 illustrates how larger receptive fields effectively lead to lower sensory thresholds and increased excitability of third-order motoneurones or thalamic neurons in the infant CNS due to convergence of inputs. The large receptive fields and dominant A-fiber input will increase the chance of central cells being excited by peripheral sensory simulation and act to increase the sensitivity of infant sensory reflexes.

The postnatal maturation of synaptic connections between afferent C fibers and SG cells takes place over a prolonged period. C-fiber activation is unable to evoke spike activity in the rat spinal cord until the second postnatal week and is unlikely to be involved in the immediate reaction to the heel stick in young infants (Fitzgerald 1988; Fitzgerald and Jennings 1999). C fibers are apparently able to produce subthreshold activation of central neurons before that time (Akagi and others 1982; Yanigasawa and others 1985) and are therefore likely to trigger the more persistent responses described in the following sections.

Persistent Central Response

The persistent pain response of the infant in intensive care will depend on the ability of the developing sensory nervous system to activate mechanisms of peripheral and central sensitization.

C-fiber-evoked activity matures after the first postnatal week in rats, and from P10, repetitive C-fiber stimulation produces a classical "wind-up" as reported in the adult dorsal horn in 18% of cells. This has increased to 40% of cells by P21 (Fitzgerald and Jennings 1999). In contrast, stimulation of the peripheral receptive field on the hind limb at twice the A-fiber threshold at a frequency of 0.5 Hz can produce considerable sensitization of the dorsal horn cells from the time of birth (Jennings and Fitzgerald 1998). This sensitization takes the form of a build-up of background activity in the cells during repetitive stimulation that outlasts the stimulation period, thereby producing a prolonged after-discharge of more than 2 min. It is particularly apparent in younger animals and gradually declines postnatally so that it is absent at P21 (Jennings and Fitzgerald 1998). The A-fiber-induced sensitization is not accompanied by an increase in the direct A-fiber-evoked spike discharge, but during the stimulation period, the sensitized units show a significant increase in activity outside of this short latency evoked burst. In a situation where there is peripheral tissue damage, this property of neonatal dorsal horn cells will contribute to a persistent excitability to both noxious and nonnoxious sensory stimulation.

Descending Inhibition

Another contributory factor will be the immaturity of descending inhibition. It is well established in adults that descending pathways originating in higher centers can modulate the output of spinal nociceptive neurons and are activated in the presence of persistent pain (Dubner and Ren 1999). Descending inhibitory controls are immature at birth (Fitzgerald 1991), and the slow maturation of descending inhibitory pathways traveling from the brainstem via the dorsolateral funiculus of the spinal cord to the dorsal horn is particularly relevant to persistent pain in infancy. Although descending axons from brainstem projection neurons grow down the spinal cord early in fetal life, they do not extend collateral branches into the dorsal horn for some time and are not functionally effective until postnatal day 10 in the rat (Fitzgerald and Koltzenburg 1986; van Praag and Frenk 1991; Boucher and others 1998). This may in part be due to deficiency of neurotransmitters, in this case, 5-HT (serotonin) and noradrenaline, but may also be due to delayed maturation of interneurones. It has been suggested that the maturation of descending inhibition is dependent on afferent C-fiber activity because rats treated neonatally with capsaicin have reduced inhibitory controls as adults (Cervero and Plenderleith 1985). The lack of descending inhibition in the neonatal dorsal horn means that an important endogenous analgesic system that might "dampen" persistent sensory inputs is lacking and their effects may therefore be more profound than in the adult.

Involvement of NMDA Receptors

There is a large body of evidence implicating the glutamate NMDA receptor system as the primary candidate for this form of plasticity (Dubner and Ruda 1992; Dickenson 1997; Woolf and Mannion 1999). Although normal spinal nociceptive processing is mediated via the ionotropic AMPA (α-amino-3 hydroxy-5-methylisoxazole) and kainate glutamate receptors, repetitive activation of nociceptors associated with tissue injury results in the activation of the NMDA (N-methyl-D-aspartate) glutamate receptor. This involvement of NMDA receptors is due to two mechanisms. First is the removal of the Mg^{2+} ions, which normally block the channel at resting membrane potential, by the cumulative depolarization arising from summation of nociceptor-evoked slow synaptic potentials. Second, neuropeptides such as CGRP and SP (acting on NK1 receptors) and growth factors such as BDNF (acting on trkB receptors) released by the C fibers may potentiate the release of glutamate and its actions on the NMDA receptor. G-protein-coupled receptors such as NK1 and mGlu receptors and receptor tyrosine kinases such as the trkB receptors may enhance NMDA currents via activation of protein kinase C (see Woolf and Slater 2000).

The developmental regulation of NMDA receptor function in relation to central sensitization has not been directly investigated. NMDA-dependent C-fiber-evoked depolarization of spinal cord cells and wind-up of cells to repeated C-fiber stimulation have been demonstrated in the young (8- to 14-day) spinal cord in vitro (Sivilotti and others 1993), but the effects on A-fiber sensitization are not known. Preliminary results show that the ability of dorsal horn cells to display enlargement of receptive fields, increased spontaneous activity, and enhanced responses to mechanical, thermal, and electrical stimulation differs in the newborn cord (Torsney and Fitzgerald 2000). A feature of the neonatal dorsal horn is the presence of synapses with NMDA receptors only, that is, no functional AMPA receptors. These "silent synapses," so called because of their inability to function at resting membrane potentials (Isaac and others 1997), disappear with age and with the onset of colocalization of AMPA and NMDA receptors (Petralia and others 1999; Baba and others 2000). However, the silence may be limited because NMDA receptors in lamina II of the postnatal rat dorsal horn can significantly affect neuron excitability even in the absence of co-activation of AMPA receptors (Bardoni and others 2000). The neonatal spinal cord has a higher concentration of NMDA receptors in the gray matter than that observed in older animals (Gonzalez and others 1993). All laminae in the dorsal horn are uniformly labeled with NMDA-sensitive [^{3}H] glutamate until day 10, 11, or 12, when higher densities gradually appear in SG so that by P30 binding is similar to that in the adult. Furthermore, the affinity of the receptors for NMDA decreases with postnatal age. NMDA-evoked calcium efflux in neonatal rat SG is very high in the first postnatal week and then declines. This is delayed by neonatal capsaicin treatment, suggesting that C-fiber afferent activity regulates the postnatal maturation of NMDA receptors (Hori and Kanda 1994). The different NMDA receptor channel subunits are independently regulated. Of particular interest are the NR2 subunits, which are modulatory in that their expression alters properties such as voltage-dependent Mg^{2+} block and deactivation kinetics of NMDA receptors. The subunit composition of the NMDA channel complex undergoes considerable rearrangement during spinal cord development (Watanabe and others 1994), which appears to be activity dependent (Audinat and others 1994).

NK1 receptor density is maximal in the first 2 postnatal weeks—at P60, the cord has one-sixth of the binding sites present at P11. Furthermore, in the newborn the receptor distribution is the inverse of the adult; that is, the superficial laminae have very few receptors and the high density observed in the adult SG is not apparent until the second week of life (Kar and Quirion 1995). In contrast to receptor levels, SP levels are low at birth in the rat. This immaturity of the SP/NK1 system will undoubtedly affect central sensitization in the infant nervous system, but it is not known how.

The developmental pharmacology of other excitatory and inhibitory systems is also important and has been reviewed elsewhere (Fitzgerald 1987; Alvares and Fitzgerald 1999).

Activity-Dependent Changes

NMDA receptors are also involved in activity-dependent changes during development, a phenomenon that is likely to be of great importance in the response of the infant nervous system to tissue damage. The role of sensory experience mediating subsequent modification, whereby appropriate connections are strengthened and inappropriate ones eliminated, fulfils the criteria for a Hebbian mechanism of synaptic plasticity. From LTP studies, the molecular basis of this plasticity is likely to involve NMDA receptor activation (Daw and others 1993).

Although the visual system and particularly the patterning of connections within the retinogeniculocortical pathway has been the most intensively studied system in terms of activity-dependent synaptic plasticity in CNS development (Katz and Shatz 1996), a similar developmental process exists in the thalamocortical projections that relay sensory information from the vibrissae to the whisker barrels of the somatosensory cortex in the rat. In both cases, diffuse afferent projections at each stage along the pathway are refined by postnatal sensory experience to create somatotopic projections of sensory stimuli to the relevant cortical area. The postnatal refinement has been shown to be activity dependent in both the visual system and the thalamocortical projections to the somatosensory cortex (Katz and Shatz 1996). This period of plasticity exists for a strictly defined critical period and can be disrupted by changing

activity patterns, either by pharmacological or physical means.

It is possible to draw an analogy between the two systems described above and the postnatal changes that occur in the dorsal horn of the spinal cord, where sensory inputs are topographically organized, initially diffuse projections are fine-tuned postnatally (Fitzgerald and others 1994; Fitzgerald and Jennings 1999), and layer/laminar specific changes occur. Preliminary data suggest that chronic NMDA receptor blockade in the dorsal horn from the day of birth halts this normal developmental process and that postnatal reorganization of afferent terminals is an NMDA-receptor-mediated activity-dependent process (Beggs and others 1999). If normal peripheral afferent input is required to fine-tune central connectivity in the dorsal horn of the spinal cord, this raises the intriguing possibility that aberrant activity in the form of tissue damage in infancy could alter the normal developmental process.

Summary of Central Events

Structural and functional differences in the neonatal spinal cord sensory connections will generally enhance and prolong the effects of both noxious and innocuous sensory inputs.

Analgesia

The pain of tissue-damaging procedures is generally abolished or reduced by the use of topical local anesthetics such as lignocaine-prilocaine (EMLA) cream or amethocaine gel. These surface analgesics prevent the transmission of painful stimuli by nociceptors, presumably by their action on sodium channels (Butterworth and Strichartz 1990). Developmental aspects of local anesthetic activity have not been intensively studied, but there is evidence that such differences exist (Benzon and others 1988; Hu and others 1997). Recently, developmentally regulated sensitivity to the antinociceptive effects of epidural analgesia in the rat has been described, in which the efficacy of local anaesthetic is clearly enhanced in the presence of peripheral inflammation, particularly in the neonate (Howard and others 2000). This activity-dependent effect may explain the confusion surrounding the use of topical local anesthetics in human neonates. EMLA and amethocaine gel are clearly effective local anesthetics in newborn infants from 29 to 42 weeks (Jain and Rutter 2000), and repeated EMLA application reduces the hypersensitivity that follows repeated heel lance injury (Fitzgerald and others 1989). However, EMLA does not block the direct response to heel lance itself (Larsson and others 1995; Taddio and others 1998).

Opioids are widely used in neonates and infants (De Lima and others 1996), but there is still a lack of knowledge on their specific effects in this population of patients (Marsh and others 1997). Differences in opioid pharmacology compared to the adult may arise from changes in drug metabolism and transport, receptor expression, and function and also from differences in information processing due to immature neuronal connections. Of importance also is the development of other transmitter systems influencing opioid effects, such as NMDA and cholecystokinin. The analgesic effectiveness of opioid agonists is likely to be different in neonates compared with adults, although this has not been directly studied in humans and animal data vary between laboratories (see Rahman and Dickenson 1999 for review). In addition, there is increasing evidence from animal studies that opioids have qualitatively different effects in the immature compared with the mature nervous system. While opioid agonists specifically depress nociceptive C- and Aδ inputs in the adult (Dickenson and others 1987), studies in vitro and in vivo suggest that non-nociceptive Aβ-mediated stimuli are also depressed in neonatal rats (Faber and others 1997; Marsh and others 1999). One possible mechanism for this lack of opioid selectivity in the young rat spinal cord could be a different pattern of expression of opioid receptors, which is regulated over the postnatal period. Studies of the postnatal development of μ-opiod receptor (MOR) and δ-opioid receptor (DOR) immunoreactivity in rat dorsal root ganglia (DRG) have shown that a greater proportion of cells are immunoreactive for MOR and DOR in P3 neonatal rat DRG compared with P21. Furthermore, MOR expression is down-regulated in the largest diameter, NF200-positive, primary sensory neurons postnatally (Beland and Fitzgerald 2000). Because these neurons are mainly non-nociceptive, this may explain previous reports of opioid agonists affecting reflex responses to both innocuous and noxious stimuli in rat pups.

The results highlight an important difference between opioid function in the immature and adult nervous system.

Summary of Developmental Analgesia

In the case of local anesthetics and opioids, it is clear that analgesic actions can be developmentally regulated not only by differences in pharmacokinetics but also as a result of differences in receptor distribution and function.

Concluding Remarks

The study of the development of pain mechanisms is, quite literally, in its infancy. Recent work has emphasized the importance of studying pain pathways in terms of developmental neurobiology. The infant pain response is not simply an immature adult one but stems from a quite different underlying structural and functional connectivity within the CNS. Currently, too few pediatric patients receive adequate pain relief, and there is little rationale for the choice of treatment. The challenge now is to unravel developing pain mechanisms for the benefit of these patients and to encourage pharmaceutical companies to support the effective evaluation of existing and new drugs for pediatric pain, thereby ful-

filling the basic right of the child to safe and effective treatments.

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