REVIEWS

THE DEVELOPMENT OF NOCICEPTIVE CIRCUITS

Maria Fitzgerald

Abstract | The study of pain development has come into its own. Reaping the rewards of years of developmental and molecular biology, it has now become possible to translate fundamental knowledge of signalling pathways and synaptic physiology into a better understanding of infant pain. Research has cast new light on the physiological and pharmacological processes that shape the newborn pain response, which will help us to understand early pain behaviour and to design better treatments. Furthermore, it has shown how developing pain circuitry depends on non-noxious sensory activity in the healthy newborn, and how early injury can permanently alter pain processing.

TYROSINE KINASE RECEPTORS (Trk). Neurotrophic factors — nerve growth factor (NGF), neurotrophin 3 (NT3), NT4/5 and brain-derived neurotrophic factor (BDNF) — act through a family of receptor proteins, the Trk receptors. TrkA is primarily the receptor for NGF, TrkB for BDNF and NT4/5, and TrkC for NT3.

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After years of neglect, the study of infant pain has emerged as one of the most interesting and important areas of pain research today. It provides a fascinating insight into how immature neurons connect together to analyse and process somatic events, and illustrates how the growing nervous system deals with events that threaten its postnatal integrity and survival. It also has the potential to provide us with a neurobiological basis for pain assessment and treatment in human infants.

Newborn infants show strong pain behaviour, but the study of the development of nociceptive pathways shows that their pain involves functional signalling pathways that are not found in the mature nervous system in healthy individuals. Recent research into the development of inhibitory and excitatory synaptic transmission has provided new insights into the crucial changes that shape pain pathways in the first postnatal weeks.

This review focuses on the underlying organization and strengthening of nociceptive circuitry in the dorsal horn during the first postnatal weeks and on recent data that show how that circuitry might be altered by sensory inputs in early life. It begins with the specification of nociceptive neurons — recent studies have identified key molecular pathways that control the genesis of spinal nociceptive circuits. It then traces the formation of functional synapses, neural circuits and nociceptive reflexes, emphasizing recent research on the postnatal developmental regulation of excitatory and inhibitory synaptic transmission in the developing dorsal horn. Finally, the role of sensory activity — from both non-noxious and excessive noxious inputs — in influencing the development of pain processing is reviewed and the implications for human infant pain discussed. Ultimately, we argue that we need to use this research to design better strategies for the relief of pain in infants and children.

Early specification of nociceptive neurons

Nociceptive neurons are specified early in development, long before they form contacts with their future peripheral or central targets. Sensory sublineages are determined even before neural crest cells become committed to neuronal or glial fates[1]. Different classes of sensory neurons in dorsal root ganglia (DRG) are generated in two waves: large-diameter TYROSINE KINASE RECEPTOR C- and B-expressing (TrkC$^+$ and TrkB$^+$) neurons are born first, followed by small-diameter TrkA$^+$ neurons[2,3]. Recently, an even later wave of nociceptive neurons has been shown to arise from boundary cap cells, which are neural crest derivatives that migrate down the root from the dorsal root entry zone[4]. All these neurons require neurogenin 1 or 2 (*NGN1/2*) — two neuronal determination genes that encode basic helix–loop–helix (bHLH) transcription factors

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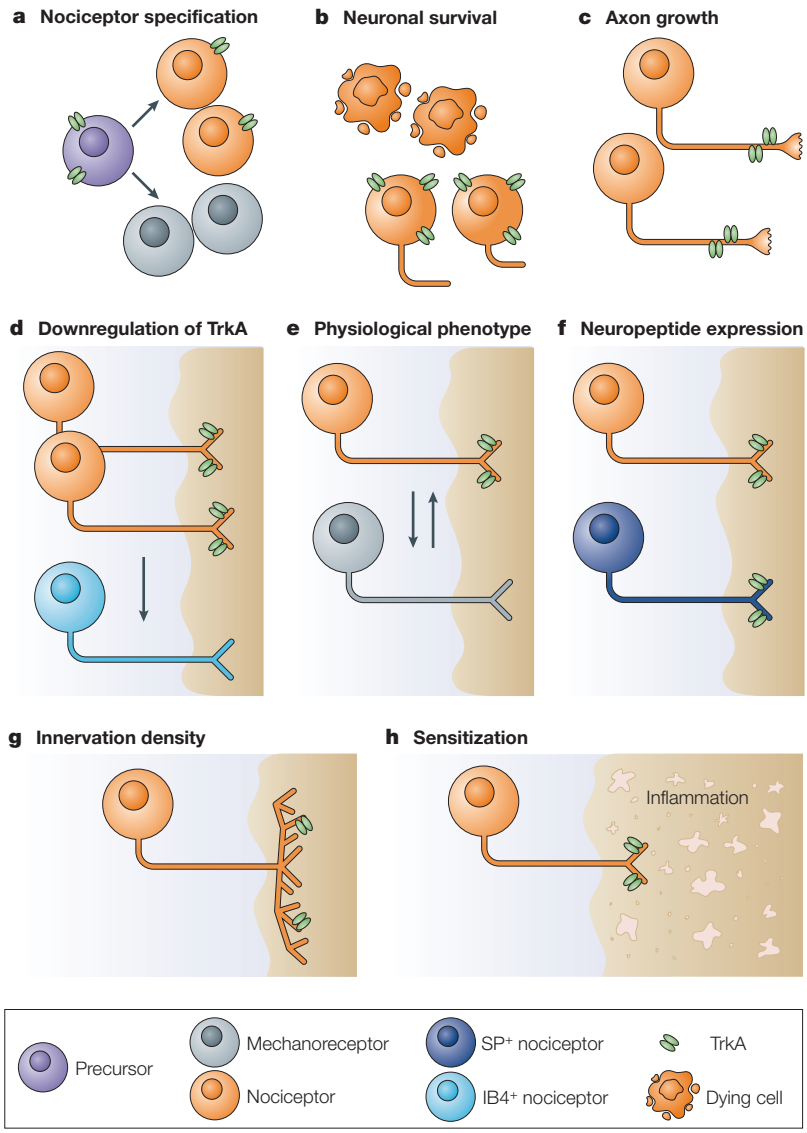

Figure 1 | **Neurotrophins and nociceptor development.** Almost every aspect of nociceptor development is influenced by neurotrophins. **a** | Determination of sensory neuron fate. Neurotrophins have an instructive role in nociceptor specification[182]. **b** | Nociceptor survival depends on access to nerve growth factor (NGF), which is produced in the target tissue and acts on the tyrosine kinase A receptors (TrkA)[6,183]. The underlying mechanisms seem to require the pro-apototic B-cell leukaemia/lymphoma 2 (BCL2) homologue, BAX, heat shock protein 27 (HSP27) and bone morphogenic protein (BMP)[184–186]. **c** | Neurotrophins regulate axon growth, independently of cell survival[189,190]. **d** | A subpopulation of nociceptors downregulates the expression of TrkA receptors around the time of birth, loses its NGF dependency and becomes responsive to glial cell line-derived growth factor (GDNF), a member of the transforming growth factor-β (TGFβ) family. These neurons form the IB4-positive (IB4$^+$) group of nociceptors[187,188]. **e** | The physiological properties of nociceptors are determined by neurotrophin levels during the postnatal period. NGF and neurotrophin 3 (NT3) regulate the differentiation of Aδ-fibre high-threshold mechanoreceptors and C-fibre nociceptors. The ability of unmyelinated nociceptors to respond to noxious heat depends on exposure to NGF during the early postnatal period[191–193]. **f** | Regulation of the chemical phenotype of nociceptors by neurotrophins. The expression of neuropeptides, such as substance P (SP) and calcitonin gene-related peptide in TrkA-expressing neurons is triggered by peripheral target innervation[194,195]. **g** | The innervation density of nociceptors in the skin is regulated by access to the local neurotrophins NGF and brain-derived neurotrophic factor (BDNF)[194–197]. Neonatal skin wounding during a critical neonatal period upregulates neurotrophin levels, which results in prolonged hyperinnervation of the skin[24,125]. **h** | Excess neurotrophins sensitize peripheral nociceptors, thereby increasing their responses to noxious stimulation, but only after postnatal day 10 (REF. 110).

— *NGN2* primarily for the generation of TrkC$^+$ and TrkB$^+$ neurons, and *NGN1* for the generation of TrkA$^+$ neurons[5]. Competitive interactions between these precursors might control the final proportions of different neuronal subtypes normally produced.

As the majority of C-FIBRE nociceptive neurons are TrkA$^+$ at this stage, and the non-nociceptive cutaneous muscle afferents are TrkC$^+$ and TrkB$^+$ (FIG.1), the two functional subpopulations of primary sensory neurons seem to be under separate transcriptional control. There is also evidence to indicate that in the TrkA$^+$ cell population future peptidergic neurons are generated before nonpeptidergic, isolectin B4-positive (IB4$^+$) cells[3], although the crucial transcription factors are unknown. The final numbers of DRG cells are determined by the balance between cell birth and subsequent cell death, and the survival of DRG cell populations is regulated by access to neurotrophic factors[6] (FIG. 1). The number of DRG neurons increases steadily until birth, and is then followed by a 15% loss during the first five postnatal days, which coincides with innervation of the skin[7].

Axon growth to peripheral and central targets

In the rat, outgrowth of sensory axons from the DRG to peripheral and central target skin occurs before birth and reflects the two waves of neurogenesis. In the hindlimb skin, larger-diameter A FIBRES form a cutaneous nerve plexus, and the formation of C fibres follows soon after[8]. Early skin nerve fibres first reach up to the epidermal surface, subsequently withdrawing as the epidermis thickens at about the time of birth, and morphologically distinct end organs develop[8,9]. Neurotrophins acting on Trk receptors have an important role in regulating this process (FIG. 1). Meanwhile, centrally directed dorsal root fibres reach the lumbar cord and, after a significant delay, penetrate the grey matter. The A fibres penetrate first, at EMBRYONIC DAY (E)15–17, and are followed by C fibres several days later at E18–20 (REFS 8,10). From the outset, each DRG innervates characteristic skin dermatomes and projects in a precise somatotopic pattern in the dorsal horn[11,12]. Functionally, this means that the lack of RECEPTIVE FIELD 'tuning' (discussed below) — whereby the immature CNS is less able to localize stimuli to a particular area of the body surface — is not the result of inaccurate afferent central connections. Grafting experiments have shown that DRGs are not matched to particular skin targets; although the peripheral target skin influences the pattern of projections in the CNS, it does not direct cutaneous axons to specific populations of neurons in the dorsal horn[13,14]. Several extracellular molecular cues might be important in directing axon growth of sensory neurons, including the inhibitory growth cone collapsing molecule semaphorin 3A and the homeodomain protein DRG11 (REFS 15–17). In addition, fetal DRG cells fire action potentials spontaneously during this period, which might play a part in the formation of appropriate synaptic connections[18].

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Functional development of nociceptors

Many of the defining features of nociceptive C fibres can be detected at an early embryonic age, such as the expression of the neurotrophin receptor TrkA and selective binding of IB4. The capsaicin receptor TRPV1, which has a key role in detecting painful thermal and chemical stimuli, is expressed in a similar percentage of DRG neurons at postnatal day (P)2 as in the adult. A subset of these neurons might undergo the switch from nerve growth factor (NGF)- to glial cell line-derived neurotrophic factor (GDNF)-dependence that occurs postnatally in DRG cells[19] (FIG. 1). At this age, many DRG cells also express the ATP-gated P2X3 receptor, which is important for thermal and mechanical hyperalgesia following inflammation or nerve injury. The tetrodotoxin (TTX)-resistant sodium channel Nav1.8 (SNS/PN3), one of a group of voltage-gated sodium channels that regulate neuronal hyperexcitability and might trigger chronic pain in adults, is expressed in developing C-fibre neurons by E17, and adult levels can be observed by P7 (REF. 20).

Consistent with these findings, physiological recordings of neonatal polymodal nociceptors reveal responses to noxious chemical, mechanical and thermal stimuli with thresholds and firing frequencies that are characteristic of mature C fibres, which indicates that the underlying transduction mechanisms are functional from the first days of postnatal life[21–23]. In addition, a group of less well-defined A-fibre 'pressure' receptors are observed in the newborn but their incidence declines with age. In contrast to C fibres, low threshold A fibres require a longer postnatal period to acquire their full stimulus-response sensitivity, which coincides with myelination and the innervation of specific end organs, such as Merkel cells. All types of afferent projection have lower conduction velocities at birth, but their absolute receptive field sizes do not appear to change with age, despite the growth of body surface area[21].

FIGURE 1 emphasizes the important role of neurotrophins and Trk receptors in the growth, establishment of phenotype and physiological sensitivity of nociceptors. The early maturation of nociceptor transduction means that nociceptive activity can be transmitted to the CNS even before birth, although any change in the normal neurotrophin profile during the perinatal period could markedly alter the nature of this activity. It is important to note that large increases in neurotrophin levels follow neonatal tissue injury[24].

Development of central connections

C-fibre projections are the last group of primary afferents to enter the dorsal grey matter, well after proprioceptive and low-threshold A fibres. In the rat lumbar cord, the first dorsal root afferents enter the dorsal horn at E13, but TrkA$^+$ C-fibre terminals are not observed in lamina II until E18–19 (REFS 8,10,11,25,26). From the outset, these fibres terminate in a somatotopically precise manner in laminae I–II of the dorsal horn and include those that express substance P, calcitonin gene-related peptide (CGRP), galanin and somatostatin[27,28]. The IB4$^+$ subset of C-fibre synaptic terminals cannot be detected here until P5, despite their presence in the DRG from E18 (REF. 20), which indicates that these fibres might form central connections later than peptidergic fibres[29]. The relatively late formation of central C-fibre synapses means that, despite the ability of polymodal nociceptors to signal noxious events in the periphery, central nociceptive processing is immature in the postnatal period. This is discussed in more detail below.

Perhaps as a result of their earlier arrival in the dorsal horn grey matter, A-fibre terminals initially grow beyond their final adult destination. In the adult, lamina II is exclusively occupied by C fibres, but in the neonatal period it is also occupied by transient A-fibre terminals. Some, but not all, in-growing cutaneous afferent A fibres extend dorsally right up into laminae I and II to reach the surface of the grey matter, and only gradually withdraw over the first 3 postnatal weeks to terminate in laminae III and IV (REFS 30–32). The functional class of afferent that gives rise to these exuberant terminals is not clear, but they are unlikely to be hair follicle afferents and might arise from the less well-defined A-fibre 'pressure' receptors that are seen in newborns[32–34]. Their presence can be observed at the electron microscopic level[35,36], and they might be responsible for the high incidence of Aβ-fibre-evoked MONOSYNAPTIC responses that are seen in neurons in the substantia gelatinosa in young rats[37,38], and the activation of c-fos expression by Aβ fibres in the substantia gelatinosa at P3 but not P21 (REF. 39). On the other hand, C fibres grow specifically to laminae I and II (REF. 11), so for a considerable postnatal period these laminae are occupied by both A- and C-fibre terminals. The functional effect of this changing postnatal profile of A- and C-fibre inputs on nociceptive processing is discussed below.

Specification of dorsal horn cells

The spinal cord develops along a ventrodorsal gradient such that deep dorsal horn neurons are born after motor neurons, and substantia gelatinosa neurons in lamina II are the last to mature[2]. During the early phase of neurogenesis in the mouse, interneurons are generated along the dorsoventral axis and settle in the deep laminae of the dorsal horn. This is followed by the development of two late-born populations in the superficial laminae, which can be distinguished by their complementary expression of the homeobox genes *Lmx1b* and *Pax2* (REF. 40). The expression of homeobox gene *Lbx1* is required for both the correct specification of substantia gelatinosa neurons and the appropriate sensory afferent innervation of the dorsal horn[41]. Recent evidence shows that post-mitotic selector genes *Tlx1* and *Tlx3* determine excitatory glutamatergic versus inhibitory GABA (γ-aminobutyric acid)-mediated cell fates in the embryonic dorsal spinal cord[42]. Interestingly, generation of lamina I projection neurons is complete before that of local circuit interneurons[43,44], which implies that direct transmission of nociceptive activity from the spinal cord to higher centres could develop before the onset of local

C FIBRES
Small diameter unmyelinated primary afferent sensory fibres, with small cell bodies in the dorsal root ganglion. Most are nociceptors. They divide into a neuropeptide-containing, Trk receptor-expressing group and a lectin IB4-binding group, although the functional implications of this are still unclear.

A FIBRES
Large diameter myelinated primary afferent sensory fibres, with large cell bodies in the dorsal root ganglion. The largest diameter Aβ fibres are mainly low-threshold mechanoceptors, and the smaller Aδ fibres are both mechanoreceptors and nociceptors.

EMBRYONIC DAY
(E). These are dated from the time of fertilization. Rat gestation is 21.5 days, mouse a little shorter. Rats are born relatively early in terms of CNS development and the early postnatal period is often paralleled with the final gestation of development in humans.

RECEPTIVE FIELD
The area on the body surface that, when stimulated, evokes action potentials in a given neuron.

MONOSYNAPTIC
A direct synaptic input from pre- to postsynaptic neurons with no involvement of interneurons in between.

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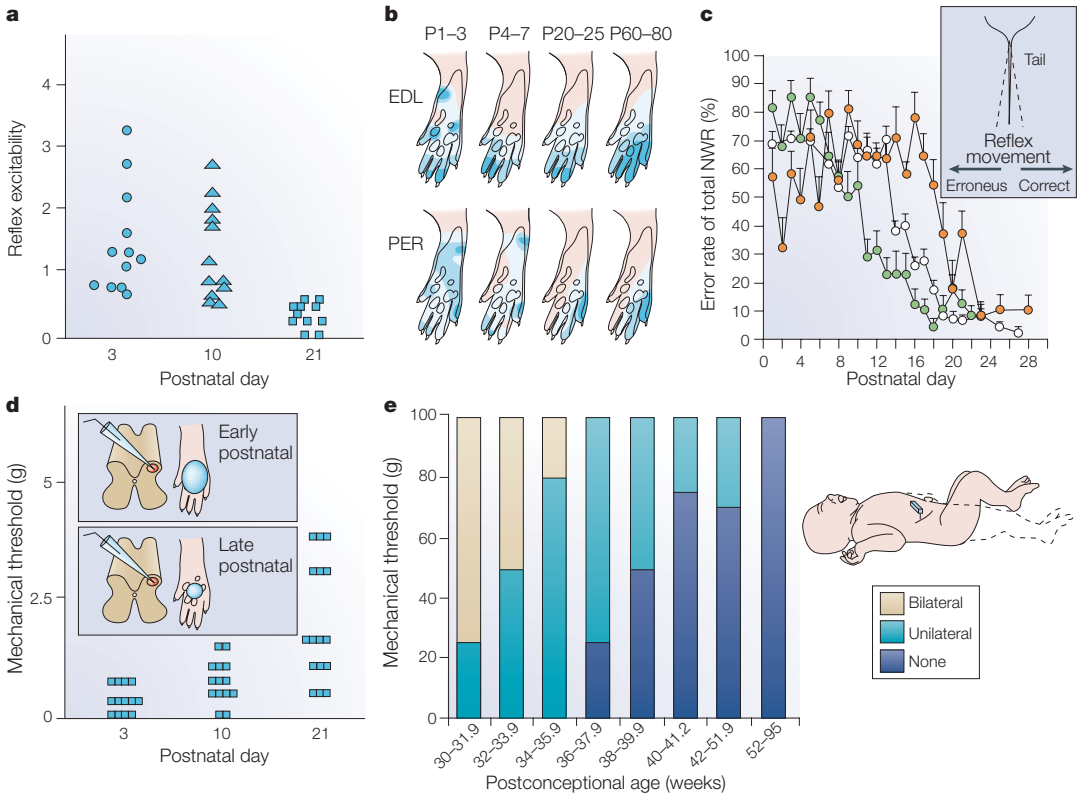

Figure 2 | **Reflex modules and nociceptive responses in neonates. a** | The flexion reflex response to mechanical skin stimulation decreases in amplitude, duration and sensitivity with age in rat pups. **b** | Cutaneous receptive fields of individual hindlimb muscles undergo postnatal tuning in rat pups[56]. Darker colour indicates higher sensitivity to cutaneous stimulation. EDL, extensor digitorum longus; P, postnatal day; PER, peroneus. **c** | Rat pup tail reflex responses to a focused laser heat stimulus become increasingly accurate with postnatal age. The graph shows the fall in error rate for three individual animals[55]. NWR, nociceptor withdrawal refex. **d** | During the first postnatal weeks in rat dorsal horn cells, cutaneous receptive fields (blue circles in inset) decrease in size (as measured by responses of the dorsal horn cells), and mechanical thresholds increase (graph)[58]. **e** | Unilateral abdominal skin stimulation in human infants evokes limb flexion, which becomes increasingly tuned with postconceptional age[50]. Although the oldest babies show no knee flexion (only abdominal muscle contraction) to abdominal skin stimulation, the majority of very young babies flex both their knees. At intermediate ages, unilateral knee flexion on the side of the stimulus predominates. Panel **b** reproduced, with permission, from REF. 55 © (2003) Society for Neuroscience; panel **c** reproduced, with permission, from REF. 95 © (2003) Macmillan Magazines Ltd; panel **e** reproduced, with permission, from REF. 50 © (2002) International Association for the Study of Pain.

modulation by interneurons. At P18–27, the miniature excitatory postsynaptic current (mEPSC) frequencies of lamina I projection cells are five times higher than those in interneurons[45]. The relatively late maturation of interneurons means that their axodendritic growth takes place postnatally, which might be important for activity-dependent shaping of nociceptive circuits (see below).

Functional maturation of nociceptive circuits

The formation of synaptic connections between sensory afferents and spinal cord neurons leads to the first functional reflex responses to tactile and noxious stimuli. Although these spinal reflexes should not be interpreted as evidence of pain awareness or 'feelings', they are an important indication of the maturation of functional neural circuits that can protect an animal from tissue damage and can trigger a range of physiological responses throughout the CNS. In isolated rat lumbar cord, hindlimb responses to touching the skin can be evoked from E15–17 — when the first connections are formed between A-fibre dorsal root afferents, interneurons and motor neurons — and nociceptive stimulation evokes a response soon afterwards[46].

At birth, cutaneous reflex responses are diffuse and untuned. Dorsal root stimulation evokes prominent INTERSEGMENTAL REFLEXES in isolated neonatal cord, which decline in magnitude with age[47]. A noxious prick on the foot of a rat pup or human infant can cause movement of the whole body and simultaneous responses from all four limbs, which only gradually become more individuated and restricted to the movement of an isolated leg or foot. Cutaneous reflexes have lower thresholds and more synchronized and prolonged muscle contractions, which become less pronounced after 29–35 POSTCONCEPTIONAL WEEKS in the human and P8 in the rat; they are also easily sensitized by repeated stimulation[48–52]. Thresholds for withdrawal from heat stimuli are also lower in younger animals and the sensitivity of neonatal rats in response to formalin is

INTERSEGMENTAL REFLEXES
Motor reponses evoked by sensory stimulation in different spinal segments.

POSTCONCEPTIONAL WEEKS
Postconceptional age is the age of a premature human infant dated from the estimated time of conception.

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tenfold higher than that of weanlings[53,54]. Until P10, the response to formalin (applied to the foot) also consists predominantly of 'nonspecific' whole body movement, whereas the targeted flexion, shaking and licking of the paw predominates from then on. Furthermore, there is a high error rate in the direction of a tail flick response to precise laser stimulation of the tail for the first 10 postnatal days, which gradually improves over three weeks[55]. This is consistent with the finding that the receptive fields of hindlimb flexor muscles are large and disorganized in young animals, such that noxious stimulus-evoked limb withdrawal is not always appropriate to the stimulus[56] (FIG. 2).

These changing behaviours arise from fine-tuning of both excitatory and inhibitory synaptic connections and neuronal circuitry over the postnatal period, and the focus for such tuning has been on the interneurons of lamina II. Lamina II displays low-frequency, spontaneous EXCITATORY POSTSYNAPTIC CURRENTS (EPSCs) and INHIBITORY POSTSYNAPTIC CURRENTS (IPSCs) from birth, which increase markedly in frequency with age. Unlike in adults, neonatal spontaneous EPSCs (sEPSCs) are TTX-sensitive, which indicates that spike activity is important for spontaneous glutamate release at this time[57]. The earlier maturation and widespread presence of functional A-fibre terminals in the first postnatal weeks has a significant influence on the physiological responses of dorsal horn neurons. In slice preparations, a high incidence of Aβ-evoked monosynaptic responses relative to C-fibre inputs has been observed in the substantia gelatinosa neurons of young dorsal horn[37,38]. *In vivo*, low-intensity electrical skin stimulation — which is sufficient to recruit A-fibres — or non-noxious tactile stimulation evokes spike activity in both superficial and deep laminae at latencies that progressively decrease in magnitude and variability with age. As a result, dorsal horn cell cutaneous mechanical thresholds are lower and a larger proportion of neurons only respond to innocuous stimulation and have no nociceptive input[58,59]. These properties are thought to contribute to the high level of cutaneous reflex excitability that is described above.

In contrast to A-fibre-evoked activity, C-fibre-evoked activity in dorsal horn cells develops gradually over the postnatal period. A lower percentage of dorsal horn neurons with nociceptive inputs are observed in the first week of life compared with in adults[60], and electrical stimulation of C-fibres fails to evoke a synchronized burst of spikes in dorsal horn neurons or motor neurons[58,60–63]. In addition, the C-fibre irritant mustard oil induces only a weak flexion reflex and c-fos expression in dorsal horn neurons, despite its ability to excite C fibres in the skin of newborn rats[64,65]. However, in spinal cord slices, application of capsaicin increases glutamate release in the superficial dorsal horn from P0, although a significant increase in the effect occurs between P5 and P10, as might be expected from the proliferation of C-fibre synaptic inputs during this period[57]. Therefore, at least some nociceptive primary afferents clearly begin to form functional synapses before birth, but neurotransmitter release is asynchronous and synaptic activation cannot readily evoke spike activity *in vivo* (FIG. 3). This indicates that rapid nociceptive responses are largely mediated through Aδ fibres in the newborn and that, to be effective, C-fibre inputs require more sustained or widespread convergent input.

EXCITATORY AND INHIBITORY POSTSYNAPTIC CURRENTS (EPSCs and IPSCs). When a neuron is voltage clamped, ion flow across a membrane can be measured as electric current while the membrane potential is controlled with a feedback amplifier. Whole-cell patch clamping has extended the technique to allow recording of excitatory and inhibitory postsynaptic currents following synaptic activation of cells in a tissue slice or even *in vivo*.

The balance of excitation and inhibition

In many respects, dorsal horn cells in newborn animals are more excitable than their mature counterparts. At birth, the cutaneous receptive fields of dorsal horn cells, particularly those in the deep dorsal horn, are relatively larger than those in adults and rapidly decrease in size during the first two postnatal weeks[58,59]. Noxious stimulation of the skin at early postnatal ages (P0–P3) often results in a prolonged after-discharge — which consists of action potential activity that lasts 30–90 s beyond the end of the stimulus — an effect that decreases in amplitude and duration with age[21]. The responsiveness of newborn dorsal horn cells is likely to contribute to the diffuse and exaggerated reflex behaviour and disorganized reflex muscle receptive fields described above (FIG. 2) and reflects the immaturity of excitatory and inhibitory synaptic transmission in neonatal spinal cord. The elimination of some synapses (such as the Aβ-fibre input to lamina II) and the strengthening of others (such as C-fibre inputs) alters the balance of postnatal transmission and might be linked to the number of silent synapses — which express only NMDA (*N*-methyl-D-aspartate) receptors (NMDARs) — in neonatal superficial dorsal horn neurons[66–68]. Many other aspects of glutamatergic excitatory synaptic transmission, such as NMDAR channel opening times and the number of calcium-permeable AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) and kainate (KA) receptor channels in the newborn — properties that are thought to be crucial for growth and synaptogenesis — are developmentally regulated in the dorsal horn and impact on nociceptive transmission (BOX 1).

The development of organized inhibitory connections is as important as the excitatory input in nociception. Recently, the need for a precise balance between excitation and inhibition in the nervous system has been emphasized, particularly at the level of dendritic inputs, at which inhibitory synapses have been shown to determine the impact of adjacent excitatory synapses only if they are co-localized on the same dendritic branch and are activated simultaneously[69]. Like excitatory synapses, inhibitory GABA-mediated and glycinergic synapses are postnatally regulated in the dorsal horn (BOX 2). Unlike in the hippocampus, these neurotransmitters are now known to be essentially inhibitory in lamina II in the newborn[70] — topical application of $GABA_A$ (GABA type A) antagonists *in vivo* causes robust disinhibition of superficial and deep dorsal horn activity, as it does in adults (L. R. Bremner and M. F., unpublished observations). Indeed, the net GABA-mediated drive might even be higher in the newborn than in the adult, as the decay time of miniature IPSCs (mIPSCs) is longer than that

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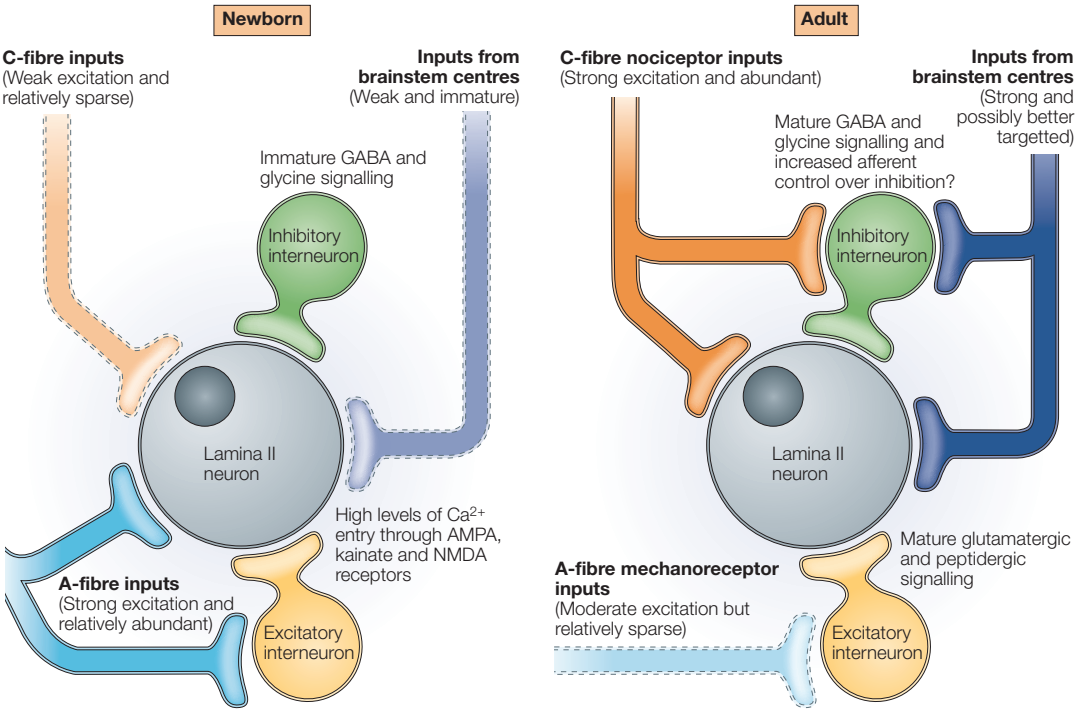

Figure 3 | **Schematic diagram of the synaptic changes that take place in the superficial laminae of the dorsal horn over the first 2–3 postnatal weeks.** The balance of excitation and inhibition differs in the neonatal dorsal horn compared with that in the adult. Although inhibitory transmission is present, it may be less targeted in the neonate than in the adult. This is depicted as a possible absence of specific afferent and descending control of inhibitory interneurons. In the neonate, C-fibre synaptic transmission is weak and the frequency of miniature excitatory postsynaptic currents (mEPSCs) is low. In addition, the Ca^{2+} entry into neurons on activation is enhanced by the unique properties and distribution of the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid), kainate and NMDA (*N*-methyl-D-aspartate) channels in neonates, which might be important for strengthening of these synapses (see BOX 2). Transmission at inhibitory synapses in the neonate is predominantly GABA (γ-aminobutyric acid)-mediated, with little glycinergic activity. The frequency of miniature inhibitory postsynaptic currents (mIPSCs) is low, but IPSC decay times are prolonged. Several properties of $GABA_A$ (GABA type A) channels change over this period (BOX 2) — for example, the intracellular concentration of Cl^- is high in the newborn and declines with age, which means that, at birth, GABA can be depolarizing but still inhibitory, as the reversal potential for Cl^- (E_{Cl}) is below the spiking threshold. In the neonate, descending fibres are present but inhibitory influences are weak or absent. This is probably due to low levels of the transmitter serotonin, as its receptors are functional in the early neonatal period. Immature inhibitory interneuronal connections might also be responsible. Myelinated A fibres have a strong input into neonatal superficial laminae (depicted as both mono- and polysynaptic), which declines substantially, presumably through synaptic weakening, during the postnatal period. Conversely, C-fibre synapses strengthen with age. These changes result in a shift in the balance of C-fibre to A-fibre input. The developmental changes in glutamatergic transmission (BOX 2) are as important for weakening A-fibre connections as for strengthening C-fibre inputs and, possibly, for excitatory inputs to inhibitory interneurons.

in adults. This is due to the production of 5α-reduced neurosteroids, which confer slow kinetics on mIPSCs in lamina II of the neonatal spinal cord[71]. However, it is important to note that the inhibition is almost entirely GABA-mediated at this time; faster glycinergic inhibition develops later in the postnatal period[70]. This affects the temporal integration of inhibitory and excitatory inputs into the neonatal dorsal horn.

Although inhibitory synaptic activity is evident in the newborn dorsal horn, this inhibition might not be appropriately targeted and, therefore, might not modulate excitatory inputs with precision. An example of this has been reported in visual tectal neurons, in which the gradual reduction in receptive field size is accompanied by a transition from disparate to matched topography of excitatory and inhibitory inputs, which is itself dependent on appropriate GABA-mediated activity[72]. Such a mechanism would explain the lack of tuning in nociceptive reflex responses, the larger receptive fields and the lower thresholds of neonatal dorsal horn cells that are described above (FIG. 3). With regard to this point, it is also interesting to note that presynaptic inhibition, as measured by dorsal root potentials, is asynchronous in the spinal cord of newborns[73].

Descending control of nociceptive circuits

Descending activity from the brainstem is another factor that contributes to excitation and inhibition in spinal nociceptive circuits. As a result of the prolonged postnatal maturation of descending fibre systems, spinal transection before P15 has markedly less impact on spinal sensory circuits than it does at older ages[74]. Serotonin-containing fibres from the brain stem

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Box 1 | Development of excitatory synaptic transmission in the neonatal dorsal horn

AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors (AMPARs) are highly expressed in the newborn dorsal horn and steadily decrease to ~20% of neonatal levels in the adult. This decrease is accompanied by changes in subunit expression, such that the glutamate RECEPTOR SUBUNITS GluR1, 2 and 4 are more highly expressed than in the adult, but that the ratio of GluR2 to GluR 1,3 and 4 is lower[155]. As the presence of GluR2 reduces the Ca^{2+}-permeability of AMPARs[156], this indicates greater AMPA-dependent Ca^{2+} influx in neonatal spinal neurons, perhaps to drive growth and synaptic plasticity. Ca^{2+}-permeable AMPARs are expressed in GABA (γ-aminobutyric acid)-mediated and neurokinin 1-expressing ($NK1^+$) embryonic dorsal horn neurons in culture and in the superficial laminae of neonatal spinal cord[157,158].

Kainate (KA) receptors also mediate excitatory glutamatergic C-fibre synaptic transmission in the neonatal spinal cord[159]. Subunits GluR5 and, to a lesser extent, GluR6 and 7 are expressed throughout the cord at birth, become restricted to the superficial dorsal horn by postnatal day (P)10, and are gone by P21. KA1 and KA2 are also expressed in the dorsal horn at birth but not at P21 (REF. 160). Presynaptic kainate receptors are also found on C-fibre terminals of the developing rat, and the application of kainate to immature spinal cord slices decreases AMPAR- and NMDA (*N*-methyl-D-aspartate) receptor (NMDAR)-mediated currents[161,162]. Ca^{2+}-permeable KA receptors are also expressed on isolectin B4-positive ($IB4^+$) C fibres, but switch to a Ca^{2+}-impermeable form early in the first postnatal week[163].

NMDAR concentration is high in the neonatal dorsal horn compared with that of older animals[164] and the distribution is widespread over all laminae, only gradually becoming restricted to lamina II as seen in the adult. Subunit expression also changes, and although the NMDA subunit NR2B mRNA is found throughout the cord prenatally, its expression moves dorsally so that by P21 it is restricted to laminae I and II (REF. 165). In many parts of the CNS, an experience-dependent postnatal shift from NR2B to NR2A expression results in an accelerated decay rate of NMDA excitatory postsynaptic currents (EPSCs), whereas EPSCs in substantia gelatinosa neurons show rapid decay time constants throughout the postnatal period[166]. NR2D is the most highly expressed NMDA subunit in the rat embryonic spinal cord and has slower offset times than any other recombinant receptor[167,168]. NMDARs in neonatal rat substantia gelatinosa neurons also have an unusually high Mg^{2+} sensitivity, which indicates that they have a novel stiochiometry[169]. Both receptor affinity for NMDA and NMDA-evoked Ca^{2+} influx decline with postnatal age in rat substantia gelatinosa neurons in an activity-dependent manner[87].

Metabotropic glutamate receptors in the spinal cord are also differentially regulated during development — the mGluR3 and mGluR5 subtypes are highly expressed at birth and this subsequently decreases during the postnatal period[170,171].

invade the grey matter at about the time of birth[75], but the pattern and density of adult termination are not achieved in the lumbar spinal cord until P21 (REF. 76). Although descending serotonin-mediated inputs to the mature spinal cord can produce both excitatory and inhibitory effects on nociceptive processing, the role of this system in modulating spinal sensory processing in the neonate is less clear. Interestingly, electrical activation of the PERIAQUEDUCTAL GREY (PAG) region does not produce analgesia until P21 (REF. 77), and stimulation of the dorsolateral funiculus cannot inhibit the firing of dorsal horn neurons until at least P10 (REFS 78,79), which strongly indicates that descending inhibition has little functional impact in the neonatal dorsal horn.

In support of this, the classic biphasic behavioural response to formalin, which, in adults, is thought to arise from descending inhibition, is also not apparent in the rat pup until P15 (REF. 80). Application of D-amphetamine at doses sufficient to enhance monoamine release does not affect pain behaviour before P10 (REF. 81), which indicates that the delayed postnatal functional maturation of this system might be due to low levels of serotonin (5-hydroxytryptamine, 5-HT) in synaptic terminals during the early postnatal period. However, intrathecal administration of serotonin agonist does not depress the formalin test response until P10 (REF. 82), which indicates that there is also a delay in receptor maturation. Although the low levels of serotonin might limit synaptic transmission, they could still influence synaptic maturation, as exposure to serotonin causes rapid insertion of AMPA receptors (AMPARs) into the synaptic membrane of neonatal substantia gelatinosa neurons[68].

The noradrenergic component of descending inhibition seems to mature before the serotonin-containing counterpart. The expression of spinal α_2-adrenergic receptor (α_2-AR) peaks during the second postnatal week[83], and α_2-AR agonists selectively depress C-fibre-evoked ventral root potentials in the immature spinal cord *in vitro*[84] and elicit analgesia in response to noxious mechanical and thermal stimuli in neonatal rats[85]. The results of recent studies show that epidural dexmedetomidine, a potent and selective α_2-AR agonist, reverses inflammatory hyperalgesia at all postnatal ages at doses that have no effect on baseline sensory processing, and that are lower than those required in the adult[86].

Shaping of nociceptive circuitry

The increasing postnatal maturation of C-fibre-evoked activity in the dorsal horn plays an important part in the organization of nociceptive circuitry. Neonatal destruction of C fibres by systemic administration of the neurotoxin capsaicin prevents or delays the development of several synaptic processes in lamina II. These include a delay in the postnatal

PERIAQUEDUCTAL GREY
An area of the brainstem that surrounds the aqueduct connecting the third and fourth ventricles. This area projects to the medullary raphe region, which, in turn, sends projections down the dorsolateral funiculus of the spinal cord to the dorsal horn. This pathway is known to strongly modulate spinal pain processing.

RECEPTOR SUBUNITS
Ion channels are generally made up of several glycoprotein subunits that surround a central pore. These subunits can confer special characteristics on a channel, such as increased calcium permeability or longer opening times. The subunits of many channels change with development, thereby altering the channel properties.

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Box 2 | **Development of inhibitory transmission in the neonatal dorsal horn**

GABA (γ-aminobutyric acid) synthesizing enzymes, the glutamic acid decarboxylases 65 and 67 (GAD65/67), are expressed in the spinal cord from an early embryonic stage[172], but GABA is not expressed in the substantia gelatinosa until embryonic day (E)17–E18 (REF. 173). GABA-positive spinal neurons increase in the first postnatal weeks and then decrease towards adulthood[174]. A mature pattern of glycine expression is present before birth, mainly in the deep dorsal horn, which coincides with the presence of glycine transporter GLYT2 (REF. 175). Nevertheless, newborn (postnatal day (P)0–P1) lamina II neurons show only GABA miniature inhibitory postsynaptic currents (mIPSCs) despite possessing functional glycine receptors (GlyRs)[70]. After P8, mixed $GABA_AR$ (GABA type A receptor)–GlyR mIPSCs are observed. These are subsequently downregulated so that mIPSCs at older (P>30) ages are mediated by either GlyRs or $GABA_A$ receptors ($GABA_ARs$), but not both[14]. Neonatal GABA mIPSCs have long decay times and the rate of GABA mIPSC decay in laminae I–II neurons increases fourfold between P8 and P23, which was recently attributed to a tonic production of 5α-reduced neurosteroids in the immature dorsal horn[71].

$GABA_AR$ activation in the developing CNS can be depolarising rather than hyperpolarising, due to high intracellular Cl^- concentrations ($[Cl]_i$) in immature neurons, which give rise to a Cl^- REVERSAL POTENTIAL (E_{Cl}) that is more positive than the resting potential and, in some cases, the action potential threshold. The onset of expression of the K^+–Cl^- cotransporter KCC2 leads to a decrease in $[Cl]_i$ during the postnatal period, which leads to a negative shift in E_{Cl}, such that GABA and glycine become progressively hyperpolarising and inhibitory[36]. In cultured embryonic dorsal horn cells, $GABA_AR$ or GlyR activation results in membrane depolarization and elevated levels of intracellular Ca^{2+} (REFS 176,177). A subset of newborn superficial dorsal horn neurons in spinal cord slices depolarize in response to $GABA_AR$ activation, a response that becomes exclusively hyperpolarizing by P6–P7. However, E_{Cl} is consistently more negative than the action potential threshold, which indicates that depolarizing GABA responses will still inhibit the firing of newborn superficial dorsal horn neurons[70]. $GABA_AR$ depolarization mediates Ca^{2+} influx through N-type and L-type voltage-gated Ca^{2+} channels[176] and relieves the Mg^{2+} block of NMDARs[178], which might be important for synapse formation and remodelling[168].

All three $GABA_BR$ (GABA type B receptor) subunits ($GABA_BR1a$, 1b, and 2) and the $GABA_CR$ (GABA type C receptor) subunits, rho1 and rho2, are also expressed in the immature spinal cord[179,180]. Significant $GABA_BR$-mediated presynaptic inhibition of primary afferent-evoked excitatory postsynaptic currents (EPSCs) is observed from birth, but the effects of postsynaptic $GABA_BR$ increase over the first postnatal week[70,181].

reduction of the NMDA-evoked calcium influx in lamina II cells[87], a failure of A-fibre synaptic withdrawal from lamina II cells[88,89], a failure in the organization of expression patterns of neurokinin 1 (NK1) receptors[90], abnormal maturation of GABA-mediated and descending inhibition[91,92], and disorganized somatotopic maps[93].

However, under normal physiological circumstances, activation of nociceptors is rare in the first postnatal weeks and, in any case, C-fibre-evoked spike activity is weak at this time. This is an important consideration, as spike activity is required for enhanced neuronal protein synthesis and EPSCs in the absence of spiking can actually inhibit protein synthesis[94]. On the other hand, A-fibre inputs evoke plenty of spike activity in the newborn dorsal horn, and repetitive A-fibre stimulation causes sensitization of dorsal horn cells in the neonate, which takes the form of a build-up of background activity in the cells and post-stimulus after-discharge that lasts for a matter of minutes[60]. Therefore, spike activity-dependent shaping of nociceptive circuits might arise from low-threshold A-fibre activity. This is supported by the finding that postnatal tuning of the nociceptive tail reflex is not affected by daily noxious stimulation, but can be prevented by blocking low-intensity tactile inputs from the tail with local anaesthetic during the critical ten-day period. So, low-intensity tactile inputs, such as those that arise from spontaneous twitching during sleep, have been proposed to be responsible for shaping nociceptive circuits in early life[55,95,96]. The limb and abdominal withdrawal reflexes of preterm human infants show an analogous lack of tuning, and it would be interesting to know if these are also influenced by tactile stimulation[49,50].

The mechanism that underlies this A-fibre-induced synaptic tuning is not known. Repeated afferent stimulation might convert silent synapses in dorsal horn cells to functional ones by rapid insertion of AMPARs[68]. The postnatal synaptic elimination of exuberant A-fibre inputs, reduction of receptive field size and the increase in mechanical threshold of the dorsal horn are all activity-dependent processes, as they can be prevented by chronic, local blockade of NMDARs in the dorsal horn[30]. Intrathecal application of low doses of the NMDAR antagonist MK801 in P0 rat pups leads to greater A-fibre-evoked responses in substantia gelatinosa neurons and reduced mechanical thresholds compared with adults, whereas the response to C-fibre inputs and noxious heat threshold are unaffected (FIG. 4). It is possible that the postnatal developmental increase in firing frequency of A-fibre mechanoreceptors[33] might lead to a gradual mismatch in timing of pre- and postsynaptic spikes in lamina II cells, which are increasingly driven by low-frequency C-fibre inputs. This could lead to a HEBBIAN WEAKENING of synapses between A-fibre terminals and lamina II cells, perhaps through long-term depression (LTD)-type mechanisms. Low-frequency Aδ-fibre stimulation does produce a robust, NMDAR-dependent LTD in young lamina II cells[97].

HEBBIAN WEAKENING
Hebb proposed that if a neuron, A, took part in firing another neuron, B, then a plastic change would occur in the synapse between neurons A and B, such that the connection between A and B would be strengthened. This has been extended to include the opposite effect — that is, failure to take part in firing leads to synaptic weakening.

REVERSAL POTENTIAL
The membrane potential at which chemical and electrical drive are equal and opposite, so there is no net flow of ions across the membrane. The direction of flow reverses above and below this potential.

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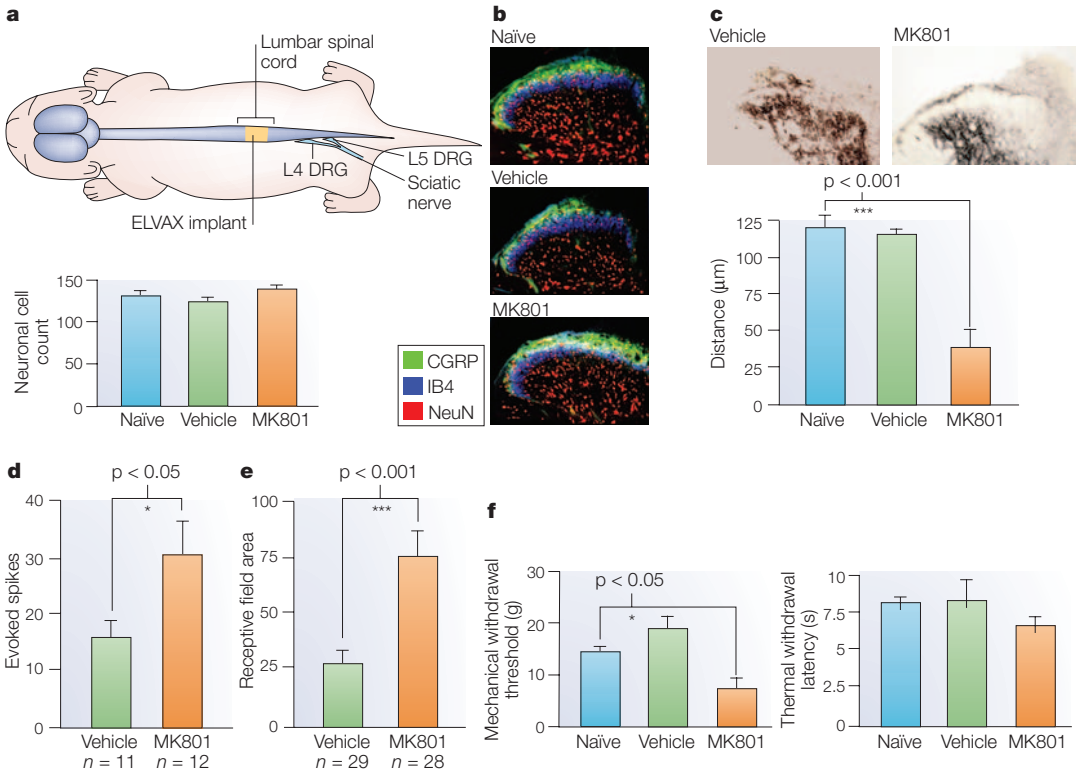

Figure 4 | **Activity-dependent development in spinal cord sensory connections. a** | Local, low-dose chronic NMDA (*N*-methyl-D-asparate) antagonist MK801 is applied to the dorsal surface of the rat spinal cord at birth and the dorsal horn circuitry is examined six weeks later. An ELVAX implant provides slow release of MK801 or vehicle. L4/L5 DRG, fourth and fifth lumbar dorsal root ganglion. **b** | The number of dorsal horn cells and termination of C fibres in lamina II are unaffected. CGRP, calcitonin gene-related peptide; IB4, isolectin B4; NeuN, neuronal nuclear antigen. **c** | A-fibre terminals are still present in lamina II in MK108- treated animals. The graph shows the length of A-fibre terminals as measured from the surface of the dorsal horn. **d** | The activity that is evoked by dorsal horn A fibres is enhanced in MK108-treated animals. **e** | Dorsal horn receptive fields are larger in MK108-treated animals. **f** | The thresholds for mechanical behavioural reflex are lower in MK108-treated animals, but thermal thresholds are normal. Adapted, with permission, from REF. 30 © (2002) Blackwell Publishing.

The effects of early tissue damage

Short-term effects. Nociceptive activity might not be a normal physiological event for newborn mammals, but in the event of tissue injury there is strong C-fibre activation over a prolonged period of time. C-fibre nociceptors can be sensitized by inflammatory chemicals from before birth[23], which further increases the afferent barrage after injury. Almost as soon as peripheral C-fibre stimulation begins to evoke spike responses in dorsal horn cells, repetitive stimulation evokes an NMDAR-dependent 'wind-up', in which the response amplitude increases with each subsequent stimulus[58,98,99]. Long-term potentiation (LTP) of lamina II cells has also been observed after repetitive high-frequency C-fibre stimulation of laminae I–II cells in juvenile rats[100]. 'Wind-up' and sensitization are known to be central mechanisms that underlie injury-induced pain.

Peripheral tissue injury not only evokes immediate nociceptive responses but can also lead to a state of hyperalgesia and allodynia, in which noxious responses are enhanced and can be evoked by previously non-noxious stimulation. Hyperalgesia that lasts for minutes (mustard oil), hours (formalin), days (carageenan) and weeks (complete Freunds adjuvant, CFA) can all be produced in young animals from P3 or earlier, although its magnitude is not always as great as in adults[54,101–104]. *In vivo* electrophysiological studies show sensitisation of dorsal horn cells after carageenan inflammation of the hindpaw at all postnatal ages, although the exact pattern of effects is age-dependent. Spontaneous activity and the magnitude of responses increase from the earliest postnatal age that has been studied (P3), but inflammation-induced expansion of mechanical receptive field size is not observed until at least the second postnatal week[59]. Hypersensitivity to tissue damage can also be measured in human infants, in which 'tenderness' or a fall in reflex thresholds is established for days or weeks in the presence of local or deep visceral tissue injury[49,50,105]. The effect is small in the youngest infants and increases with age in both rats and humans. Secondary hyperalgesia, which spreads into an area that surrounds the original injury site, seems to develop later than primary hyperalgesia in newborn rats[50,73].

The postnatal maturation of hyperalgesia might relate to the development of signalling by substance P, which is known to be important for this form of central sensitization. Substance P is released in very low quantities before P10 (REF. 64), although it is clearly involved in C-fibre-evoked LTP in lamina I projection neurons in

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older rat pups (P17–21)[106]. Application of exogenous substance P causes substantial depolarization of neonatal dorsal horn neurons[107], which shows that they have functional NK1 receptors, but NK1 receptor distribution undergoes marked postnatal changes in laminar distribution, and lamina II expression is not established for some weeks postnatally[108].

It is important to emphasize that the neonatal response to tissue injury is not simply a weak form of that found in the adult. Although we have only a poor appreciation of the signalling systems that underlie neonatal inflammatory hyperalgesia, evidence is accumulating for a profile that changes during the postnatal period. Unlike mature C fibres, exposure of neonatal C-fibre terminals to inflammatory mediators accelerates the development of the $IB4^+$ phenotype and increases CGRP expression in both small and large sensory neurons during early postnatal development[109]. The ability of NGF to induce sensitization in DRG cells is not evident in the rat before P10 (REF. 110), although it is likely to occur earlier in mice[111] (BOX 1). This indicates that there is a developmental switch in TrkA signal transduction cascades — from those involved in survival, growth and phenotypic development to those involved in hyperalgesia. Early inflammation also causes an acute expansion of the C-fibre central termination field and denser CGRP-positive terminals in lamina II of the dorsal horn, which is not observed in older animals[112,113], and induces a central protein kinase C (PKC)-signalling profile that is different from that in adults[114].

Long-term effects. Increasing awareness of activity-dependent and injury-related plasticity in the newborn CNS has highlighted the possibility that early tissue injury can affect future pain processing through developmental alterations in nociceptive circuitry[115]. Many preterm infants receive numerous invasive procedures in intensive care and it is not always possible to achieve adequate levels of analgesia. There is evidence from both animal models and humans that these early pain experiences might alter subsequent CNS function[116,115,117]. Although many of the nervous system responses to local tissue damage resolve after the injury has healed, tissue damage during a critical period in newborn rodents can cause prolonged alterations in somatosensory function, which last into adult life.

The consequences of neonatal injury in rodents depend on the type of injury and the modality of sensation under investigation. Repetitive paw needle prick in the first postnatal week produces heat hyperalgesia several weeks later[118,119]. Neonatal hindpaw inflammation has a pronounced effect on the behavioural and dorsal horn cellular response to a second inflammatory challenge well into adulthood[120–122], but does not produce heat or mechanical hyperalgesia beyond the first week[113,123]. Chemical or mechanical irritation of the colon in P8–21 rats, on the other hand, produces a persistent visceral hypersensitivity in the adult[124]. Skin wounds in the newborn also have prolonged effects: the skin remains hypersensitive long after the wound has healed[125] and the size of the dorsal horn receptive field increases for at least six weeks following injury[126].

Neonatal injury can also have the opposite effect. Both repetitive foot shock[127] and repeated formalin injections into neonatal paws[128] lead to a generalized heat hypoalgesia in adulthood. Furthermore, hindpaw inflammation causes a generalized and slowly-developing reduction in baseline sensitivity all over the body in response to mechanical and thermal stimuli, which provides a background to the enhanced inflammatory responses described above[120]. The early onset inflammatory hyperalgesia and the later onset baseline hypoalgesia only occur if the original inflammatory stimulus is applied within the first 10 days of life and both responses last into adulthood.

Although it is not known how these long-term changes in pain behaviours develop, candidate mechanisms might include alterations in synaptic connectivity and signalling in postnatal nociceptive pathways, and changes in the balance of inhibition versus excitation (BOXES 1,2; FIG. 3). Tissue injuries also release much higher concentrations of growth factors in neonates than in adults[24], which can have a myriad of potential effects on peripheral nociceptor development (FIG. 1). For example, excessive NGF at a critical period could increase the terminal density of nociceptors in the injured region and alter nociceptive phenotype — neonatal skin wounding results in a prolonged hyperinnervation of the wound site independent of neural activity[125,129]. Increased access to trophic factors might also trigger long-lasting expansion of C-fibre terminals in the dorsal horn[121]. Affected synapses might be strengthened and/or weakened by activity-dependent mechanisms, which could change the balance of excitation and inhibition in the CNS. The permanent expansion of dorsal horn receptive fields in a neonatally wounded area[126] indicates a failure to develop a targeted inhibitory control in nociceptive circuits (FIG. 3).

Long-term hypoalgesia that affects the whole body is likely to arise from an alteration or resetting of the stress response[120], as exposure to stress during the perinatal period is known to influence adult nociceptive behaviour[130,131]. This could be viewed as a useful adaptive behaviour in response to early trauma. Any long-term sensitization that occurs at a segmental level could be masked and require a strong stimulus, such as reinflammation, to uncover it.

A clear-cut example of a central adaptive response to neonatal injury is seen following peripheral nerve damage. Although partial peripheral nerve damage in adult rodents causes significant and prolonged neuropathic pain behaviour, which is characterized by marked allodynia, this does not occur in rat pups up to the age of P21 (REF. 132). During the first two postnatal weeks, tight ligation of the fifth and sixth lumbar spinal nerves produces only transient mechanical allodynia[133], whereas no change in sensitivity occurs in the SPARED NERVE INJURY (SNI) and the CHRONIC CONSTRICTION INJURY (CCI) models until P28 (REF. 132). This is consistent with the observation that no chronic long-term

SPARED NERVE INJURY AND CHRONIC CONSTRICTION INJURY
(SNI and CCI). Animal models of neuropathic pain aim to produce a partial denervation and/or inflammation around a nerve, as this seems to trigger characteristic allodynia or touch-evoked pain. SNI involves ligation of two nerves that supply the lateral hind paw, while leaving one intact; CCI involves tying loose ligatures around a major hindlimb nerve.

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pain follows brachial plexus avulsion at birth, in contrast to the severe pain that accompanies this type of injury in adults[134]. Neonatal nerve lesions are accompanied by substantial DRG cell death[135–138] due to lack of peripheral neurotrophic support (FIG. 1), followed by collateral sprouting of adjacent intact afferent terminals and the formation of new functional connections in the dorsal horn[139,140]. This central sprouting, which involves substance P- and CGRP-expressing fibres, is only observed if the injury occurs during the first postnatal week[141] and, therefore, cannot explain the lack of neuropathic pain behaviour in animals with nerve damage performed up to 3–4 weeks of age. This raises the intriguing possibility that some of the key signalling pathways for neuropathic pain[142,143] might not be active for a considerable postnatal period.

Concluding remarks and future perspectives

This review has concentrated on the maturation of nociceptive processing at the peripheral and spinal level. Although necessary, these processes are not sufficient to produce a true 'pain experience', which requires functional maturation of higher brain centres. Despite the existence of sensory reflexes from the first trimester of human fetal life, it is unlikely that the fetus is ever awake or aware and, therefore, able to truly experience pain, due to high levels of endogenous neuroinhibitors, such as adenosine and pregnanolone, which are produced in the feto-placental unit and contribute to fetal sleep states[144]. In preterm infants below 32 weeks most pain responses, including facial expressions, seem to be largely subcortical[145]. Stronger nociceptive reflexes in infants should not, therefore, be interpreted as a greater pain experience, but might be protective and beneficial to an organism that is unable to perceive and organize a more directed response to pain.

Nevertheless, the approach taken here to the development of nociceptive circuitry presents possibilities for translation to paediatric pain relief in clinical practice. Infant pain has traditionally been poorly understood and undertreated, and its assessment, treatment and functional consequences present a considerable unmet challenge[146,147]. The research discussed in this review could help elucidate the physiological and pharmacological mechanisms that underlie pain in infants. Furthermore, they could contribute to the design of better strategies for analgesia. Although pharmacokinetics and drug metabolism are major influences on analgesic efficacy, the neurobiological mechanisms of analgesia in immature pain pathways are also important. For example, the fact that neonates aged 7 days or younger require significantly less morphine postoperatively than older neonates[148] parallels the finding that morphine reduces mechanical sensitivity at lower doses in the neonatal rat pup compared with older animals[149,150] and is consistent with the high levels of μ-opiod receptor expression in the postnatal DRG and superficial dorsal horn[108,151,152]. The functional expression of μ-opioid receptors in both large A-fibre nociceptors and small C-fibre nociceptors in the newborn, which only gradually become restricted to small diameter C-fibre nociceptors by P21, might play a part in this extra sensitivity to morphine[150]. Other laboratory studies have shown that sensitivity to epidural local anaesthetics[101], α2 agonists[86], systemic codeine[153] and cyclooxygenase 1 (COX1) inhibitors[154] are all developmentally regulated. The fact that regulation of analgesia can frequently be separated from other central pharmacodynamic effects, such as sedation, indicates that investigating underlying synaptic pharmacology is as important as studying drug metabolism and distribution[81].

In conclusion, there is a promising future ahead for a better understanding of infant pain. The study of the developmental neurobiology of pain processing could contribute to a rational scientific design of analgesic regimes that are specific to young infants.

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Competing interests statement
The author declares no competing financial interests.

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