

## REVIEW ARTICLES

# Spinal cord mechanisms of pain

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The spinal cord is the first relay site in the transmission of nociceptive information from the periphery to the brain. Sensory signals are transmitted from the periphery by primary afferent fibres into the dorsal horn of the spinal cord, where these afferents synapse with intrinsic spinal dorsal horn neurones. Spinal projection neurones then convey this information to higher centres in the brain, where non-noxious and noxious signals can be perceived. During nociceptive transmission, the output of the spinal cord is dependent on various spinal mechanisms which can either increase or decrease the activity of dorsal horn neurones. Such mechanisms include local excitatory and inhibitory interneurones, *N*-methyl-D-aspartate receptor activation, and descending influences from the brainstem, which can be both inhibitory and excitatory in nature. After nerve injury or conditions of inflammation, shifts can occur in these excitatory and inhibitory mechanisms which modulate spinal excitability, often resulting in the heightened response of dorsal neurones to incoming afferent signals, and increased output to the brain, a phenomenon known as central sensitization. In this review, we consider the ways in which spinal cord activity may be altered in chronic pain states. In addition, we discuss the spinal mechanisms which are targeted by current analgesics used in the management of chronic pain.

*Br J Anaesth* 2008; **101**: 8–16

**Keywords:** analgesics non-opioid; pain; spinal cord

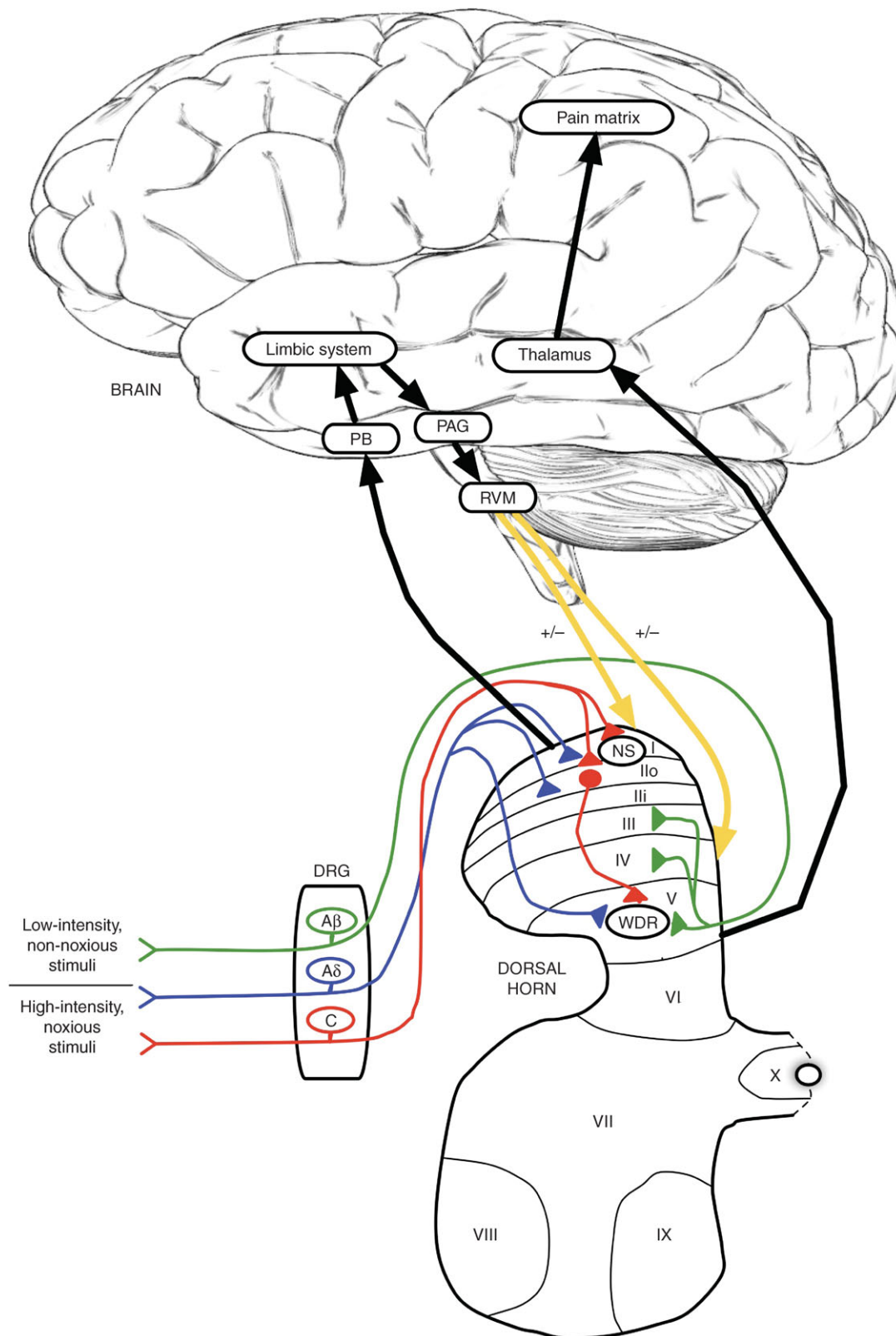
According to the International Association for the Study of Pain (IASP), pain is defined as 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage'. This definition reminds us that pain involves a significant psychological component which can alter its perception and therefore, undergoes extensive processing through the nervous system, and particularly in the brain. This account considers the ways in which the spinal cord, the first relay in the pathways from the periphery to the brain, can be sensitized by noxious stimuli, and thus allows a minor peripheral input to now be amplified. In addition, we will explore the ways in which current and future drugs may target spinal mechanisms in the treatment of chronic pain states.

## Peripheral mechanisms of sensory transmission

The sensory experience begins in the periphery, where the peripheral terminals of primary afferent fibres respond to a myriad of stimuli and translate this information into the dorsal horn of the spinal cord, where the central ends of these fibres terminate (Fig. 1). There are three main types of sensory fibre in the peripheral nervous system, A $\beta$ -fibres,

A $\delta$ -fibres, and C-fibres. Each has different properties allowing them to respond to and transmit different types of sensory information. A $\beta$ -fibres are large in diameter and highly myelinated, thus allowing them to quickly conduct action potentials from their peripheral to central terminals. These fibres have low activation thresholds and normally respond to light touch and are responsible for conveying tactile information. A $\delta$ -fibres are smaller in diameter and thinly myelinated, making them slower-conducting than A $\beta$ -fibres, and they also possess higher activation thresholds. They respond to both thermal and mechanical stimuli. C-fibres are the smallest type of primary afferents and are unmyelinated, thus making them the slowest conducting. They have the highest thresholds for activation and therefore detect selectively nociceptive or 'painful' stimuli. Collectively, both A $\delta$ - and C-fibres can be termed as nociceptors or 'pain fibres', responding to noxious stimuli which may be mechanical, thermal, or chemical.

A number of polymodal receptors on C-fibres can be selectively activated by noxious thermal and mechanical stimuli. In the case of noxious heat for example, it is widely believed that the transient receptor potential vanilloid receptor-1 (TRPV1)<sup>13</sup> receptor-channel, which responds to capsaicin, the extract of chilli peppers, may also be responsible for the generation of action potentials after application



**Fig 1** Pain pathways from periphery to brain. Primary afferent fibres (A $\beta$ -, A $\delta$ -, and C-fibres) transmit impulses from the periphery, through the dorsal root ganglion (DRG) and into the dorsal horn of the spinal cord. Nociceptive specific (NS) cells are mainly found in the superficial dorsal horn (laminae I–II), whereas most wide dynamic ranges (WDRs) are located deeper (lamina V). Projection neurones from lamina I innervate areas such as the parabrachial area (PB) and periaqueductal grey (PAG) and such pathways are affected by limbic areas. From here descending pathways (yellow arrows) from brainstem nuclei such as the rostral ventromedial medulla (RVM) are activated and modulate spinal processing. Lamina V neurones mainly project to the thalamus (spinothalamic tract), and from here the various cortical regions forming the ‘pain matrix’ (primary and secondary somatosensory, insular, anterior cingulate, and prefrontal cortices) are activated.

of heat. The endogenous ligand for this receptor is unclear, although the cannabinoid, anandamide, is one potential candidate.<sup>44–89</sup> The peripheral terminals of small diameter neurones are excited by a number of endogenous chemical mediators, especially in conditions of tissue inflammation. These can be released from local non-neuronal cells, the afferent fibres themselves, and from products triggered by activation of the body's defence mechanisms. These chemical mediators then act to sensitize nociceptors so that afferent activity to a given stimulus is increased. This is known as primary hyperalgesia. Pain hypersensitivity due to peripheral sensitization has been shown to occur after inflammation, via activation of intracellular signalling pathways such as protein kinase A (PKA)<sup>6</sup> and protein kinase C (PKC).<sup>5</sup> Activated kinases bring about sensitization through phosphorylation and activation of receptors such as TRPV1. It has been shown that phosphatidylinositol-3 kinase (PI3K) is also activated by inflammation and is able to mediate heat hyperalgesia through sensitization of TRPV1 in an extracellular signal-regulated kinase (ERK)-dependent manner.<sup>88</sup>

Neuropathy elicits a number of changes in nerves in terms of activity, properties, and transmitter content. The recent advent of a number of animal models of neuropathic pain states has facilitated our understanding of the peripheral mechanisms involved. Damaged nerves may start to generate ongoing ectopic activity due to the accumulation and clustering of sodium (Na<sup>+</sup>) channels around the damaged axons and there is also evidence that mechanoreceptors become highly sensitive to applied stimuli.<sup>55</sup> This aberrant activity can then start to spread rapidly to the cell bodies in the dorsal root ganglia. Nerve fibres can start to cross-excite each other (ephaptic transmission) and the same occurs in the cell bodies. In addition to changes within sensory nerves, sympathetic efferents become able to activate sensory afferents via, as yet, poorly characterized  $\alpha$ -adrenoceptors. These interactions between adjacent sensory and autonomic nerve axons and between ganglion cells results in excitation spreading between different nerve fibres. These peripheral ectopic impulses can cause spontaneous pain and prime the spinal cord to exhibit enhanced evoked responses to stimuli, which themselves have greater effects due to increased sensitivity of the peripheral nerves.

### Sensory transmission in the dorsal horn

The central terminals of primary afferent fibres terminate in the dorsal horn of the spinal cord, which is organized into different laminae, extending from the superficial to the deep dorsal horn. Most nociceptive A $\delta$ - and C-fibres terminate superficially in laminae I–II, with a smaller number reaching deeper laminae, whereas A $\beta$ -fibres predominantly innervate laminae III–VI.<sup>70</sup> The spinal cord itself contains various neuronal cell types which make connections with primary afferents, and depending on

their specific synaptic inputs, have different properties, and respond to different types of sensory information (Fig. 1). Nociceptive-specific (NS) cells are mostly found superficially and synapse with A $\delta$ - and C-fibres only. These cells fire action potentials when a painful stimulus is detected at the periphery. Cells which receive input exclusively from A $\beta$ -fibres are proprioceptive and only respond to touch. A third type of neurone, termed wide dynamic range (WDRs), receive input from all three types of sensory fibre, and therefore respond to the full range of stimulation, from light touch to noxious pinch, heat, and chemicals. WDRs fire action potentials in a graded fashion depending on stimulus intensity, and also exhibit 'wind-up', a short-lasting form of synaptic plasticity. During wind-up, repetitive stimulation of WDR neurones induces an increase of their evoked response and post-discharge with each stimulus.<sup>18</sup> There are also excitatory, glutamatergic, and inhibitory, GABAergic interneurons within the spinal cord and these can increase or decrease the response of NS cells and WDRs, thus influencing the output of the dorsal horn. Recent evidence has shown that non-neuronal cell types within the spinal cord, namely astrocytes and microglia, are also able to influence pain transmission through the dorsal horn, particularly under pathological conditions.<sup>16–45–79</sup> In this review, we focus specifically on neuronal mechanisms. However, the role of non-neuronal cells and their interactions with neurones should not be underestimated, and have recently been reviewed elsewhere.<sup>29–58–80</sup>

### Glutamatergic mechanisms of spinal excitability modulate nociceptive transmission

The spinal cord is an important site at which the various incoming sensory and nociceptive signals undergo convergence and modulation. Spinal neurones, which respond to these peripherally generated signals, are under ongoing control by peripheral inputs, interneurons, and descending controls. One consequence of this modulation is that the relationship between stimulus and response to pain is not always straightforward. The response of output cells could be greatly altered via the interaction of various neurotransmitter systems in the spinal cord, all of which are subject to plasticity and alterations, particularly during pathological conditions.

Glutamate is an excitatory amino acid and is the major excitatory neurotransmitter found throughout the whole of the nervous system, and is therefore essential for pain signalling at every anatomical level. Thus, as expected, the vast majority of primary afferents synapsing in the dorsal horn of the spinal cord, regardless of whether they are small or large diameter, utilize this transmitter. Glutamate exerts an excitatory effect on a number of receptors found on post-synaptic spinal neurones,<sup>8–71</sup> leading to membrane depolarization via three distinct receptor subclasses, the  $\alpha$ -amino-3-hydroxy 5-methyl-4-isoxazepropionic acid (AMPA) receptor,<sup>32–50–72</sup> the *N*-methyl-D-aspartate

(NMDA) receptors,<sup>48 71</sup> and the G-protein coupled metabotropic (mGluR) family of receptors.<sup>77 85 86</sup> In addition, pre-synaptic kainate receptors for glutamate have been described in the spinal cord.<sup>26 30 31 37</sup> Most is known about the role of ionotropic AMPA and NMDA receptors in pain, both of which are named after chemical analogues of glutamate with selective actions at these sites.

Glutamate is released from sensory afferents in response to acute and more persistent noxious stimuli, and it is fast AMPA receptor activation that is responsible for setting the initial baseline response of spinal dorsal horn neurones to both noxious and tactile stimuli. However, if a repetitive and high-frequency stimulation of C-fibres occurs, there is then an amplification and prolongation of the response of spinal dorsal horn neurones to subsequent inputs, so-called wind-up.<sup>18</sup> This enhanced activity results from the activation of the NMDA receptor. If there are only acute or low-frequency noxious or tactile inputs to the spinal cord, then activation of the NMDA receptor is not possible, since under normal physiological conditions the ion channel of this receptor is blocked by the normal levels of magnesium ions ( $Mg^{2+}$ ) found in nervous tissues. This unique  $Mg^{2+}$  plug of the channel requires a sustained depolarization of the membrane in order to be removed and allow the NMDA receptor-channel to be activated and opened. Here, it is likely that the co-release of peptidergic transmitters, such as substance P and CGRP, which are found in C-fibres along with glutamate, is responsible for a prolonged slow depolarization of the neurone and subsequent removal of the NMDA block, thus permitting wind-up to occur.<sup>10 33 66</sup> AMPA receptor antagonists have little effect on wind-up,<sup>59 63</sup> and the brief depolarization produced by this receptor would not be expected to produce any prolonged removal of the NMDA block, unlike the long-lasting, slow (several seconds) activations caused by peptides. The lack of peptides in large A $\beta$  afferent fibres explains the lack of wind-up after low-threshold stimuli. NMDA receptor activation has been clearly shown to play a key role in the hyperalgesia and enhancement of pain signalling seen in more persistent pain states including inflammation and neuropathic conditions.<sup>17 51 61</sup>

The major mechanism by which the NMDA receptor acts is through the large influx of calcium ions ( $Ca^{2+}$ ) occurring when the channel is activated. Once inside the cell,  $Ca^{2+}$  can activate various effectors and promote downstream changes. Such effectors include neuronal nitric oxide synthase,<sup>11 35</sup> calcium/calmodulin-dependent kinases (CaMKI/II),<sup>39 40</sup> and ERK<sup>28 84</sup> which can promote mechanisms of plasticity such as long-term potentiation (LTP). Similar plastic mechanisms occur after acute high-intensity C-fibre stimulation, peripheral nerve damage, and inflammation, and can result in the elevated responsiveness and activity of dorsal horn neurones.<sup>18 83</sup> This phenomenon, termed central sensitization, manifests in the patient as an increased response to painful stimuli (hyperalgesia), and pain resulting from normally non-painful tactile

stimuli (allodynia). Therefore, the targeting of NMDA signalling with pharmacological interventions has been explored as an analgesic strategy in great depth.

There are a number of antagonists to the multiple regulatory sites found on the NMDA receptor and its channel, including the licensed drugs, ketamine, a potent channel blocker, and the weaker agents, dextromethorphan and memantine. These drugs have been shown to be antinociceptive in a number of animal models of inflammation and nerve damage and there is also evidence from human volunteer and clinical studies to support this.<sup>17 51 61</sup> Overall, these studies indicate that it is likely that aberrant peripheral activity is amplified and enhanced by NMDA receptor-mediated spinal mechanisms in tissue damage and neuropathic pain and that the receptor is critical for both the induction and the maintenance of the pain. Thus, therapy after the initiating damage can still be effective. Despite there being good clinical evidence for effectiveness of agents acting as antagonists at the NMDA receptor complex, especially ketamine, and although some individual patients do get good pain relief in nerve injury situations, the majority cannot achieve complete pain control. This is partly because adequate dosing is prevented by the narrow therapeutic window of the existing drugs. This is largely due to the widespread distribution and functionality of NMDA receptors, meaning that the introduction of an antagonist will not only target the pathology, but will also disrupt normal essential NMDA signalling within the central nervous system, and this explains why such drugs are commonly associated with numerous unavoidable and unacceptable side-effects. Ultimately, the broad use of NMDA antagonists in the treatment of chronic pain will therefore depend on strategies that increase their therapeutic window over existing drugs. These may include drugs acting on specific sub-types of the receptor (NR2B receptor antagonists are analgesic with a better side-effect profile),<sup>9 14</sup> drugs with different use-dependent block of the channel, or more practically, the use of low-dose NMDA blockers in combination with another agent.

## Spinal projections to higher centres in the brain

The output from the dorsal horn to higher centres in the brain is carried by spinal projection neurones along ascending pathways (Fig. 1). A large population of projection neurones is found superficially in lamina I. It is estimated that 80% of these cells express the neurokinin 1 (NK1) receptor for substance P,<sup>70</sup> a neuropeptide which is released by nociceptive afferents, meaning that these cells respond to noxious stimulation.<sup>19 42</sup> NK1-positive cells in lamina I have been shown to project to areas in the brain such as the thalamus, the periaqueductal grey (PAG), and in particular the parabrachial area (PB).<sup>70</sup> In addition to transmitting pain signals up to higher centres in the brain, these cells also project into brainstem areas such as the



rostral ventromedial medulla (RVM), a region which has descending projections back to the dorsal horn. Therefore, lamina I NK1-expressing cells can modulate spinal processing by activation of descending pathways from the brainstem.<sup>42–67</sup> These descending pathways can be influenced by limbic regions in the brain and so incorporate the emotional, affective component of the pain experience. A large number of projection neurones are also found deeper in the dorsal horn from lamina III–VI and these project predominantly to the thalamus, thereby making up a significant proportion of the spinothalamic tract. This ascending pathway carries primarily sensory information and so provides the sensory component of the pain experience.

From the thalamus, nociceptive information is transmitted to cortical regions. There does not exist a single pain centre within the cortex, but rather there are various cortical regions which may or may not be activated during a particular painful experience. These regions make up what is commonly referred to as a 'pain matrix' and include primary and secondary somatosensory, insular, anterior cingulate, and prefrontal cortices.<sup>73</sup>

### **Brainstem modulation of dorsal horn excitability via descending monoaminergic pathways and implications for therapy**

Descending pathways from brainstem structures are able to influence nociceptive signalling in the dorsal horn of the spinal cord. Such descending influences are both facilitatory and inhibitory in nature (Fig. 1). Descending facilitatory pathways from the RVM in the brainstem have been shown to be involved in the maintenance, but not the initiation, of nerve injury-induced pain.<sup>12 36 78</sup> Injection of the local anaesthetic lidocaine, into the RVM reverses established behavioural hypersensitivity in nerve-injured animals, but does not prevent the expression of this hypersensitivity.<sup>12</sup> In an electrophysiological study, injection of lidocaine into the RVM reduced dorsal horn neuronal responses to noxious stimuli in normal animals. This effect of lidocaine was greater in nerve-injured animals, and in these animals, it was observed that descending facilitation from the RVM now influenced neuronal responses to non-noxious tactile stimulation, thus suggesting a possible mechanism for mechanical allodynia.<sup>4</sup> The origin of such modulation from nuclei in the brainstem is in fact located in the superficial dorsal horn itself, thus forming a spino-bulbo-spinal loop which can modulate spinal nociceptive transmission. Suzuki and colleagues<sup>67</sup> showed that the ablation of lamina I/III NK1-expressing neurones with a substance P and saporin conjugate (SP–SAP) reduces the excitability of deep dorsal horn WDRs, indicating that descending influences are predominantly facilitatory, and act via spinal 5-HT<sub>3</sub> receptors, since the effect of SP–SAP could be mimicked by spinally administered ondansetron, a selective 5-HT<sub>3</sub> antagonist.

Pharmacological block of spinal 5-HT<sub>3</sub> receptors reveals a role for a serotonergic descending facilitatory influence in the modulation of spinal nociceptive transmission. These 5-HT<sub>3</sub> receptors are predominantly expressed on nerve terminals of small diameter afferents<sup>87</sup> and exert pronociceptive effects at the spinal level.<sup>1 25 49</sup> The contribution of such descending serotonergic facilitation to neuropathic pain was further confirmed by the fact that the efficacy of ondansetron to suppress spinal responses to mechanical punctuate stimuli was significantly enhanced after spinal nerve ligation, suggesting an increase in descending serotonergic facilitatory drive to the spinal cord.<sup>68</sup> In accordance, depletion of spinal 5-HT attenuates signs of behavioural hypersensitivity after nerve ligation.<sup>53</sup> Additionally, it has been shown that spinal SP–SAP treatment attenuates behavioural hypersensitivity and also abnormal neuronal coding exhibited after both nerve ligation<sup>69</sup> and intraplantar injection of capsaicin.<sup>33 42</sup> Interestingly, although the spinal administration of SP–SAP can both block wind-up and prevent spinal LTP in deep WDRs, a phenomenon related to central sensitization, ondansetron does not reduce these events.<sup>56</sup> However, it does mimic all other effects of SP–SAP in terms of reducing neuronal coding to mechanical and thermal stimuli in both normal and neuropathic animals and also attenuates chemical coding. This indicates that wind-up and LTP are intrinsic spinal events using spinal mechanisms but that the brain can further facilitate responses of dorsal horn neurones. Thus targeting of this facilitatory spino-bulbo-spinal loop may provide novel therapeutics for analgesia. A small double-blinded, placebo-controlled, crossover study has suggested an antinociceptive effect of ondansetron in humans which merits further investigation,<sup>43</sup> whereas SP–SAP, although not tested in humans, has been found to be without toxic effects when administered in dogs.<sup>2</sup>

Gabapentin is used as a first line treatment for neuropathic pain.<sup>3 54 60</sup> Although originally designed as a GABA analogue, it has no significant interaction with GABA mechanisms, but binding data have revealed possible interactions with the auxiliary  $\alpha_2\delta$  subunit of voltage-dependent Ca<sup>2+</sup> channels (VDCCs).<sup>23</sup> Interestingly, gabapentin is effective in one in three patients suggesting that there are yet unknown factors that govern its effectiveness. Given the similar presynaptic localization of the gabapentin binding site on calcium channels and the spinal 5-HT<sub>3</sub> receptor,<sup>34 47</sup> we have employed *in vivo* electrophysiological approaches to study whether disruption of the spino-bulbo-spinal loop, through ablation of lamina I/III NK1 positive neurones, induces alterations in neuronal sensitivity to gabapentin after peripheral nerve injury. Spinal SP–SAP was able to block the antinociceptive actions of gabapentin after nerve injury.<sup>69</sup> Furthermore, the responses of deep dorsal horn neurones were characterized to a wide range of natural and electrical stimuli to reveal the role of NK-1 expressing neurones in the development of neuropathic pain and associated plasticity in the spinal cord. We then manipulated the

5-HT<sub>3</sub> receptor to show that the actions of gabapentin are dependent on activation of this receptor. Blocking the receptor prevented the actions of gabapentin after nerve injury and even more remarkably, activation allowed the drug to now work in normal animals. Thus, activation of the excitatory 5-HT<sub>3</sub> receptor enhances pain processing, but at the same time produces a state-dependent or permissive interaction that allows treatment.<sup>69</sup> These results further support a role for descending serotonergic (5-HT<sub>3</sub> receptor-mediated) pathways in the development of injury-related hypersensitivity. Superficial NK1 positive neurones can trigger descending facilitation mediated through parabrachial–RVM connections and regulate the sensitivity of deeper lying neurones to gabapentin through activation of spinal 5-HT<sub>3</sub> receptors. These excitatory influences promote spinal central sensitization and facilitating nociceptive reflexes and their inappropriate tonic activation contributes to the pathology of neuropathic pain. These supraspinal descending facilitatory systems are likely to represent a central mechanism by which the loss of sensory input resulting from the nerve damage is compensated.<sup>17</sup>

Descending inhibition largely involves the release of norepinephrine (NE) in the spinal cord from brainstem nuclei such as the locus coeruleus (LC), acting predominantly at the  $\alpha_2$ -adrenoceptor subclass, and inhibiting transmitter release from primary afferent terminals and suppressing firing of projection neurones in the dorsal horn.<sup>46</sup> Clonidine, which has been clinically successful in the alleviation of neuropathic pain<sup>21</sup> and which is licensed for the treatment of cancer pain in the USA,<sup>22</sup> acts by partial agonism at spinal  $\alpha_2$ -adrenoceptors. Again, it has recently been shown that NK1-expressing cells project to the brainstem and initiate inhibitory descending noradrenergic projections, although descending facilitation via 5-HT<sub>3</sub> receptors seems to predominate.<sup>67</sup> Like descending facilitation, inhibitory noradrenergic pathways from the brainstem to the dorsal horn may also undergo plastic changes in chronic pain states. Several studies after peripheral inflammation indicate an increase in descending noradrenergic inhibition,<sup>24 62 74–76 81</sup> coupled with an enhanced efficacy of spinally administered  $\alpha_2$ -adrenoceptor agonists.<sup>27 41 65</sup> This increased inhibitory drive is presumably a homeostatic mechanism initiated in an attempt to counteract an enhanced facilitatory drive and increased spinal hyperexcitability. It has also been suggested that there is increased noradrenergic innervation to the dorsal horn after nerve injury,<sup>38</sup> again analogous to the enhanced facilitatory drive to the dorsal horn mediated by spinal 5-HT<sub>3</sub> receptors. Studies have shown nerve injury-induced changes in noradrenergic pathways, including up-regulation of spinal  $\alpha_{2A}$ -adrenoceptors<sup>7 15 64</sup> and increased spinal NE content.<sup>57</sup> Again, the enhanced potency of  $\alpha_2$ -adrenergic agonists after nerve injury points to an enhancement of descending inhibition, or at least to an increased noradrenergic innervation and sensitivity of the dorsal horn, but these two mechanisms are not necessarily the same thing. The use of

agonists shows that a system or pathway can be activated. However, in order to show that a particular system or pathway is in fact active during a particular physiological function, the use of antagonists is required. It is plausible that the increased noradrenergic receptor density and innervation of the dorsal horn observed after nerve injury is the result of compensatory mechanisms which occur to counteract the actual loss of a tonic descending noradrenergic inhibitory drive. Therefore, it would be expected that  $\alpha_2$ -adrenoceptor agonists would increase in potency and efficacy after nerve injury. In support of this, a recent study from our laboratory has utilized the selective  $\alpha_2$ -adrenoceptor agonist atipamezole, to show that there is an apparent loss of descending noradrenergic influences after spinal nerve ligation, but only in specific sensory modalities.<sup>52</sup> Atipamezole enhanced evoked responses of dorsal horn neurones to low-intensity mechanical stimulation. This observation only occurred in control sham-operated animals, and was absent in nerve-ligated animals, suggesting a selective control of descending inhibition, via spinal  $\alpha_2$ -adrenoceptors, on low-intensity mechanical neuronal responses. No effects of atipamezole were seen after noxious mechanical or both non-noxious and noxious thermal stimulation, in either sham-operated or nerve-injured animals. Further evidence for this differential control of stimulus modalities was shown previously with the use of a selective and potent  $\alpha_2$ -adrenoceptor agonist S18616.<sup>65</sup> This compound suppressed dorsal horn neuronal responses to thermal and high-intensity mechanical stimulation equally in both sham-operated and nerve-ligated rats. However, the suppression of low-intensity mechanically evoked responses was greatly enhanced after nerve injury, supporting the loss of descending controls of this sensory modality, but also supporting the up-regulation of adrenergic receptor density and enhanced sensitivity to  $\alpha_2$ -adrenoceptor agonists. Interestingly, atipamezole also enhanced spontaneous activity of dorsal horn neurones, again exclusively in sham-operated rats.<sup>52</sup> Overall, these results suggest that there is a loss of tonic descending inhibitory control of neuronal responses to low-intensity mechanical stimulation and also of spontaneous neuronal activity in the dorsal horn. Coupled with the enhancement of descending serotonergic facilitation, this decrease in descending noradrenergic inhibition would result in an overall enhancement of dorsal horn excitability, which manifests as mechanical hypersensitivity and allodynia, and spontaneous pain, common complaints of neuropathic pain patients.

This dual control of the spinal cord by monoamine systems in the brain, whereby 5-HT appears to enhance spinal processing and NE acts to inhibit activity may be one way in which the brain can alter pain processing, and may be the route by which sleep, anxiety, coping, and catastrophizing can impact upon the level of pain perceived. In this context, the use of antidepressants to control pain relates to activity in these systems. Agents that block the reuptake of either or both of these neurotransmitters, such

as tricyclic antidepressants (TCAs), selective-serotonin reuptake inhibitors (SSRIs), and serotonin-NE reuptake inhibitors (SNRIs) provide benefit in the treatment of pain.<sup>20</sup> Antidepressants are used to increase either 5-HT- or NE-mediated neurotransmission, or both. Studies have also shown that inhibiting both these monoamines is more effective than inhibiting just 5-HT alone, and in this regard, the ability of NE to inhibit pain through  $\alpha_2$ -adrenoceptor activation, whereas 5-HT can enhance pain, is a basis for the need for increased NE levels as a determinant of efficacy of antidepressant drugs in pain.<sup>20</sup>

## Conclusions

In this review, we have discussed mechanisms of pain transmission centred around the spinal cord, the first relay site in pain pathways from periphery to brain, with respect to mechanisms of pain processing and how intrinsic events at spinal levels have the ability to enhance pain. We have aimed to explain how current drugs employed in the treatment of chronic pain states interact with these systems including the use of NMDA receptor blockers such as ketamine. TCAs, such as amitriptyline, and SNRIs are often part of the pharmacotherapy for neuropathic pain, and we describe how these agents can interact with descending pathways that link the brain with the modulation and enhancement of pain. The ability of drugs such as gabapentin/pregabalin to alter excitability is also discussed. It should also be remembered that these drugs may also have a significant supraspinal mechanism of action, in particular antidepressants, which may act on the significant psychological component of pain perception, and thus allow patients to better cope with their pain.

Drugs that may have a more local, peripheral mechanism of action are also used in the pain clinic. For example, carbamazepine, an anti-epileptic which is effective in trigeminal neuralgia, targets  $\text{Na}^+$  channels, which have been shown to have an extensive role in peripheral pain transmission.<sup>82</sup> The local anaesthetic lidocaine can be applied directly to the surface of the skin in patches (Lidoderm®). Capsaicin can be applied in a similar way by the use of a topical cream (Zostrix®) and acts to desensitize peripheral nociceptors which express TRPV1 channels.

The extensive pain literature now contains details of numerous peripheral and central mechanisms, including various receptors, channels, and also intracellular signalling pathways, which may provide novel, future drug targets for the clinical management of chronic pain. We envisage that a large proportion of targets will employ spinal mechanisms to combat the as yet, unsolved problem of chronic pain.

## Funding

We are supported by the Wellcome Trust London Pain Consortium.

## References

- 1 Ali Z, Wu G, Kozlov A, Barasi S. The role of 5HT<sub>3</sub> in nociceptive processing in the rat spinal cord: results from behavioural and electrophysiological studies. *Neurosci Lett* 1996; **208**: 203–7
- 2 Allen JW, Mantyh PW, Horais K, et al. Safety evaluation of intrathecal substance P-saporin, a targeted neurotoxin, in dogs. *Toxicol Sci* 2006; **91**: 286–98
- 3 Backonja M, Beydoun A, Edwards KR, et al. Gabapentin for the symptomatic treatment of painful neuropathy in patients with diabetes mellitus: a randomized controlled trial. *JAMA* 1998; **280**: 1831–6
- 4 Bee LA, Dickenson AH. Rostral ventromedial medulla control of spinal sensory processing in normal and pathophysiological states. *Neuroscience* 2007; **147**: 786–93
- 5 Bhawe G, Hu HJ, Glauner KS, et al. Protein kinase C phosphorylation sensitizes but does not activate the capsaicin receptor transient receptor potential vanilloid 1 (TRPV1). *Proc Natl Acad Sci USA* 2003; **100**: 12480–5
- 6 Bhawe G, Zhu W, Wang H, et al. cAMP-dependent protein kinase regulates desensitization of the capsaicin receptor (VR1) by direct phosphorylation. *Neuron* 2002; **35**: 721–31
- 7 Birder LA, Perl ER. Expression of alpha2-adrenergic receptors in rat primary afferent neurones after peripheral nerve injury or inflammation. *J Physiol* 1999; **515**(Pt 2): 533–42
- 8 Bleakman D, Alt A, Nisenbaum ES. Glutamate receptors and pain. *Semin Cell Dev Biol* 2006; **17**: 592–604
- 9 Boyce S, Wyatt A, Webb JK, et al. Selective NMDA NR2B antagonists induce antinociception without motor dysfunction: correlation with restricted localisation of NR2B subunit in dorsal horn. *Neuropharmacology* 1999; **38**: 611–23
- 10 Budai D, Larson AA. Role of substance P in the modulation of C-fiber-evoked responses of spinal dorsal horn neurons. *Brain Res* 1996; **710**: 197–203
- 11 Budai D, Wilcox GL, Larson AA. Effects of nitric oxide availability on responses of spinal wide dynamic range neurons to excitatory amino acids. *Eur J Pharmacol* 1995; **278**: 39–47
- 12 Burgess SE, Gardell LR, Ossipov MH, et al. Time-dependent descending facilitation from the rostral ventromedial medulla maintains, but does not initiate, neuropathic pain. *J Neurosci* 2002; **22**: 5129–36
- 13 Caterina MJ, Schumacher MA, Tominaga M, et al. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* 1997; **389**: 816–24
- 14 Chizh BA, Headley PM, Tzschentke TM. NMDA receptor antagonists as analgesics: focus on the NR2B subtype. *Trends Pharmacol Sci* 2001; **22**: 636–42
- 15 Cho HJ, Kim DS, Lee NH, et al. Changes in the alpha 2-adrenergic receptor subtypes gene expression in rat dorsal root ganglion in an experimental model of neuropathic pain. *Neuroreport* 1997; **8**: 3119–22
- 16 Coyle DE. Partial peripheral nerve injury leads to activation of astroglia and microglia which parallels the development of allodynic behavior. *Glia* 1998; **23**: 75–83
- 17 Dickenson A, Suzuki R, Matthews EA, et al. Balancing excitations and inhibitions in spinal circuits. In: Villanueva L, Dickenson AH, Ollat H, eds. *The Pain System in Normal and Pathophysiological States: A Primer for Clinicians*. Seattle: IASP Press, 2004; 79–105
- 18 Dickenson AH, Sullivan AF. Evidence for a role of the NMDA receptor in the frequency dependent potentiation of deep rat dorsal horn nociceptive neurones following C fibre stimulation. *Neuropharmacology* 1987; **26**: 1235–8
- 19 Doyle CA, Hunt SP. Substance P receptor (neurokinin-1)-expressing neurons in lamina I of the spinal cord encode for the

- intensity of noxious stimulation: a c-Fos study in rat. *Neuroscience* 1999; **89**: 17–28
- 20 Dworkin RH, O'Connor AB, Backonja M, et al. Pharmacologic management of neuropathic pain: evidence-based recommendations. *Pain* 2007; **132**: 237–51
  - 21 Eisenach JC, De Kock M, Klimscha W. Alpha(2)-adrenergic agonists for regional anesthesia. A clinical review of clonidine (1984–1995). *Anesthesiology* 1996; **85**: 655–74
  - 22 Eisenach JC, DuPen S, Dubois M, Miguel R, Allin D. Epidural clonidine analgesia for intractable cancer pain. The Epidural Clonidine Study Group. *Pain* 1995; **61**: 391–9
  - 23 Gee NS, Brown JP, Dissanayake VU, et al. The novel anti-convulsant drug, gabapentin (Neurontin), binds to the alpha2delta subunit of a calcium channel. *J Biol Chem* 1996; **271**: 5768–76
  - 24 Green GM, Lyons L, Dickenson AH. Alpha2-adrenoceptor antagonists enhance responses of dorsal horn neurones to formalin induced inflammation. *Eur J Pharmacol* 1998; **347**: 201–4
  - 25 Green GM, Scarth J, Dickenson A. An excitatory role for 5-HT in spinal inflammatory nociceptive transmission; state-dependent actions via dorsal horn 5-HT(3) receptors in the anaesthetized rat. *Pain* 2000; **89**: 81–8
  - 26 Hwang SJ, Pagliardini S, Rustioni A, Valtchanoff JG. Presynaptic kainate receptors in primary afferents to the superficial laminae of the rat spinal cord. *J Comp Neurol* 2001; **436**: 275–89
  - 27 Hylden JL, Thomas DA, Iadarola MJ, Nahin RL, Dubner R. Spinal opioid analgesic effects are enhanced in a model of unilateral inflammation/hyperalgesia: possible involvement of noradrenergic mechanisms. *Eur J Pharmacol* 1991; **194**: 135–43
  - 28 Ji RR, Baba H, Brenner GJ, Woolf CJ. Nociceptive-specific activation of ERK in spinal neurons contributes to pain hypersensitivity. *Nat Neurosci* 1999; **2**: 1114–9
  - 29 Ji RR, Suter MR. p38 MAPK, microglial signaling, and neuropathic pain. *Mol Pain* 2007; **3**: 33
  - 30 Kerchner GA, Wilding TJ, Huettner JE, Zhuo M. Kainate receptor subunits underlying presynaptic regulation of transmitter release in the dorsal horn. *J Neurosci* 2002; **22**: 8010–7
  - 31 Kerchner GA, Wilding TJ, Li P, Zhuo M, Huettner JE. Presynaptic kainate receptors regulate spinal sensory transmission. *J Neurosci* 2001; **21**: 59–66
  - 32 Kerr RC, Maxwell DJ, Todd AJ. GluR1 and GluR2/3 subunits of the AMPA-type glutamate receptor are associated with particular types of neurone in laminae I–III of the spinal dorsal horn of the rat. *Eur J Neurosci* 1998; **10**: 324–33
  - 33 Khasabov SG, Rogers SD, Ghilardi JR, et al. Spinal neurons that possess the substance P receptor are required for the development of central sensitization. *J Neurosci* 2002; **22**: 9086–98
  - 34 Kia HK, Miquel MC, McKernan RM, et al. Localization of 5-HT3 receptors in the rat spinal cord: immunohistochemistry and in situ hybridization. *Neuroreport* 1995; **6**: 257–61
  - 35 Kitto KF, Haley JE, Wilcox GL. Involvement of nitric oxide in spinally mediated hyperalgesia in the mouse. *Neurosci Lett* 1992; **148**: 1–5
  - 36 Kovelowski CJ, Ossipov MH, Sun H, et al. Supraspinal cholecystokinin may drive tonic descending facilitation mechanisms to maintain neuropathic pain in the rat. *Pain* 2000; **87**: 265–73
  - 37 Lucifora S, Willcockson HH, Lu CR, et al. Presynaptic low- and high-affinity kainate receptors in nociceptive spinal afferents. *Pain* 2006; **120**: 97–105
  - 38 Ma W, Eisenach JC. Chronic constriction injury of sciatic nerve induces the up-regulation of descending inhibitory noradrenergic innervation to the lumbar dorsal horn of mice. *Brain Res* 2003; **970**: 110–8
  - 39 Malinow R, Madison DV, Tsien RW. Persistent protein kinase activity underlying long-term potentiation. *Nature* 1988; **335**: 820–4
  - 40 Malinow R, Schulman H, Tsien RW. Inhibition of postsynaptic PKC or CaMKII blocks induction but not expression of LTP. *Science (New York, NY)* 1989; **245**: 862–6
  - 41 Mansikka H, Pertovaara A. Influence of selective alpha 2-adrenergic agents on mustard oil-induced central hyperalgesia in rats. *Eur J Pharmacol* 1995; **281**: 43–8
  - 42 Mantyh PW, Rogers SD, Honore P, et al. Inhibition of hyperalgesia by ablation of lamina I spinal neurons expressing the substance P receptor. *Science (New York, NY)* 1997; **278**: 275–9
  - 43 McClean GJ, Suzuki R, Dickenson AH. Does a single intravenous injection of the 5HT3 receptor antagonist ondansetron have an analgesic effect in neuropathic pain? A double-blinded, placebo-controlled cross-over study. *Anesth Analg* 2003; **97**: 1474–8
  - 44 Melck D, Bisogno T, De Petrocellis L, et al. Unsaturated long-chain N-acyl-vanillyl-amides (N-AVAMs): vanilloid receptor ligands that inhibit anandamide-facilitated transport and bind to CB1 cannabinoid receptors. *Biochem Biophys Res Commun* 1999; **262**: 275–84
  - 45 Meller ST, Dykstra C, Grzybycki D, Murphy S, Gebhart GF. The possible role of glia in nociceptive processing and hyperalgesia in the spinal cord of the rat. *Neuropharmacology* 1994; **33**: 1471–8
  - 46 Millan MJ. Descending control of pain. *Prog Neurobiol* 2002; **66**: 355–474
  - 47 Miquel MC, Emerit MB, Nosjean A, et al. Differential subcellular localization of the 5-HT3-A5 receptor subunit in the rat central nervous system. *Eur J Neurosci* 2002; **15**: 449–57
  - 48 Nagy GG, Watanabe M, Fukaya M, Todd AJ. Synaptic distribution of the NR1, NR2A and NR2B subunits of the N-methyl-D-aspartate receptor in the rat lumbar spinal cord revealed with an antigen-unmasking technique. *Eur J Neurosci* 2004; **20**: 3301–12
  - 49 Oyama T, Ueda M, Kuraishi Y, Akaike A, Satoh M. Dual effect of serotonin on formalin-induced nociception in the rat spinal cord. *Neurosci Res* 1996; **25**: 129–35
  - 50 Polgar E, Watanabe M, Hartmann B, Grant SG, Todd AJ. Expression of AMPA receptor subunits at synapses in laminae I–III of the rodent spinal dorsal horn. *Mol Pain* 2008; **4**: 5
  - 51 Price DD, Mao J, Frenk H, Mayer DJ. The N-methyl-D-aspartate receptor antagonist dextromethorphan selectively reduces temporal summation of second pain in man. *Pain* 1994; **59**: 165–74
  - 52 Rahman W, D'Mello R, Dickenson AH. Peripheral nerve injury-induced changes in spinal alpha(2)-adrenoceptor-mediated modulation of mechanically evoked dorsal horn neuronal responses. *J Pain* 2008; **9**: 350–9
  - 53 Rahman W, Suzuki R, Webber M, Hunt SP, Dickenson AH. Depletion of endogenous spinal 5-HT attenuates the behavioural hypersensitivity to mechanical and cooling stimuli induced by spinal nerve ligation. *Pain* 2006; **123**: 264–74
  - 54 Rowbotham M, Harden N, Stacey B, Bernstein P, Magnus-Miller L. Gabapentin for the treatment of postherpetic neuralgia: a randomized controlled trial. *JAMA* 1998; **280**: 1837–42
  - 55 Roza C, Laird JM, Souslova V, Wood JN, Cervero F. The tetrodotoxin-resistant Na<sup>+</sup> channel Nav1.8 is essential for the expression of spontaneous activity in damaged sensory axons of mice. *J Physiol* 2003; **550**: 921–6
  - 56 Rygh LJ, Suzuki R, Rahman W, et al. Local and descending circuits regulate long-term potentiation and zif268 expression in spinal neurons. *Eur J Neurosci* 2006; **24**: 761–72
  - 57 Satoh O, Omote K. Roles of monoaminergic, glycinergic and GABAergic inhibitory systems in the spinal cord in rats with peripheral mononeuropathy. *Brain Res* 1996; **728**: 27–36



- 58 Scholz J, Woolf CJ. The neuropathic pain triad: neurons, immune cells and glia. *Nat Neurosci* 2007; **10**: 1361–8
- 59 Seagrove LC, Suzuki R, Dickenson AH. Electrophysiological characterisations of rat lamina I dorsal horn neurones and the involvement of excitatory amino acid receptors. *Pain* 2004; **108**: 76–87
- 60 Serpell MG. Gabapentin in neuropathic pain syndromes: a randomised, double-blind, placebo-controlled trial. *Pain* 2002; **99**: 557–66
- 61 Sindrup SH, Jensen TS. Efficacy of pharmacological treatments of neuropathic pain: an update and effect related to mechanism of drug action. *Pain* 1999; **83**: 389–400
- 62 Stanfa LC, Dickenson AH. Enhanced alpha-2 adrenergic controls and spinal morphine potency in inflammation. *Neuroreport* 1994; **5**: 469–72
- 63 Stanfa LC, Dickenson AH. The role of non-N-methyl-D-aspartate ionotropic glutamate receptors in the spinal transmission of nociception in normal animals and animals with carrageenan inflammation. *Neuroscience* 1999; **93**: 1391–8
- 64 Stone LS, Vulchanova L, Riedl MS, et al. Effects of peripheral nerve injury on alpha-2A and alpha-2C adrenergic receptor immunoreactivity in the rat spinal cord. *Neuroscience* 1999; **93**: 1399–407
- 65 Suzuki R, Green GM, Millan MJ, Dickenson AH. Electrophysiologic characterization of the antinociceptive actions of S18616, a novel and potent alpha 2-adrenoceptor agonist, after acute and persistent pain states. *J Pain* 2002; **3**: 234–43
- 66 Suzuki R, Hunt SP, Dickenson AH. The coding of noxious mechanical and thermal stimuli of deep dorsal horn neurones is attenuated in NK1 knockout mice. *Neuropharmacology* 2003; **45**: 1093–100
- 67 Suzuki R, Morcuende S, Webber M, Hunt SP, Dickenson AH. Superficial NK1-expressing neurons control spinal excitability through activation of descending pathways. *Nat Neurosci* 2002; **5**: 1319–26
- 68 Suzuki R, Rahman W, Hunt SP, Dickenson AH. Descending facilitatory control of mechanically evoked responses is enhanced in deep dorsal horn neurones following peripheral nerve injury. *Brain Res* 2004; **1019**: 68–76
- 69 Suzuki R, Rahman W, Rygh LJ, et al. Spinal-supraspinal serotonergic circuits regulating neuropathic pain and its treatment with gabapentin. *Pain* 2005; **117**: 292–303
- 70 Todd AJ. Anatomy of primary afferents and projection neurones in the rat spinal dorsal horn with particular emphasis on substance P and the neurokinin 1 receptor. *Exp Physiol* 2002; **87**: 245–9
- 71 Tolle TR, Berthele A, Zieglansberger W, Seeburg PH, Wisden W. The differential expression of 16 NMDA and non-NMDA receptor subunits in the rat spinal cord and in periaqueductal gray. *J Neurosci* 1993; **13**: 5009–28
- 72 Tolle TR, Berthele A, Zieglansberger W, Seeburg PH, Wisden W. Flip and Flop variants of AMPA receptors in the rat lumbar spinal cord. *Eur J Neurosci* 1995; **7**: 1414–9
- 73 Tracey I, Mantyh PW. The cerebral signature for pain perception and its modulation. *Neuron* 2007; **55**: 377–91
- 74 Tsuruoka M, Arai YC, Nomura H, Matsutani K, Willis WD. Unilateral hindpaw inflammation induces bilateral activation of the locus coeruleus and the nucleus subcoeruleus in the rat. *Brain Res Bull* 2003; **61**: 117–23
- 75 Tsuruoka M, Willis WD. Descending modulation from the region of the locus coeruleus on nociceptive sensitivity in a rat model of inflammatory hyperalgesia. *Brain Res* 1996; **743**: 86–92
- 76 Tsuruoka M, Willis WD, Jr. Bilateral lesions in the area of the nucleus locus coeruleus affect the development of hyperalgesia during carrageenan-induced inflammation. *Brain Res* 1996; **726**: 233–6
- 77 Valerio A, Paterlini M, Boifava M, Memo M, Spano P. Metabotropic glutamate receptor mRNA expression in rat spinal cord. *Neuroreport* 1997; **8**: 2695–9
- 78 Vera-Portocarrero LP, Zhang ET, Ossipov MH, et al. Descending facilitation from the rostral ventromedial medulla maintains nerve injury-induced central sensitization. *Neuroscience* 2006; **140**: 1311–20
- 79 Watkins LR, Martin D, Ulrich P, Tracey KJ, Maier SF. Evidence for the involvement of spinal cord glia in subcutaneous formalin induced hyperalgesia in the rat. *Pain* 1997; **71**: 225–35
- 80 Watkins LR, Milligan ED, Maier SF. Glial activation: a driving force for pathological pain. *Trends Neurosci* 2001; **24**: 450–5
- 81 Weil-Fugazza J, Godefroy F, Manceau V, Besson JM. Increased norepinephrine and uric acid levels in the spinal cord of arthritic rats. *Brain Res* 1986; **374**: 190–4
- 82 Wood JN, Boorman JP, Okuse K, Baker MD. Voltage-gated sodium channels and pain pathways. *J Neurobiol* 2004; **61**: 55–71
- 83 Woolf CJ. Evidence for a central component of post-injury pain hypersensitivity. *Nature* 1983; **306**: 686–8
- 84 Xia Z, Dudek H, Miranti CK, Greenberg ME. Calcium influx via the NMDA receptor induces immediate early gene transcription by a MAP kinase/ERK-dependent mechanism. *J Neurosci* 1996; **16**: 5425–36
- 85 Young MR, Blackburn-Munro G, Dickinson T, et al. Antisense ablation of type I metabotropic glutamate receptor mGluR1 inhibits spinal nociceptive transmission. *J Neurosci* 1998; **18**: 10180–8
- 86 Young MR, Fleetwood-Walker SM, Mitchell R, Munro FE. Evidence for a role of metabotropic glutamate receptors in sustained nociceptive inputs to rat dorsal horn neurons. *Neuropharmacology* 1994; **33**: 141–4
- 87 Zeitz KP, Guy N, Malmberg AB, et al. The 5-HT3 subtype of serotonin receptor contributes to nociceptive processing via a novel subset of myelinated and unmyelinated nociceptors. *J Neurosci* 2002; **22**: 1010–9
- 88 Zhuang ZY, Xu H, Clapham DE, Ji RR. Phosphatidylinositol 3-kinase activates ERK in primary sensory neurons and mediates inflammatory heat hyperalgesia through TRPV1 sensitization. *J Neurosci* 2004; **24**: 8300–9
- 89 Zygmunt PM, Petersson J, Andersson DA, et al. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature* 1999; **400**: 452–7