Neurotrophic influences on neuropathic pain

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Abstract. Damage to peripheral nerves following trauma or disease has a number of consequences including the emergence of neuropathic pain. Commonly, neuropathic pain sufferers experience spontaneous burning pain in and radiating from the area innervated by the damaged nerves, and an exquisite sensitivity to light touch stimuli, which are now perceived as painful. These neuropathic pains are often refractory to conventional analgesic therapy, with most patients obtaining only partial relief. Unfortunately, neuropathic pains are frequently also very persistent and do not resolve with time. Thus, neuropathic pain is often an extremely debilitating condition with a bleak outlook. In this paper we review the pathophysiological mechanisms that underlie these neuropathic pain states with particular emphasis on the therapeutic role of neurotrophic factors.


The International Association for the Study of Pain defines Neuropathic pain as ‘Pain initiated or caused by a primary lesion or dysfunction in the nervous system’ (Merskey & Bogduk 1994). Clearly, this is a very broad definition and gives very little insight into the neurobiological mechanisms of such pains. Commonly, neuropathic pain sufferers experience spontaneous burning pain within and radiating from the area innervated by the damaged nerves, and many report an exquisite sensitivity to light touch stimuli, which are now perceived as painful — a condition known as allodynia. These neuropathic pains are often refractory to conventional analgesic therapy, with most patients obtaining only partial relief. Unfortunately, neuropathic pains are commonly very persistent and do not resolve with time. Thus, neuropathic pain is often an extremely debilitating condition with a bleak outlook.

The diverse causes of neuropathic pain also offer only limited mechanistic understanding. Perhaps the most common form of neuropathic pain is that associated with metabolic abnormality, notably diabetes. Many diabetics, especially those with poor blood sugar control, ultimately develop a distal
symmetrical and painful neuropathy that initially affects the longest peripheral axons, but with time spreads proximally. Another large and growing group of neuropathic patients have pain secondary to infection. Many AIDS sufferers, perhaps up to 50%, develop painful neuropathy similar to those seen in diabetics. The incidence of HIV-induced neuropathy is increasing with improvements in antiretroviral drug therapy (Moyle & Sadler 1998) partly because patients are living longer with the disease. The pain experienced in the wake of an attack of Herpes Zoster is another example of neuropathic pain and the intensity and persistence of post-herpetic neuralgia increase with age. A third important cause of neuropathic pain is iatrogenic, as a side-effect of several drug treatments, including some anticancer drugs (where neuropathy may be the dose-limiting factor) and some of the drugs currently used to treat HIV infection. A further cause of neuropathic pain is that associated with traumatic nerve injuries. While such injuries are not very common in peacetime, the advent of high velocity firearms means gunshot wounds have added significantly to the burden of neuropathic pain around the world. All of the above causes are associated with damage or disease to the peripheral nervous system. However, neuropathic pain can be associated with damage to central structures. The clearest examples are pains associated with spinal cord injury and pains associated with vascular lesions of the thalamus. It seems unlikely that neuropathic pain of central and peripheral origin has a common underlying mechanism, but it is also unclear whether there are multiple contributing mechanisms of neuropathic pain of peripheral origin. This is of considerable practical as well as academic interest, since, as we will review below, most of our understanding of mechanisms arises from the study of a very limited type of animal model.

In total, neuropathic pain is likely to affect some 1.8 million people in the USA and for only a few of these (e.g. those with pain associated with carpal tunnel syndrome) is there a straightforward and effective treatment.

Animal models of neuropathic pain

Many of the disease states causing neuropathic pain can be modelled in animals. For instance, there are reasonably well-characterized models of diabetic neuropathy and several animal models of neuropathies associated with anticancer drug treatments. There have also been attempts to induce HIV neuropathy in rats (Milligan et al 2001), and several reports of abnormal pain sensitivity after experimental spinal cord injury in rats (e.g. see Yezierski & Burchiel 2002). However, for reasons of reproducibility and simplicity, most studies of neuropathic pain use traumatic nerve injury, usually in rodents. One important caveat, as previously mentioned, is that it is not clear whether understanding derived from the study of such models will be applicable to neuropathic pain
associated with other causes. There are several such models in fairly common use, and the nature of the nerve lesions is illustrated in Fig. 1. The different models share the common feature of degeneration of some but not all sensory fibres in a major peripheral nerve, so that a peripheral target is partially denervated and conversely, partially innervated. One model introduced by Selzter et al (1990) involves partial nerve ligation (Fig. 1A). Typically one-third to one-half of the sciatic nerve is tightly ligated with a silk suture. Since sensory fibres in the sciatic nerve exhibit considerable mixing as they travel distally, this procedure does not result in total denervation of a confined area, but a partial denervation throughout much of the sciatic innervation territory. A more recent derivation of this approach is to tie off one or more of the branches of the sciatic nerve (Doesterd & Woolf 2000, Fig. 1B). The damaged sensory fibres do innervate a more restricted area in this case, but because of the overlap of nerve territories, there are broader zones of partial innervation which exhibit neuropathic signs (i.e. altered sensibility to sensory stimuli). A third extensively used model is that of chronic nerve constriction (Fig. 1C, Bennett & Xie 1988). Here several chromic ligatures are loosely tied around the sciatic nerve at mid-thigh level. The sutures are only tight enough to partially restrict blood flow in superficial vessels in the nerve. But the nerve swells and a marked constriction results. Anatomical studies (Coggeshall et al 1993) show that a substantial fraction but not all fibres undergo Wallerian degeneration distal to the ligation site. This model appears to have a greater inflammatory component than the others. The presence particularly of chromic suture material may exacerbate the inflammatory response (Maves et al 1993). As we review below, the liberation of cytokines or other factors (e.g. nerve growth factor) from

FIG. 1. Schematic illustration of different experimental models of neuropathic pain. In each case the sciatic nerve and its projection through dorsal root ganglia are shown. (A) A portion, typically about 50% of the sciatic nerve is tightly ligated (from Seltzer et al 1990). (B) Ligatures are loosely tied around the sciatic nerve (from Bennett et al 1988). (C) One or more branches of the sciatic nerve are tightly ligated and cut (from Deosterd & Woolf 2000). (D) One or more spinal nerves are ligated and cut (from Kim & Chung 1992). See text for more details.
immunocytes at the site of constriction is also likely to contribute to neuropathic symptoms.

The most commonly used model today involves the ligation of one or two spinal nerves (usually L5 or L5 and L6), just distal to the dorsal root ganglion (DRG) (Fig. 1D, Kim & Chung 1992). Since the sciatic nerve carries large numbers of sensory fibres from the L4 and L5 spinal nerves (and smaller numbers from the L6 nerve), this lesion results in degeneration of about 50% of the fibres in the sciatic nerve, and these project throughout the normal sciatic innervation territory. One advantage of this model for mechanistic studies is that in a particular DRG virtually all sensory neuron cell bodies are either axotomized or intact. This contrasts with other models, where the cell bodies of injured and uninjured neurons are mixed together in one or more DRGs.

In all of these animal models pronounced sensory changes are seen, similar to those observed in many neuropathic pain patients. Thus, neuropathic animals will guard and avoid weight-bearing on the affected paw, consistent with the existence of some ongoing pain. Animals also show escape behaviour to very light tactile stimulation of the paw, indicating allodynia. Cold stimuli also trigger greatly exaggerated responses, and this state is frequently referred to as cold allodynia. In response to tests of noxious heating, these models also show increased sensitivity, but this thermal hyperalgesia is usually quite modest. An example of the degree and time-course of these sensory changes seen in one group of animals subjected to spinal nerve ligation lesions (as in Fig. 1D) is shown in Fig. 2. Note that mechanical allodynia emerges very rapidly, and is fully developed within one or two days. It seems quite possible that the mechanical allodynia and thermal hyperalgesia may have different underlying causes. For instance, treating neuropathic animals with the C-fibre neurotoxin resiniferatoxin reportedly abolishes the thermal hyperalgesia whilst leaving mechanical allodynia (Ossipov et al 1999).

Traumatic injury models of neuropathic pain have been used in many different studies aimed at elucidating the factor or factors that might contribute to the emergence of neuropathic symptoms. These studies have demonstrated a series of pathophysiological reactions to the injuries that sweep forward from the site of the injury, involving considerable changes in gene expression in the cell bodies of sensory neurons in DRG and also marked alterations in the central processing of sensory information, particularly within the dorsal horn of the spinal cord. These changes are reviewed below.

Which sensory neurons are responsible for neuropathic pain behaviour?

In the traumatic nerve injury models described above, one common feature is that some but not all of the sensory axons running in a major peripheral nerve are
axotomized. The distal portions of those damaged axons begin very rapidly to undergo Wallerian degeneration. The axotomized proximal stumps cannot regenerate in these models, because they are trapped at the site of a nerve ligation. These fibres form a distinct functional group that have lost contact with the peripheral targets that they normally innervate, and consequently any target-derived trophic factors that are normally provided to them (for instance, NGF, see below).

However, there is a second functionally distinct group of sensory neurons. These are intact neurons, ‘spared’ from injury, but running in the same peripheral nerves. These ‘spared’ sensory neurons have axons running through an area of Wallerian degeneration and may be subject to the altered chemical environment of the degenerating nerve and alterations in target-derived factors (see below).

The existence of two such groups of sensory neurons can plausibly be suggested for all neuropathic pain states associated with peripheral nerve injuries. In diabetic neuropathy (and in several other causes of neuropathic pain) the nerve damage may be very distal and some axons may only die back relatively modest distances from peripheral targets. Nonetheless, some neurons may be injured and disconnected from their normal innervation fields, while others are intact but present amongst degenerating fibres.

In one traumatic model — that produced by spinal nerve ligation (Fig. 1D) — there is considerable anatomical separation of these two subgroups, because all neurons of one dorsal root ganglion and dorsal root, will be either injured or ‘spared’. The anatomical advantage of this model has permitted a number of experiments aimed at identifying the contribution of intact versus ‘spared’ neurons in the evolution of neuropathic pain. Surprisingly, perhaps, because the

![FIG. 2. Typical sensory changes seen in neuropathic pain models. Here the responses of rats were studied before and after an L5 spinal nerve ligation (SNL). (A) and (B) show changes in the mechanical and thermal threshold, respectively, necessary to elicit withdrawal reflexes. (C) Shows the number of paw flinches on exposure to cold stimulus of about 1 °C for 30 seconds (T. J. Boucher, S. B. McMahon, unpublished data).]
experiment would seem rather straightforward, there is a great deal of controversy about the result. Cutting the L5 dorsal root (after L5 spinal nerve ligation) prevents activity from reaching the CNS from the damaged neurons in the L5 dorsal root ganglion (DRG), and is reported to abolish signs of neuropathic pain by some (Sheen & Chung 1993, Yoon et al 1996, Sukhotinsky et al 2004), but not all (Li et al 2000) groups.

These studies are further complicated by reports that dorsal rhizotomy (i.e. a section of dorsal roots) may itself produce behavioural hyperalgesia (Colburn et al 1999, Li et al 2000), and so it might be that this lesion abolishes one form of neuropathic pain behaviour only to be replaced with another. The infusion of local anaesthetic onto the L5 DRG (after L5 spinal nerve ligation) blocks sensory nerve activity and is reported to reverse neuropathic pain behaviour (Sukhotinsky et al 2004).

Li et al (2000) reported on a number of apparently well-controlled behavioural studies of neuropathic pain generated by spinal nerve ligation. They found that signs of neuropathic pain were selectively abolished when sensory activity in ‘spared’ afferents was prevented from reaching the cord. They also reported that interrupting signalling from injured axons was without effect. These authors have suggested that influences arising from Wallerian degeneration are crucial in altering responsiveness of ‘spared’ neurons. In support, the same group have reported that ventral rhizotomy (which damages motoneurons but also causes Wallerian degeneration in the sciatic nerve) also leads to neuropathic pain behaviour.

The reasons for the discrepancies in these reports are not obvious. They do not seem related to animal strain, time points studied or other simple methodological variables. One interpretation of these data is that it would be prudent to consider both these groups of sensory neurons as potentially important contributors to neuropathic pain.

Changes in peripheral sensory neurons in neuropathy models

A logical question to ask is ‘What aspect or consequence of nerve injury is important for neuropathic pain?’ There is now good evidence to suggest that post-injury sequelae are dictated by at least two principal processes. The first is an alteration in the availability of target derived neurotrophic factors, and the second is the generation of injury-induced factors, such as cytokines and chemokines. The former constitutes a ‘negative’ signal to some sensory neurons. That is, a factor normally supplied to some sensory neurons is lost or diminished. But both the former and the latter can be ‘positive’ signals to some neurons—becoming available de novo or at increased levels. Both these groups of factors are responsible for inducing very marked changes in gene expression in sensory
neurons, which in turn lead to the emergence of abnormal electrical activity, known to be essential for the manifestation of neuropathic pain behaviour. We will consider each of these changes in turn.

**Trophic factor availability**

Sensory neurons depend upon limited amounts of neurotrophic factors produced by target tissues during development to maintain an appropriate peripheral innervation. Expression of high affinity neurotrophic factor receptors by functionally distinct sub-populations of sensory neurons ensures physiological connectivity (see Fig. 3). While expression levels of neurotrophic factors are maintained into adulthood, albeit at a low level, these factors are not necessary to maintain the survival of sensory neurons. Nonetheless, they can exert very profound effects on sensory systems. For instance, there is now substantial evidence that highlights the pro-nociceptive role of the proto-typical neurotrophic factor, nerve growth factor (NGF) (see McMahon & Bennett 1999).

**Damaged sensory fibres.** CX Disconnection of damaged sensory axons from peripheral targets interrupts the retrograde trophic support these neurons normally receive from peripheral targets (Heumann 1987, Raivich 1991). There is now good evidence for a greatly reduced retrograde transport of at least three important trophic factors in damaged sensory axons. These are NGF, which normally supports about one-half of the small diameter (very largely nociceptive) sensory neurons; NT3, which supports most large diameter (mostly
mechanosensitive) sensory neurons; and glial cell-derived neurotrophic factor (GDNF), which supports the other half of small sensory neurons as well as a subgroup of some large neurons (see Fig. 3). The loss of retrograde supply of these factors to the cell bodies of sensory neurons causes dramatic alterations in the expression of neuropeptides, ion channels and receptors (see below). Many studies have shown that exogenous delivery of appropriate neurotrophic factors rescues or ameliorates many of these changes.

After nerve injury, there is increased expression of NGF and GDNF by Schwann cells distal to the injury site in areas undergoing Wallerian degeneration (Heumann 1987, Herzberg et al 1997, Naveilhan et al 1997). However, these factors do not appear to be available to the proximally damaged axons, or at least not available in sufficient amounts to compensate for the lost target-derived supply. After peripheral axotomy, there is also increased expression of NGF and NT3 in satellite cells surrounding sensory neuron cell bodies in dorsal root ganglia (DRG) (Zhou et al 2000). While it remains an open and intriguing question of what signal is responsible for this change, it too does not appear to be sufficient to substitute for lost target-derived supplies. (It may, however, be sufficient to trigger the sprouting of sympathetic fibres within the DRG after peripheral nerve injury and in this way contribute to neuropathic pain—see Ramer et al 1999.) Direct measurement of NGF protein levels in DRG after nerve injury confirms the net reduction in bioavailability of this factor.

Other members of the neurotrophin family have been shown to be key modulators in the maintenance of neuropathic pain and therefore remain intriguing therapeutic targets. Brain-derived neurotrophic factor (BDNF) appears an important target-derived factor for many placode-derived sensory neurons, such as vagal afferents innervating visceral structures. However, for the neural crest derived sensory neurons of the DRG, this role seems less important. Unlike NGF, BDNF is synthesized by sensory neurons themselves (Ernfors et al 1990, 1993) and its expression is subject to alteration after nerve injury (Michael et al 1997). Both L5 spinal nerve ligation (Fukuoka et al 2001) and chronic constriction nerve injury (CCI) (Obata et al 2003) precipitate a net loss in BDNF expression levels in small diameter TrkA-expressing neurons, presumably due to a loss of target-derived NGF. BDNF is thought to modulate sensory processing via its accumulation (Michael et al 1997) and subsequent release with activity from primary afferent terminals in the dorsal horn (Lever et al 2001). Several studies have illustrated that sequestering centrally released BDNF can attenuate behavioural signs of neuropathic pain (Theodosiou et al 1999, Yajima et al 2002). After partial nerve injury, intact or ‘spared’ neurons face less competition for target-derived factors owing to partial target denervation. Expression of BDNF is up-regulated in ‘spared’ DRG neurons in L4 after L5 spinal nerve ligation and after chronic constriction injury of the sciatic nerve (Fig. 1). Not all of these
‘spared’ neurons express TrkA and therefore alteration in BDNF expression cannot be due entirely to increased availability of NGF. This suggests a role for a currently unknown injury induced factor.

Neurotrophin 3 (NT3), another target-derived neurotrophic factor, maintains the adult phenotype of large diameter myelinated mechanoreceptors. These cells express the high affinity TrkC receptor (Fig. 3) and are subject to modification after peripheral nerve injury owing in part to their loss of target-derived support. Treatment of damaged nerves with exogenous NT3 has been shown to ameliorate some of these changes (Ohara et al 1995, Munson et al 1997). Despite the observation that nerve injury induces the expression of NT3 mRNA within satellite cells in the DRG (Zhou et al 1999), application of NT3 antisera and or TrkC fusion proteins failed to elicit a profound alteration in pain thresholds after L5 spinal nerve ligation (Zhou et al 2000, Deng et al 2000), thereby highlighting a more subtle role of NT3 as a possible therapy for neuropathic pain.

Another neurotrophic factor, structurally unrelated to the neurotrophins, is GDNF (see Fig. 3). This supports the survival of the non-peptidergic small diameter nociceptive C-fibres (Naveilhan et al 1997). As is the case with NGF and NT3, nerve injury-induced interruption of target derived GDNF alters the expression of neuromodulators and receptors within the cell bodies of damaged sensory neurons, alterations that can be reversed by the exogenous delivery of GDNF (Bennett et al 1998, Cummins et al 2000, Boucher et al 2000). Together these data clearly show that interruption of target-derived trophic support precipitates many of the phenotypic changes seen in sensory neurons after nerve injury. However, experimental approaches that aim to replenish neurotrophic support to neurons disconnected from their target may fail to address additional maladaptive consequences that neurotrophic support can have on intact neurons.

Intact sensory fibres. Few experimental models of nerve injury fully transect an entire nerve fascicule; therefore, many intact fibres are closely opposed to injured fibres and consequently share the same environmental consequences of nerve injury. There are two ways in which these ‘spared’ sensory neurons may be exposed to increased levels of neurotrophic factors. First, the peripheral targets they innervate are partially denervated. Since the expression of target-derived factors does not seem to depend on innervation density, ‘spared’ fibres will have fewer others to compete with for these factors. Second, Schwann cells reacting to Wallerian degeneration and other cells invading the nerve as a part of the process start to express several of the factors normally expressed by peripheral targets. The best examples are NGF and GDNF. Spared axons running through this environment are exposed to these factors and we have direct and indirect evidence that the net result is an increased retrograde supply of some factors to the cell bodies of spared axons. Fukuoka et al (2001) measured NGF protein
levels in the L4 DRG after a spinal nerve ligation of L5. They found a progressive increase in NGF protein in this ganglion, but not in the (axotomised) L5 DRG. There have also been several studies on the expression of specific genes known to be regulated by NGF and GDNF. These studies (see below) report changes consistent with increased NGF and GDNF levels in these spared neurons.

It is important to consider the likely consequences of increased trophic factor supply to ‘spared’ afferents. NGF has a potent algogenic effect on intact TrkA-expressing sensory neurons, causing robust thermal and mechanical hyperalgesia within hours of systemic administration (Lewin et al 1993). Local administration of NGF sensitizes cutaneous nociceptors to thermal and mechanical stimuli via direct action on the afferent fibres, but also via an indirect action on resident non-neuronal cellular elements, such as mast cells. Mast cells also express TrkA (Horigome et al 1993) and in response to NGF proliferate, degranulate and release inflammatory mediators such as interleukin (IL)10, serotonin (5HT) and tumour necrosis factor (TNF)α (Woolf et al 1995). Furthermore, the delivery of function blocking molecules has demonstrated that NGF contributes to abnormal pain sensitivity in several animal models (e.g. McMahon et al 1995).

As previously mentioned, NGF stimulates expression of BDNF in small-diameter peptidergic C-fibres and the intrathecal administration of BDNF has been shown to cause mechanical and thermal hyperalgesia (Zhou et al 2000). It is known that BDNF levels increase in ‘spared’ sensory neurons after CCI (Obata et al 2003) and in intact L4 DRG after L5 spinal nerve ligation (SNL) (Fukuoka et al 2001), in an NGF-dependent manner, and this may contribute to neuropathic pain behaviour. One would predict that ‘spared’ neurons that express TrkC may experience less competition for target derived NT3. If this is indeed the case there is no evidence to suggest that NT3 is directly algogenic.

Injury-induced factors

Peripheral nerves have an immune privilege maintained by the blood–nerve barrier, which allows for minimum immune surveillance, mainly by activated T lymphocytes. Nerve injury dissolves this privilege and the nerve is subject to invasion from dedifferentiating and proliferating fibroblasts, macrophages and Schwann cells. Broadly speaking, injury-induced cytokines initiate a loop of self-promoting activity; by increasing vascular permeability at the site of trauma and concomitant up-regulation of endothelial adhesion molecules, thereby enhancing leukocyte adhesion and extravasation. While the key action of recruited cells is to remove cellular debris and facilitate axonal regeneration, it is clear that these cells produce a variety of pro-inflammatory cytokines and chemokines (summarized in Fig. 4) which have been implicated in the generation of neuropathic pain either via direct sensitizing actions on nociceptors, or indirectly by stimulating the release of
FIG. 4. Neural inflammatory response. Summary of injury-induced neural mediators that initiate and maintain the inflammatory response. TNFα released locally stimulates the release of cytokines IL1, IL6 and LIF (arrows) from resident macrophages and Schwann cells. Subsequent release of chemokines (CCL2) from activated macrophages and Schwann cells initiates the recruitment of further phagocytic cells, which infiltrate and continue the release of cytokines. Resident Mast cells degranulate in response to injury-induced stimuli and release prostaglandins, NGF and histamine. The locations of action of these mediators are indicated by broken arrows. Cytokines (such as TNFα) directly influence the axon via interactions with sodium and calcium channels. Prostaglandins (PGs), bradykinin and NGF released from mast cells sensitize axons directly. Injury-induced chemokines (CCL2) directly increase vascular permeability thereby enhancing leukocyte extravasation.
agents that act on neurons (reviewed by Watkins & Maier 2002). The expression of injury-induced factors is not limited to the distal stump of transected axons or areas undergoing Wallerian degeneration. Therefore both injured and intact neurons are subject to their influence. Overall, the cytokine response to nerve injury is highly complex, involving the up-regulation of pro- and anti-inflammatory factors that act and interact on a broad number of neuronal and non-neuronal cells producing transcription-dependent and -independent alterations in sensory processing.

Nerve trauma initiates a potent immune response typified by the early release of TNFα from infiltrating and resident macrophages (George et al 1999) and Schwann cells. Within 5 hours of nerve injury, TNFα levels are elevated within resident Schwann cells, which owing to their intimate proximity can directly sensitize nearby neurons (Shamash et al 2002). Subsequently TNFα stimulates the sequential production and release of IL1 and IL6 from infiltrating macrophages and dedifferentiating Schwann cells (Wagner & Myers 1996, Bolin et al 1995, Sommer 1999) along the entire length of the degenerating nerve. Simply delaying the infiltration of macrophages after nerve injury delays the development of neuropathic pain (Myers et al 1996), while delivering neutralizing antibodies to TNFα and IL1 reduces behavioural signs of experimental neuropathic pain (Shafer et al 2001, Sommer et al 1999). Furthermore IL6−/− mice fail to exhibit neuropathic pain after nerve injury (Ramer et al 1998, Murphy et al 1999). Much of the evidence to suggest a role for cytokines and chemokines in the initiation and maintenance of neuropathic pain come from studies such as these that have utilized tools that block cytokine function after experimental injury, or have been conducted in mice that experience delayed Wallerian degeneration (Ramer et al 1997). These mice fail to show signs of mechanical and thermal hypersensitivity after chronic constriction injury, highlighting a crucial role of degeneration-induced factors, such as cytokines and chemokines.

Intact and injured sensory neurons are known to express receptor components capable of transducing extracellular TNFα (Pollock et al 2002), IL1 and IL6 (Gardiner et al 2002). Indeed intraneuronal (Wagner & Myers 1996) and intraplantar injection of TNFα induces mechanical (Cunha et al 1992) and thermal hyperalgesia (Perkins et al 1994), via the TNFα1 receptor (Sommer et al 1998). While sensory neurons are a substrate for direct sensitization by TNFα, the underlying mechanism remains to be fully determined. Evidence from non-neuronal cells indicates an interaction with endogenous sodium and calcium channels (Wilkinson et al 1996). Intriguingly, trimers of TNFα have been reported to insert into membranes and form functional voltage-dependent sodium channels (Kagan et al 1992), which may allow for a generalized sensitization of sensory neurons in the absence of functional TNFα receptors.
It is clear that TNFα initiates a cascade of nerve injury-induced cytokine production (Woolf et al 1997, Shamash et al 2002), a self-promoting loop that also recruits production and release of IL1 and IL6. Intradermal injection of IL1 causes both mechanical and thermal hyperalgesia within minutes (Fukuoka et al 1994), suggesting a direct role on nociceptors. However, a dependence of IL1-induced hyperalgesia on bradykinin receptors 1 and 2 (Davis & Perkins 1994), prostaglandins (Schweizer et al 1988) and production of NGF (Lewin et al 1994) has also been observed.

The chemokine CCL2 (formerly known as monocyte chemoattractant protein 1) is another injury-induced product that accumulates within sensory neurons and contributes to macrophage recruitment. Recent data from our laboratory have implicated CCL2 in the maintenance of neuropathic pain: exogenous application of CCL2 to the sciatic nerve results in transient mechanical and thermal hyperalgesia (M. Thacker, B. J. Cafferty, S. Thompson, S. B. McMahon, unpublished observations). IL6, the prototypical member of the gp130 cytokines is absent from the adult peripheral nervous system, but is rapidly up-regulated by neurons (Murphy et al 1999) and Schwann cells at the site of nerve injury (Bolin et al 1995, Kurek et al 1996) probably via injury-induced TNFα release. Along with its related cytokine, LIF (leukaemia inhibitory factor; Thompson et al 1996), IL6 has been shown to promote touch-evoked allodynia after exogenous application (DeLeo et al 1996). The precise role of gp130 cytokines is complicated by the observation that some studies have highlighted an anti-inflammatory role for LIF and IL6 in models of cutaneous inflammation (reviewed by Gadient & Patterson 1999). However, their roles in nerve injury are better defined, having been shown to be crucial in the up-regulation of key modulators of sensory processing such as BDNF (Murphy et al 2000), galanin and substance P (Sun & Zigmond 1996) after peripheral nerve injury.

Electrophysiological changes

There is considerable evidence that activity in sensory neurons after injury is necessary for the elaboration of neuropathic symptoms. For instance, blocking sensory inflow by cutting dorsal roots, or applying local anaesthetics or the sodium channel blocker TTX, reportedly prevents the emergence of neuropathic pain in some circumstances in animal models (Lyu et al 2000, Liu CN et al 2000, Liu X et al 2000, Sheen & Chung 1993, Sukhotinsky et al 2004, Yoon et al 1996). Clinical observations also support the idea that abnormal sensory inputs trigger neuropathic pain (Price et al 1989, Campbell et al 1988). Electrophysiological recordings of more than a quarter of a century ago showed that damaged peripheral nerves became the source of abnormal activity (Wall & Gutnick 1974). Some of this activity appears to arise from the damaged sensory nerve
terminals (particularly those trapped in the neuroma that forms at the site of peripheral nerve injury). Some activity also clearly arises at the level of the cell body in the dorsal root ganglion (Wall & Devor 1983). However, it is only in the last few years that a clear picture has emerged as to which particular type of fibre becomes abnormally active.

Primary sensory neurons can be divided crudely into two functional subgroups. First, a group of small diameter cells with slowly conducting axons (so called Aδ and C axons). More than 90% of these cells are nociceptors. The second group are large diameter neurons with rapidly conducting (Aβ) axons, most of which are innocuous mechanoreceptors. One can easily see how ectopic or abnormal activity arising in nociceptors would provide a ready explanation for the ongoing pain seen in many neuropathic states. But there is also considerable evidence that activity in Aβ fibres can elicit pain in the presence of central sensitization — that is an enhanced excitability of central neurons. It is generally accepted that most of the mechanical hyperalgesia seen following peripheral nerve injury arises because of this reason. For instance, in human neuropathic pain states, activation of these Aβ afferents is capable of inducing pain (Campbell et al 1988). A matter of considerable debate, however, is the event(s) responsible for inducing the central nervous system (CNS) sensitization that allows Aβ afferent activity to produce pain. One clear possibility is that C-fibre activity initiates central sensitization and Aβ activity plays on this to maintain neuropathic touch-evoked pain. A second issue that now appears to be of central importance is between those fibres that are damaged in neuropathic conditions, and those that are intact but run alongside the damaged ones.

**Damaged sensory fibres.** Following nerve injury, some axotomised afferent neurons begin to discharge spontaneously (see Devor & Seltzer 1999). This afferent barrage provides constant input to the CNS, and thus may induce central sensitization. In many circumstances it is clear that only nociceptor activity is capable of inducing central sensitization (see Coderre et al 1993). Following L5 spinal nerve ligation, however, spontaneous activity arises almost exclusively in myelinated fibres (at least during the first week or two after injury, when neuropathic pain behaviour starts and becomes well established) (Boucher et al 2000, Li et al 2000, Liu X et al 2000). This is perhaps surprising but has been repeatedly determined by independent groups. There are conflicting reports on the importance of these ectopic discharges in damaged nerves to neuropathic pain behaviours (see above).

**‘Spared’ sensory fibres.** Damage to some afferents in a peripheral nerve leaves the remaining, intact, neighbouring fibres facing less competition for target-derived factors and subject to putative degeneration factors in the peripheral nerve. Recent work has shown that these intact ‘spared’ afferents (such as those running through
L4 after L5 SNL) show remarkable plastic changes, including the development of spontaneous activity. Myelinated fibres show very similar changes to those seen in damaged afferents, albeit slightly less well developed. That is, many Aβ afferents begin to generate relatively high frequency bursts or trains of action potentials that bombard the spinal cord (Boucher et al 2000, Michaelis et al 2000). It is interesting that myelinated afferents innervating muscle rather than skin seem to show a much greater propensity to generate these ectopic discharges (Proske et al 1995, Michaelis et al 2000). It is not clear what the functional significance of this observation might be, but one might imagine that specialized length and tension detectors in muscle would be the least likely group of afferents to generate or maintain neuropathic pain. We have observed that these ectopic discharges are reduced by GDNF treatment (Boucher et al 2000).

In ‘spared’ afferents (and not damaged ones) there are also reports of spontaneous activity arising in unmyelinated, nociceptive afferents (Koltzenburg et al 1994, Ali et al 1999). This activity has not been seen by all workers (e.g. Boucher et al 2000), but this may be because it occurs at very low rates, typically in the order of fewer than 0.1 Hz (Ali et al 1999). Indeed it is not clear what the consequences are of such low rates of C-fibre activity. However it has been reported that low level nociceptor activation (not eliciting pain) is sufficient to produce manifestations of central sensitization (Cervero et al 1993). Thus, it is possible that the key precipitating event in the development of neuropathic sensory abnormalities is the emergence of these C-fibre ectopic discharges in fibres spared by the injury, but running in the same peripheral nerves. The discharges in myelinated fibres (overwhelmingly innocuous mechanoreceptors originally) may only produce pain because they impinge on a CNS sensitized by the nociceptor inputs. In support, there are some behavioural data suggesting that blocking the spared afferent input can block the development of mechanical allodynia (Li et al 2000).

**Altered gene expression in sensory neurons**

In addition to the electrophysiological changes described above, models of experimental neuropathy lead to striking changes in gene expression in primary sensory neurons. Again it is important to distinguish between damaged and spared sensory neurons and to address which factors are responsible for these alterations.

**Damaged sensory fibres.** As discussed above, damaged neurons lose target-derived support. The damaged sensory neurons show changes in gene expression that affect virtually all aspects of the neurons' function, as summarized in Fig. 4. From the perspective of neuropathic pain, there are two types of change in gene
expression that may be particularly important. One is in the type and level of the neurotransmitters/neuromodulators that are produced by the damaged afferents, and released in the spinal cord with activity. Since, among the damaged afferents, it is myelinated fibres that become spontaneously active, changes here may be of particular importance. Some damaged A fibres (i.e. those with myelinated axons) appear to undergo a phenotypic shift, and begin to express transmitters normally associated with nociceptors, that is, substance P and BDNF. These factors are now released with A fibre activity (Malcangio et al 2000). Since there is good evidence that these factors are important contributors to central sensitization (see Woolf & Slater 2000), one can easily envisage that this contributes to neuropathic pain states. Further, many damaged fibres, including a large number of myelinated ones, begin to express the neuropeptide galanin. Traditionally, galanin has been thought of as an inhibitory neuropeptide in the dorsal horn of the spinal cord. However, it now emerges that different galanin receptors may be coupled to excitatory and inhibitory mechanisms and, using mice with null mutations in the galanin gene, we have directly observed reduced neuropathic pain behaviour in the absence of galanin expression (Kerr et al 1999).

A second observation relates to alterations in the expression of ion channels in damaged nerves. Clearly, there has to be a molecular correlate of the emergence of ectopic activity in damaged myelinated fibres. The most ready explanation is an altered expression of ion channels. Most studies have focused on changes in expression of sodium channels, the overexpression of which could alone lead to ectopic activity. One particular transcript, that encoding the Brain III sodium channel (now known as Na\textsubscript{v1.3}) is up-regulated in damaged sensory neurons. Other known subtypes are all down-regulated. Na\textsubscript{v1.3} channels have rapidly repriming characteristics appropriate to maintain high frequency spontaneous activity. We have further correlative data in that GDNF treatment (that prevents neuropathic pain behaviour) largely reverses the up-regulation of Na\textsubscript{v1.3} channels in damaged afferents (Boucher et al 2000). The potential role of other channels is considered in the following section.

**Spared sensory neurons.** Sensory neurons running alongside injured fibres in neuropathic models also show marked changes in gene expression (Fig. 4). Increased bioavailability of target-derived neurotrophic factors and the abnormal expression of several chemokines and cytokines arising in damaged nerves (as described above) are likely triggers for transcriptional regulation. Spared afferent fibres frequently show the opposite pattern of gene changes to that seen in damaged axons and many of the examples of altered gene expression in this group can most parsimoniously be explained by increased availability of NGF (see Fukuoka et al 2000). Thus, substance P and TRPV1 (both increased in spared C-fibres) are known to be strongly regulated by NGF. And these changes are likely
to increase the sensitivity of C fibres or increase their central effectiveness. There are also increases of P2X\textsubscript{3} expression in spared neurons in some but not all neuropathy models (Tsuzuki et al 2001, Fukuoka et al 2002) most readily explained by increased availability of GDNF.

The molecular basis for the increased ectopic activity in spared afferents is not well understood. On the one hand, there is no or only minor up-regulation of Na\textsubscript{v}1.3 (Boucher et al 2000) (the altered expression of which is well correlated with ectopic activity in damaged axons — see above). There has been some interest in the notion that the TTX resistant channel Na\textsubscript{v}1.8 (formally SNS), might play a critical role in the generation of neuropathic pain behaviour. Antisense treatment targeting this protein reportedly reduces neuropathic pain (Lai et al 2002). This protein is normally confined to nociceptive neurons, and it is known to be down-regulated after injury. Thus, it is unlikely to play a role in damaged afferents. However, it is up-regulated in spared afferents (Boucher et al 2000), presumably C-fibres (although this is not formally established). It would be expected to increase the excitability of these neurons and could therefore account for the low levels of spontaneous activity seen in the spared nociceptors. However, a further confounding factor is the observation that Na\textsubscript{v}1.8 knockout mice do not show any appreciable loss of neuropathic pain behaviour (Kerr et al 2001). In short, the molecular basis of this increased excitability of spared myelinated and unmyelinated afferents is currently unknown.

The relative contribution of ectopic inputs from damaged and spared afferents remains a contentious issue presently. However, the shift in focus to the undamaged afferents in neuropathic pain states provides new (and testable) hypotheses about the mechanisms underlying neuropathic pain.

**Neurotrophic factor treatment for neuropathic pain**

From the foregoing discussion, it is clear that several or many of the pathophysiological features associated with neuropathic pain appear to be secondary to altered neurotrophic factor availability. While the precise role (if any) of each of these observed changes to neuropathic pain itself is not established, a testable hypothesis is that normalizing neurotrophic factor availability will be of some use in the treatment of neuropathic pain.

The administration of NGF can induce strong neuroprotective effects on damaged neurons (reviewed in McMahon & Bennett 1999). However, the equally strong algogenic actions of NGF (McMahon & Bennett 1999) are likely to compromise the usefulness of this approach. There is one report of a beneficial effect of NGF in HIV neuropathy in human (Schifitto et al 2001). It is not clear if all subjects remained blinded on this trial and other attempts to use NGF clinically for the treatment of neuropathic pain states have been unsuccessful (see Apfel 2002).
The need to keep doses of NGF below pain-producing levels clearly limits its usefulness. Of course, if ‘spared’ rather than ‘damaged’ sensory neurons are more important for the evolution of neuropathic pain, then strategies aimed at limiting NGF availability to these neurons might be therapeutically useful, a suggestion for which there is supporting evidence (Theodosiou et al 1999).

We have assessed the effects of GDNF in several animal models. GDNF maintains the development of the non-peptidergic C fibres, and its exogenous delivery is able to reverse many of the alterations in gene expression induced by nerve injury that are crucial for the manifestation of neuropathic pain (Boucher et al 2000). Although many intact neurons express receptor components for GDNF and its related members (Bennett et al 1998), delivery of GDNF to intact animals failed to cause hyperalgesia or alter sensory processing when delivered to normal animals (Boucher et al 2000). However, GDNF does affect experimental neuropathic pain. We observed that intrathecal treatment with GDNF relieved neuropathic symptoms, and dramatically reduced the afferent barrage arising from damaged myelinated sensory neurons (Boucher et al 2000). The up-regulation of Na\textsubscript{v}1.3 in damaged sensory neurons was also reversed by GDNF treatment, further supporting a pivotal role for this channel in the generation of ectopic activity and subsequently neuropathic pain.

Artemin is a neurotrophic factor structurally related to GDNF. Artemin and GDNF bind to different receptors. The binding protein for artemin (so called GFR\textsubscript{a}3) is expressed in many small diameter sensory neurons (but not large diameter neurons, unlike GDNF). Recently, it has been reported that this factor can also reverse many of the changes in gene expression that occur in damaged sensory neurons with appropriate receptors—that is, small but not large diameter ones (Gardell et al 2003). Artemin was also reported to prevent and reverse neuropathic pain behaviour in animals models. Since the distribution of artemin receptors is very restricted (with almost none in the CNS), side effects of treatment might be limited. Artemin, unlike NGF, does not appear to be algogenic.

Changes in spinal sensory processing in neuropathy models

In this chapter, we have focused primarily on changes that occur in peripheral sensory neurons. But the fact that pain can be evoked by activation of innocuous mechanosensitive fibres with A\textsubscript{β} axons in many neuropathic patients, clearly indicates an altered state of sensory processing in these subjects. The animal models described above all exhibit mechanical allodynia—pain-related behaviour to very gentle tactile stimuli. Touch-evoked pain can emerge very quickly in patients, and in the models too allodynia is seen within one or two days. The animal models also show a marked increase in the sensitivity to cold
stimuli, again a common finding in neuropathic patients, and again an indicator of altered central processing of sensory information (since we have no evidence for an increased sensitivity of cold-sensitive primary sensory neurons in these conditions). Together, these observations suggest that the animal models do indeed accurately reflect at least some of the symptoms typically seen in patients, and it is reasonable to assume that mechanisms identified in experimental work will have some relevance to those occurring in humans. In fact, a great number of abnormalities have been identified in the central processing of sensory information in these models and one problem is in identifying those that might contribute significantly to neuropathic pain. A discussion of these central factors is beyond the scope of this chapter, but is dealt with in several other contributions to this volume.
Conclusions

Our understanding of the neuronal mechanisms contributing to neuropathic pain has advanced significantly during the past few years. Myriad changes occur following nerve injury as summarized in Fig. 5. Several independent groups have reported that among damaged sensory neurons, ectopic activity initially appears only in myelinated afferents, most of which, of course, are erstwhile innocuous mechanosensitive afferents. There is also new recognition that a critical role may be played not only by damaged afferents, but also by their spared neighbours. Rather remarkably, there are major changes in gene expression in these afferents, and consequential changes in anatomy and physiological function. The signal for change in these intact neighbours has not been revealed. A partial denervation of target tissue will lead to increased availability of target-derived factors, such as NGF, for the remaining afferents. These factors are known to powerfully regulate sensory neuron phenotype (McMahon et al 1995), and may be involved in the ectopic activity generation seen in spared unmyelinated afferents (Ali et al 1999). An alternative source of signal may arise from the process of Wallerian degeneration of damaged axons. This is associated with a rapid and massive invasion of degenerating nerves by macrophages, a ready source of neuroactive molecules such as cytokines. Schwann cells around degenerating axons also up-regulate their expression of trophic factors. The increased understanding of the roles of these target-derived and injury-induced factors offers the opportunity to develop novel therapeutic strategies for treatment of neuropathic pain states.

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**DISCUSSION**

Baron: Have you shown that application of GDNF prevents fibre death in the dorsal horn of IB4-positive neurons?

McMahon: We don’t think these cells are dying after the peripheral nerve injury. We think they are just down-regulating the sugars that bind the lectin IB4.

Baron: Did you correlate these findings with the behavioural findings?

McMahon: The data I showed you were from preparations with sciatic nerve axotomy, and we are unable to do meaningful behavioural analysis in these cases. But we have looked at IB4 binding in neuropathic models — spinal nerve ligation — and we see the same changes in damaged sensory neurons. There is a general correlation in that IB4 binding is reduced at one and two weeks after injury, when neuropathic behaviour is present. And GDNF treatment prevents both the immunohistochemical and behavioural changes in this time frame. We have not examined the correlation on an animal-by-animal basis.

Belmonte: We have an interesting observation in the cornea. When we damaged corneal nerve endings located in the superficial corneal epithelium where they do not have anymore Schwann cells, we don’t see c-jun expression in the cell bodies. Whereas if the corneal lesion is deeper, affecting the stroma where the fibres are
covered by Schwann cells, there is a marked \( c \)-jun expression in the soma of corneal neurons. What is your speculation about the role played by Schwann cells in the effects of growth factors seen after axotomy?

McMahon: Well, it’s a bit more than speculation because there are some data that quiescent Schwann cells don’t make a lot of NGF. However, Schwann cells in the presence of degenerating axons start churning it out. There is evidence that this occurs secondary to IL1\( \beta \) stimulation of the Schwann cells. But I think you are asking ‘what are the consequences of these changes?’ If a peripheral nerve is crushed, fibres start regenerating into the degenerating distal nerve almost immediately (within a day or two). Under those circumstances many of the candidate genes that are regulated by NGF don’t change. The simple explanation is that NGF is replenished in the crushed fibres as they regrow in an NGF-rich environment. But if a nerve is cut and prevented from regenerating, while the Schwann cells at the site of nerve damage start making excess NGF, it is not enough to compensate for what the sensory axon would normally get from its peripheral terminals. The corollaries of your question are also very interesting. That is, in neuropathies that are associated with dying back of axons, how far do they have to die back before they lose target-derived trophic support? I don’t know the answer to this. There are indications from intradermal and topical capsaicin studies. Just the terminals are lost with these treatments and there does not seem to be extensive retrograde degeneration of axons. Under these circumstances, C-fibres look as if they have lost their peripheral neurotrophic support.

Devor: When you have this dying back into the nerve trunk, are these dying back axon ends like a distributed microneuroma? Do you have a Tinel sign along the nerve in diabetic neuropathy? Is this something that has been tested?

McMahon: I think the dying back occurs without any Tinel sign. Studies using quantitative evaluation of C fibre epidermal innervation in humans show reduced regenerative capacity of C fibres challenged with capsaicin in diabetic patients or HIV patients when the patient is completely asymptomatic and before they start to lose innervation from the epidermis.

Devor: But their symptoms might begin when these dying back axon ends start to become hyperexcitable, one of the important symptoms being an ongoing burning pain, for example.

Wood: Have you looked in behavioural experiments at neurturin and artemin, and do all GDNF family members have the same effects?

McMahon: Yes, we have repeated them with both neurturin and artemin, two other members of the GDNF family. Neurturin has been difficult to use, because of its limited solubility and we have no convincing data. We have studied neuropathic behaviour in several experiments using artemin. In about a half of these we have seen a good neuroprotective action, but in other, apparently identical experiments we have seen nothing. The experiments are done blind and
we don’t understand the lack of reproducibility. The effects we have seen emerge later than with GDNF treatment. Subsequently a large study by Frank Porecca and others found that artemin did produce a strong but delayed behavioural recovery of neuropathic behaviour. So, several members of the GDNF family may be effective. But this in itself is quite perplexing, since different groups of sensory neurons have receptors for artemin and GDNF. This doesn’t help us understand the mechanisms.

Noguchi: What is the mechanism underlying the effect of GDNF on the L4 ganglia following L5 spinal nerve injury?

McMahan: In animals with an L5 spinal nerve ligation, we don’t think there is any deficit in GDNF or NGF in the L4 dorsal root ganglion. If you look at markers that are induced or supported by either of these factors, they don’t decrease dramatically. So there is no need to propose that tropic factors work by offering neuroprotection to these intact afferents. But there is a problem: I told you that intact myelinated afferents become spontaneously active, as do axotomised ones, and GDNF treatment dampens down activity in both these groups. But when we looked for ionic changes that might contribute to the spontaneous activity, we could only see an up-regulation of Nav1.3 in the damaged afferents. We don’t see a change in Na\textsubscript{v}1.3 by PCR in the spared afferents. So the relationship between ectopic activity and channel expression in those two sets of neurons is unclear. We don’t have a simple explanation.

Apkarian: Could you explain what the roots of your scepticism are about the lack of central anatomical reorganization?

McMahan: It is not just my view: four separate groups now have data suggesting that the anatomical reorganization (sprouting) that has been reported after peripheral axotomy is based on a methodology that isn’t sound. The classic method used to identify this sprouting is the bulk transport of CTB (the \(\beta\) subunit of cholera toxin). Several groups have now shown that after peripheral nerve injury, C fibres start to transport CTB. Therefore the change previously interpreted as sprouting may not be sprouting at all, but rather \textit{de novo} transport. That suggests that the anatomical data are more difficult to interpret than we would like. If one asks whether there is positive evidence against sprouting, then there is. One line of evidence comes from studies where a peripheral nerve is labelled with CTB and subsequently damaged peripherally. \textit{De novo} transport by damaged C fibres is not possible here, and one does not see any signs of ‘sprouting’. In a recent study Hughes et al (2003) labelled A\(\beta\) fibres with very small injections of CTB into dorsal columns. This labelled small numbers of A fibres that could be studied anatomically, again without significant contamination by \textit{de novo} transport in C fibres. They too found no evidence of sprouting.

Baron: I thought there was electrophysiological evidence showing an activation of nociceptive neurons in the dorsal horn by A\(\beta\) fibres.
McMahon: There clearly are functional changes that take place in the spinal cord, but the explanation for these is uncertain. I should say that in addition to the bulk labelling experiments, there are data from anatomical reconstructions of single A fibres, some of which do and some of which do not suggest sprouting. Both the bulk labelling and the single fibre fills throw some doubt on the simple conclusion that second order cells in the spinal cord are beginning to receive de novo monosynaptic connections from $A\beta$ fibres. There are other possible explanations, such as the unmasking of existing $A\beta$ connections, or perhaps the strengthening of such connections.

Yoshimura: We have tested a change of synaptic connections in the spinal dorsal horn following inflammation in my present talk, but we have also reported the reorganization of the synaptic transmission in the spinal cord in sciatic nerve transected rats. What we found is that in the early stage of inflammation, $A\beta$ afferent fibres made synaptic connections with interneurons which had already established synaptic contacts with substantia gelatinosa neurones. Therefore, there were many polysynaptic inputs from $A\beta$ afferents to substantia gelatinosa neurons. After 7–10 days of inflammation, the $A\beta$ fibres then made a direct synaptic contact with substantia gelatinosa neurons. Similar to the peripheral inflammation, the sprouting is also observed in sciatic nerve transected rats originally reported by Woolf’s group (Woolf et al 1992, Okamoto et al 2001). Although $A\beta$ fibres make synaptic contact with superficial dorsal horn neurons, only a few neurons have a direct input from $A\beta$ fibres, and many of the inputs from $A\beta$ are polysynaptic. Thus, the sprouting patterns of the $A\beta$ afferent fibres are distinct in different pain models.

Devor: I also wonder whether this could happen within the 24 h time frame that Jin Mo has set for us. Also, if there is a hardwiring change of that sort, how can you turn the allodynia on and off by stopping the ectopic activity in the ganglion and neuroma?

Malmberg: I would like to hear your comments on your other questions, and the relationship between two of your comments, namely does NGF promote neuropathic pain and is there a need for ectopic activity in C fibres? Given that NGF-positive neurons are C fibres, is it possible that NGF is promoting ectopic activity in C fibres?

McMahon: These are related questions. One hypothesis is that the critical peripheral event is C fibre activity. The only candidate appears to be intact C fibres. And there does seem to be an up-regulation of genes in these afferents that are likely to be controlled by NGF. A consistent hypothesis would be that C fibre activity is important and arises because of increased bioavailability of NGF to those intact afferents. This is testable, but the necessary reagents (anti-NGF) are not freely available.
Malmberg: The groups that have performed these studies have found that anti-NGF has some effect, particularly in inflammatory models, but the effect on neuropathic pain is less convincing.

Devor: On a similar issue, is there any sign that these altered L4 C afferents begin to respond to very light stimuli? I’m thinking of the sort of stimuli that evoke the tactile allostynia — the response to weak von Frey hairs. I don’t think that this happens. It is a misconception that many people have had. It would be easy to interpret tactile allodynia if that happened: if C fibres became sensitive in the skin. If anything, the role of the very low rate of abnormal C fibre activity would be to contribute to the central sensitization, which raises an alternative question: Is it possible that injured A fibres, which now have changed expression of many peptides and other molecules, could have acquired the ability to turn on and maintain central sensitization, an ability which normally they don’t have.

Dray: There is an important role for neurotrophins in the regulation of ion channels and recent reports of enhanced dorsal root reflexes suggest neurotrophins regulate redistribution of ion channels and ionic transport mechanisms in neuropathic pain. Could you comment on the relevance of neurotrophins in this respect?

McMahon: I don’t think there is any direct evidence that differential trafficking is controlled by trophic factor availability. The claim is that after injury there may be translocation of channels, but the causative agents are unknown. My own prejudice is that such translocation is an unlikely explanation for neuropathic pain, because of the continuing neuropathic behaviour in Na_\text{v}1.8 knockout mice.

Dray: There has been another discussion about neurotrophin regulation of chloride channels. Is there a relationship between GDNF and chloride channels?

McMahon: Most of the interest that I am aware of relates to regulation by other trophic factors, most notably BDNF, which has been suggested to regulate a chloride transporter in dorsal horn neurons.

Chung: I have a question regarding the activity of the spared intact nerve after spinal nerve ligation. You showed a picture of the Remak bundle with a reduced number of unmyelinated fibres. This tells me that there is plenty of opportunity for interaction between the degenerating and the intact fibres. What I don’t see is clear evidence that there is strong interaction. If there is a strong interaction, I would expect to see a whole bunch of intact C fibres firing like crazy, which I do not see.

McMahon: What one sees probably depends on what is being produced. Several putative factors won’t necessarily induce high rates of C fibre active. NGF itself, if given in large measures, can activate some deep afferents, but mainly is associated with sensitizing the peripheral terminals of intact C fibres. Secondly, if one asks whether there are other signs of increased trophic factor bioavailability, there is indeed considerable evidence that this is the case, as seen by changes in gene
expression in spared sensory axons. Some of these will be reviewed in other papers at this meeting. The fact that there are such changes in gene expression itself is highly suggestive of altered availability of neurotrophic factors or cytokines that have neurotrophic effects.

**Chung:** The activity that you report is different from that of Dick Meyer’s group. Do you know whether your activity is coming from damaged or undamaged afferents?

**McMahon:** I don’t know what causes that activity. One issue is whether spared afferents are really intact. But simply doing the surgery to make a spinal nerve ligation of L5 threatens to damage L4, which sits alongside. We recently undertook a study in which we used the marker ATF-3 (a transcription factor that marks cells that have been axotomised). We found that in some preparations there was very little ATF-3 in L4 after L5 spinal ligation, but in other preparations, up to 30% of L4 DRG neurons appeared to have been axotomised. Interestingly, there was no correlation between ATF3 expression in L4 after L5 ligation and L4 ectopic activity in the same animals. We still don’t know what causes the damage, but it appears to be different from what causes the ectopic activity in these spared afferents.

**Devor:** The activity reported by the Baltimore group in residual C fibres in L4 is something like 3–5 spikes every five minutes. We are talking about exceedingly low rates of firing. Many of us who have done recording would have thought of artefacts, that maybe this has to do with the refrigerator turning on in the next door lab! I should add, though, that the claim is that quite a high percentage of fibres show this very low level activity.

**Apkarian:** I had a similar question related to the issue of anatomic reorganization. The other issue that comes up repeatedly is central cell death. Where does this stand?

**McMahon:** Recent evidence from the Woolf lab suggests that dorsal horn cell death is a very active phenomenon that may explain some of the disinhibition phenomena seen in neuropathic models. The difference from the original claim is that cell death only arises in models of partial nerve injury, those associated with neuropathic pain.

**Apkarian:** If that is believable how could anatomical reorganization not happen, if you also have apoptosis happening at the same time?

**McMahon:** You could have anatomical reorganization which does not affect or involve sprouting of Aβ afferents.

**Devor:** The loss of inhibitory neurons in spinal cord also has the problem (along with Aβ sprouting) of explaining the reversal of tactile allodynia and of spontaneous pain with peripheral nerve block, which is an almost universal report from the clinicians. If you find the source of the ectopic firing in the peripheral nerve and you block it with local anaesthetic, the pain goes away until
the block vanishes. If the key change was happening in the spinal cord and the pain signal originated there, this wouldn’t be possible.

McMahon: You may have a disinhibited spinal cord that allows weak peripheral inputs to generate abnormal pain sensations.

Devor: But would the residual peripheral inputs remain if you blocked the injured area? Again, we go back to the question of whether we need the ectopic firing coming from the injured nerve, or whether the pain has become centralized. As I said earlier, I don’t see the evidence of frank centralization.

Aphkarian: It can still be driven from the periphery but magnified centrally.

Devor: Tactile alldynia means you are driving tactile receptors in the skin. Their signal is amplified in the spinal cord. But if the amplification is due to a change in the spinal cord that doesn’t require ectopic input from the periphery, then blocking the ectopic input shouldn’t stop the alldynia. But it does (Gracely et al 1992).

Mantyh: In light of your data, would you say that IB4 neurons are uniquely involved in neuropathic pain? What are they normally doing?

McMahon: There are some data from selective ablations using the saporin conjugates, although we have had no luck with this approach. Since it is always easier to believe one’s own data, I am not clear the approach provides any compelling insight into the selective role of IB4 fibres. You could turn the question round and say what do we know about those fibres from their normal physiology? There we end up with a clear answer. In rodents, half of the IB4-binding cells are capsaicin sensitive and half are not, and half are heat sensitive and half are not. In our hands, quite a few appear to be ATP sensitive. They don’t seem to have unusual properties compared to non-IB4-binding C fibres. The simple conclusion would be that they are just a bunch of nociceptors, but that they have a unique central connectivity. But what this means to the animal, I don’t know.

Dray: I concur with what you said with respect to the selective ablation. However, an important question is raised from human microneurography studies showing the existence of a specific population of mechano-insensitive C fibres called ‘silent nociceptors’. Amongst other characteristics these fibres respond more dramatically to capsaicin exposure and make an extraordinary contribution to the initiation of spinal sensitization. Little or nothing is known of their phenotype.

Devor: What do we know about how activity in C fibres turns on central sensitization?

McMahon: Those fibres produce flare responses. They are presumably peptidergic in nature, and we predict that they would be NGF sensitive, and not GDNF sensitive. But this is indirect evidence and somewhat speculative.
Devor: Do we know which peptide it is, if it is a peptide that is turning on the central effect? These C fibres, whether they are originally silent or not, never become responsive to these very light stimuli that drive tactile allodynia. Until someone can find C fibres that are really sensitized, we have to talk about \( \text{A} \beta \) activity as being misinterpreted by a sensitized spinal cord. Now the question becomes how do these C fibre inputs sensitize the cord? One popular idea is that a peptide that is released — perhaps substance P — produces a tonic depolarization in post-synaptic neurons and therefore enables NMDA receptors, which now become responsive to the \( \text{A} \beta \) input. However, from the work of Dr Noguchi and others, we know that after a nerve injury substance P is down-regulated and there is much less of it in the C fibres, while there is much more of it in the A fibres. This happens quite soon after axotomy. Is it possible that the injured \( \text{A} \beta \) fibres, due to this phenotypic switch, are now able to induce central sensitization? If so, the injured A fibres not only fire and produce an input, but also maintain the central sensitization that amplifies this input.

Reeh: Shouldn’t we ask the author the first of your series of questions? That is, whether low threshold C or \( \text{A} \delta \) mechanoreceptors contain neuropeptides that eventually could be released. He is the only one who could comment on that.

Perl: The evidence is incomplete for the low threshold C afferent fibres. We labelled few, and never studied them at the electron microscope level. Low threshold myelinated mechanoreceptive fibres in general do not have dense core vesicles in their synaptic terminals. The issue is half-answered. We do know that there are many peripheral C fibres in mammals that act as low threshold mechanoreceptors. They are remarkably sensitive; if they were involved, their input could easily explain tactile allodynia.

Devor: Yes, if they were nociceptors. But they have a low tactile threshold, so they are not nociceptors.

Perl: C-mechanoreceptors are not nociceptors. They have peculiar characteristics and they are reported to have important functions in human beings. They have been shown to be involved in a peculiar emotional experience by a patient with no functioning myelinated fibres below the neck (Olausson et al 2002).

Devor: If low threshold C fibres were capable of turning on central sensitization, just brushing the skin lightly for a minute or two would turn on central sensitization, and it doesn’t.

Reeh: That is not the sort of stimulus that would evoke any flare reaction or CGRP release in the periphery. We could assume that those fibres are not peptidergic.

Devor: There is an interesting observation from Molander et al (1994) in Stockholm. Normally \( e \)-fos is turned on in dorsal horn neurons only by C fibre stimulation of the peripheral nerve. However, if there has been a prior nerve
injury, now Aβ fibre stimulation will activate c-fos in the spinal cord. This is another piece of evidence suggesting that Aβ fibres may acquire the ability to trigger central sensitization after injury, a capability that they didn’t have before.

Mao: I’d like to ask a different type of question. If we imagine that whatever the mechanisms so far we have proposed for neuropathic pain, whether central or peripheral mechanisms, the end point is a common pathway, for example, activating the spinal projection pathway to the brain. If this were the case, in terms of peripheral mechanisms we would have a new generator, and in terms of central mechanisms we would have an increased gain of input. But why then do patients with neuropathic pain often describe the pain as having a different quality from physiological pain. They don’t use the same words as those used to describe physiological pain. Whatever the mechanisms involved, if it is simply to turn on the common pathway or to enhance the gain of this common pathway, why do they choose different pain descriptors? Why has the quality of the pain changed? With similar nerve injury, some patients will have allodynia and others have hyperalgesia, and this can change dynamically over time.

Devor: This might be a good point to mention recent results by Frank Porreca and his group (Porreca et al 2002). Central sensitization refers to a gain in amplification in the spinal cord. This amplifier is controlled by many different things. Afferent input, and in particular afferent C fibre input, is clearly one of them. I have raised the possibility that Aβ input along injured Aβ fibres might be another. Porreca points out the possibility that this spinal amplifier might also be controlled by descending pathways from the brainstem. He shows that cutting some of the descending pathways can eliminate tactile allodynia. This is one more thing that can control this amplifier. When we talk about individual variability in neuropathic sensation, or changes in the quality of the sensation from time to time in a given patient, this could be dependent on the diversity of inputs to the amplifier, including descending control from the head.

Belmonte: We have done experiments in the human cornea using a gas aesthesiometer that allows us to stimulate polymodal nociceptors alone or in combination with mechano-nociceptors (Acosta et al 2001). The quality of the pain sensation is completely different from one case to another. In my view, the final quality of the sensation depends on the degree of activation of the various classes of sensory receptors. In the above mentioned experiment where two subpopulations of nociceptors are activated to a variable degree, different qualities of pain were experienced. In neuropathic pain, the sensation felt by patients may be particular because many types of fibres are being simultaneously and abnormally activated.

McMahon: Why assume there is only one single pathway leading to one unique sensation?
Apkarian: The other issue here is that the discussion keeps centring on allodynia. Chronic pain can clearly lead to spontaneous pain, which is the most common form of chronic pain but it is difficult to measure and design experiments to study. Perhaps the slow acting C fibres are critical for the perception of spontaneous pain.

Devor: Perhaps, but if there is central sensitization for whatever reason, because of these abnormal L4 C fibres, or because injured Aβ fibres now are able to turn on central sensitization, or because the central sensitization is induced by descending control from the head, the spontaneous activity that many of us have been pointing out will also be amplified. This is an obvious potential source of spontaneous painful sensation. The neuroma and the DRG activity are now amplified by central sensitization which, parenthetically, may be maintained by that same ectopic input. I wanted to raise the topic of microarray experiments. Steve McMahon showed a slide from Dr Zhang’s work (Xiao et al 2002) on mRNA expression from axotomized DRG. Of the 8000 odd transcripts on the array, 167 were significantly up- or down-regulated after axotomy. This experiment has now been done by several other groups. It is safe to say that at least 1000 transcripts in the DRG are significantly regulated after nerve axotomy. This is only in the DRG. If we were to do the same arrays in spinal cord, we may find another couple of thousand mRNAs changed, and who knows what happens in the brain and skin. I think we are facing a crisis in our understanding: we began with having no theories of neuropathic pain and now we have many thousands. We will need to come up with strategies to figure out which are central to pain and which aren’t.

Zhou: I think that in the future there will be a requirement for researchers using more integrated approaches to address pain mechanisms. Personally, I think in the future more collaborations will help to solve this problem.

Devor: The problem is that we are talking about thousands of transcripts — it’s not a regular collaboration.

Mantyh: There have been a couple of very nice studies in ovarian cancer by the group led by Dr Bagnato in Italy. What she showed was that if she blocked the endothelin A receptor in precancerous cells, it could block most of the downstream events. She used microarrays to show this. In arrays, the change between precancerous and cancerous cells involved thousands of genes. I am wondering if you ran microarrays and looked at the effect of GDNF, how many of these changes in gene expression would you see?

McMahon: The London Pain Consortium is undertaking studies on genes regulated in sensory neurons by trophic factors. These are ongoing and we hope to post data on a publically available website.

Gintzler: Thousands of transcripts changing doesn’t mean thousands of theories. More importantly, it needs to be pointed out that a change in transcript level doesn’t mean there is a change in protein level. Until one knows the protein
that is encoded by all of these transcripts, we can’t validate that there are meaningful changes in protein level.

Zhou: There are lots of proteins which Western blots are not sensitive enough to detect. In most cases, biochemical analysis uses samples with mixed populations of cells from brain regions.

Wood: The subcellular localization of proteins is also important.

Devor: An important feature is threshold discontinuity in a response function. This is very characteristic of the abnormal firing that Jin Mo was talking about. If you are operating just above repetitive firing threshold, a small inhibitory stimulus will kick you from a substantial rate of firing to zero. Since you are at threshold it could be that all of these various things turn off allodynia despite the fact that each makes only a small contribution overall.

Wood: Just to turn that argument on its head, if there is cooperativity between large numbers of different mediators which summate to change thresholds, this can explain why so many different drugs, antisense and knockouts all have dramatic effects. If we can form a reasonable theory based on temporal analysis of how these things change then we may be able to home in on the kind of bottleneck which could be a globally interesting target.

References