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Review

Pathobiology of neuropathic pain

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Abstract

This review deals with physiological and biological mechanisms of neuropathic pain, that is, pain induced by injury or disease of the nervous system. Animal models of neuropathic pain mostly use injury to a peripheral nerve, therefore, our focus is on results from nerve injury models. To make sure that the nerve injury models are related to pain, the behavior was assessed of animals following nerve injury, i.e. partial/total nerve transection/ligation or chronic nerve constriction. The following behaviors observed in such animals are considered to indicate pain: (a) autotomy, i.e. self-attack, assessed by counting the number of wounds implied, (b) hyperalgesia, i.e. strong withdrawal responses to a moderate heat stimulus, (c) allodynia, i.e. withdrawal in response to non-noxious tactile or cold stimuli. These behavioral parameters have been exploited to study the pharmacology and modulation of neuropathic pain. Nerve fibers develop abnormal ectopic excitability at or near the site of nerve injury. The mechanisms include unusual distributions of Na^+ channels, as well as abnormal responses to endogenous pain producing substances and cytokines such as tumor necrosis factor α (TNF-α). Persistent abnormal excitability of sensory nerve endings in a neuroma is considered a mechanism of stump pain after amputation. Any local nerve injury tends to spread to distant parts of the peripheral and central nervous system. This includes erratic mechano-sensitivity along the injured nerve including the cell bodies in the dorsal root ganglion (DRG) as well as ongoing activity in the dorsal horn. The spread of pathophysiology includes upregulation of nitric oxide synthase (NOS) in axotomized neurons, deafferentation hypersensitivity of spinal neurons following afferent cell death, long-term potentiation (LTP) of spinal synaptic transmission and attenuation of central pain inhibitory mechanisms. In particular, the efficacy of opioids at the spinal level is much decreased following nerve injury. Repeated or prolonged noxious stimulation and the persistent abnormal input following nerve injury activate a number of intracellular second messenger systems, implying phosphorylation by protein kinases, particularly protein kinase C (PKC). Intracellular signal cascades result in immediate early gene (IEG) induction which is considered as the ouverture of a widespread change in protein synthesis, a general basis for nervous system plasticity. Although these processes of increasing nervous system excitability may be considered as a strategy to compensate functional deficits following nerve injury, its by-product is widespread nervous system sensitization resulting in pain and hyperalgesia. An important sequela of nerve injury and other nervous system diseases such as virus attack is apoptosis of neurons in the peripheral and central nervous system. Apoptosis seems to induce neuronal sensitization and loss of inhibitory systems, and these irreversible processes might be in common to nervous system damage by brain trauma or ischemia as well as neuropathic pain. The cellular pathobiology including apoptosis suggests future strategies against neuropathic pain that emphasize preventive aspects.

Keywords: Neuropathy; Nerve trauma; Nerve injury; Transcription; Sensitization; Apoptosis; Animal pain model; Hyperalgesia; Autotomy; Allodynia; Phantom pain; Opioid; Deafferentation; TNF-α (tumor necrosis factor-α); Na^+ channel

1. Introduction

Mitchell (1872) was the first to provide a systematic description of various kinds of posttraumatic neuropathic pain as seen and treated by him in Philadelphia during the US Civil War. Intensive research has resulted in major progress in the understanding of the basic mechanisms of neuropathic pain. During the past 25 years, animal models of various neuropathies have been used, but more recently, research on patients suffering from neuropathic pain has contributed as well to the knowledge of the underlying pathophysiology which, usually, is rather complex.

Neuropathic pain may be due to a primary insult to the peripheral or central nervous system, and hence, the clinical classifications differentiate between neuralgias of

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peripheral nerves (e.g. trigeminal neuralgia) or central neuropathic pain (e.g. thalamic syndrome). However, the peripheral or central site of underlying pathophysiology can no longer be used as a discriminandum, because neuropathic mechanisms may expand during the disease to imply both peripheral and central pathophysiology. For example, following peripheral nerve injury pain signals originate first from the axonal site of the lesion but over time, other parts of the afflicted primary sensory dorsal root ganglion (DRG) neuron and even the postsynaptic dorsal horn and higher order neurons up to the cortical level will contribute. Eventually, the pain is all coming from a central generator, as, e.g. in phantom pain, although the lesion was clearly peripheral. Such spread of pain generating mechanisms is due to the (slow) biochemical reactions of the nervous system, and therefore, neuropathic pain should be considered as a progressive nervous system disease. Similar slow expansions of disease mechanisms have been identified in chronic pain of non-neuropathic origin, and the term "pain memory" is now being used to indicate such processes. Accordingly, any treatment of neuropathic pain should include preventive measures against secondary nervous system pathobiology and this requires an early multifaceted approach.

A phenomenological classification relates to the type of damage or related pathophysiology causing a painful neuropathic disorder:

- Mechanical nerve injury, e.g. carpal tunnel syndrome, vertebral disk herniation;
- metabolic disease, e.g. diabetic polyneuropathy;
- neurotropic viral disease, e.g. herpes zoster, human immunodeficient virus (HIV) disease;
- neurotoxicity, e.g. by chemotherapy of cancer or tuberculosis;
- inflammatory and/or immunologic mechanisms, e.g. multiple sclerosis;
- nervous system focal ischemia, e.g. thalamic syndrome (anesthesia dolorosa);
- multiple neurotransmitter system dysfunction, e.g. complex regional pain syndrome (CRPS).

Neurotransmitter system dysfunction is not generally recognized as a basic etiological mechanism of neuropathic pain, but it is logical to include such diseases where the pain is clearly related to neurotransmitter failure or dysregulation.

More specific information on painful neuropathic disorders according to etiology and the differential diagnosis will be contained in the clinical contribution to this volume by Dr. Troel Jensen. I will present an overview on the pathophysiological mechanisms as they appear now from animal models of neuropathic pain. Most of the basic science researches have been conducted on mechanical types of nerve injury; therefore, my review will focus on the related mechanisms.

2. Pain behavior in animals following nerve injury

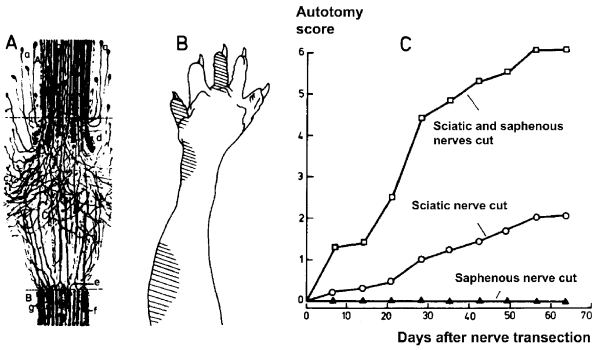
Animal models for neuropathic pain intensively studied so far are those in which a mechanical trauma is produced on a peripheral nerve, the most straightforward one being the transection and ligation of the sciatic nerve. This lesion results in immediate and irreversible interruption of electrical nerve conduction, followed by Wallerian degeneration of the axons distal to the lesion and sprouting of the proximal axonal stumps in an attempt to regenerate the nerve fiber. Apart from these local effects, there are several distant reactions which appear after days, such as the cell body response in the dorsal root ganglion involving chromatolysis of the nucleus (Cragg, 1970). Recent research had its focus on these delayed processes as they are of utmost importance for the emergence of a chronic pain syndrome and also for its prevention.

A behavioral analysis of rats following transection of nerves to the hind leg (Wall et al., 1979) is shown in Fig. 1. In this study, self-attack of the denervated leg was observed. Authors coined the label "autotomy" for this behavior, a term commonly used in studies where a form of excessive self-care or self-grooming occurs that results in bite wounds and eventually in the self-amputation of digits. Wall et al. (1979) assessed the extent of self-mutilation by counting the number of wounds on the denervated extremity and by accounting for the size of wounds. The autotomy score, defined on these criteria, was used to show the time course of abnormal behavior as well as the factors modulating it. For instance, in Fig. 1, the autotomy score continually increased during the observation time of 70 days and the score was highest when both the sciatic and the saphenous nerves had been transected. Incidentally, saphenous nerve transection alone did not induce autotomy behavior in these experiments.

There have been some controversies on whether or not autotomy behavior is a sign of pain (Rodin and Kruger, 1984). Similar behaviors can be induced in animals by skin irritations or skin diseases assumed to induce itching. In addition, it has been argued that the absence of sensory feedback from the denervated leg may result in the self-attack because the animal does not recognize the anesthetic limb as part of its body. However, although such factors may contribute to the behavior under specific circumstances, it is widely assumed now that excessive grooming and autotomy following a nerve lesion reflect chronic neuropathic pain (Coderre et al., 1986; Kauppila, 1998).

Four main animal models for nerve injury-associated pain are now in use, each with some variations (Bennett, 1994a,b; Kauppila, 1998):

- Total nerve transection and ligation, as described above, simulating the clinical conditions of amputation.
- Partial nerve lesion with a tight ligation around part (about 50%) of the nerve fascicles (Seltzer et al., 1990), simulating the clinical condition of an accidential nerve bruise or gunshot-induced nerve injury.



A: Histology of neuroma after nerve transection, by Ramon y Cajal, 1928
B: Wounds by excessive self-grooming with automutilation ("autotomy") of denervated leg
C: Time course of "Autotomy score" following nerve transections, redrawn from Wall et al., 1979

Fig. 1. Autotomy behavior in rats following sciatic and/or saphenous nerve transection. (A) Neuroma visualised by Golgi staining a few days after nerve transection. (B) Schematic drawing of rat foreleg with wounds induced by excessive self-grooming of the rat, indicated by hatched areas. (C) Autotomy score (ordinate) assessed by counting the number of wounds on hind leg of rats after transection of saphenous or sciatic nerve alone or in combination. (A) From Ramon y Cajal about 1900; (C) redrawn from Wall et al. (1979).

• Chronic constriction injury (CCI) by placing several loose ligatures around the nerve leaving a lumen of less than the diameter of the original nerve (Howe et al., 1977; Bennett and Xie, 1988), simulating the clinical condition of chronic nerve compression such as the one that occurs in nerve entrapment neuropathy or spinal root irritation by a lumbar disk herniation.

• Tight ligation of a spinal nerve (Kim and Chung, 1992; Carlton et al., 1994) or transection of one or several dorsal roots (Lombard et al., 1979; Albe-Fessard and Lombard, 1983; Brinkhus and Zimmermann, 1983) resulting in complete deafferentation of one or several spinal segments, simulating nerve plexus and dorsal root injury.

There are three behavioral indices of neuropathic pain which are regularly seen in animals with a partial nerve lesion or a peripheral or spinal nerve constriction injury:

- Autotomy, as described above;
- hyperalgesia, i.e. a stronger or earlier withdrawal response than in healthy animals to a noxious stimulus, e.g. noxious skin heating;
- allodynia, a withdrawal response to repeatedly touching with a von Frey hair, which is not painful in tests on humans and does not evoke withdrawal in animals without nerve injury. Cold allodynia has also been described.

Behavioral assessment of autotomy, hyperalgesia and allodynia following nerve injury has been multiply exploited in different directions of research, e.g. for the effects of:

• Analgesic treatment by systemic or intrathecal drugs such as morphine (Xu and Wiesenfeld-Hallin, 1991), antidepressants (Abad et al., 1989; Seltzer et al., 1989), alpha2-adrenoceptor agonists clonidine and dexmedetomidine (Puke and Wiesenfeld-Hallin, 1993), anticonvulsants (Christensen et al., 2001), NMDA-receptor antagonists (Burton et al., 1999; Suzuki et al., 2001), (*R*)-ketoprofen (Ossipov et al., 2000), magnesium sulphate (Feria et al., 1993) or cannabinoids (Fox et al., 2001). These treatments attenuated one or several of the behavioral indicators of neuropathic pain, i.e. autotomy, heat hyperalgesia and allodynia.

• Topical treatments at the neuroma or nerve level, e.g. by an implanted delivery system to administer glycerol or alcohol (Seltzer et al., 1985), a local anesthetic (Seltzer et al., 1991) or a NO-Synthase (NOS) inhibitor (Thomas et al., 1996), all of which attenuated autotomy.

• Grafting of a piece of adrenal medulla or immortalized chromaffin cells to the spinal cord which attenuated autotomy (Ginzburg and Seltzer, 1990; Yu et al., 1998; Eaton et al., 2000).

• Prolonged stress by lowering ambient temperature which increased autotomy (Wiesenfeld and Hallin, 1980; Sato et al., 2000).

• Social interaction with a cospecies of the opposite sex in the same cage, attenuating the pain behavior (Berman and Rodin, 1982).

• Genetic diversity of the degree of autotomy in different strains of rats (Shir et al., 2001b), which has led to the identification of several genes that encode for neural functions related to neuropathic pain and may disclose targets for the development of new treatments (Seltzer et al., 2001). This concept likely explains that preoperative behavior predicts neuropathic pain behavior (Vatine et al., 2000). These results corroborate the outcome of an earlier project aimed at producing inbread strains of rats with high

or low degrees of autotomy following nerve transection (Inbal et al., 1980).

• Diet, showing specifically that a soy-bean containing diet, suppresses autotomy behavior (Shir et al., 2001a).

Local anesthesia of the transected nerve, glycerol/alcohol superfusion or resection of a neuroma usually attenuate but do not completely terminate autotomy behavior (Seltzer et al., 1985). Such persistence of pain behavior following anesthesia of the neuroma suggests a central focus of hyperexcitability and ongoing discharges, which has been localized in the spinal dorsal horn at the segments of projection of the injured nerve. According to today's concept of neuropathic pain, peripherally originating impulses induce stump pain, whereas centrally arising nerve impulses induce phantom pain. In a clinical setting, stump pain can be modulated by peripheral intervention, e.g. local anesthesia of the neuroma or the peripheral nerve, while phantom pain is not affected by such peripheral interventions. Thus, there is evidence that peripheral and central mechanisms contribute to the abnormal behavior, i.e. autotomy, hyperalgesia and allodynia, as will be described in the following sections.

3. Local pathophysiology of nerve injury-induced neuropathic pain

Following nerve transection, a neuroma is developing at the proximal nerve stump, consisting of regenerative nerve sprouts growing into all directions (see Fig. 1A). Electronmicroscopy has shown that multiple unmyelinated sprouts grow out from each transected axon. Electrophysiological recording shows ongoing activity, abnormal excitability and discharge characteristics, particularly in C-fibers, and it is conceivable that these pathophysiological impulses originate at the neuroma sprouts (Culp and Ochoa, 1982; Zimmermann, 1985; Jänig, 1988; Chul Han et al., 2000). A few hours after transection of an axon, nerve impulses can be elicited by mechanical stimulation from the site of the lesion, and after a few days, spontaneous impulse activity originates predominantly in C-fibers. Mechanical sensitivity is pronounced for a long time (i.e. months) following the injury, irrespective of whether the sprouting fibers are confined to a neuroma or whether longitudinal growth occurred. The mechano-sensitivity of the sprouts is the basis of Tinel's sign used by neurologists to test the progress of the longitudinal growth of a transected nerve in patients. However, sprouts of regenerating nerves also exhibit a remarkable sensitivity to noxious heating (Dickhaus et al., 1976; Zimmermann, 1985; Zimmermann and Herdegen, 1996): many C-fibers are activated to tonic discharges, graded with temperature, while some Aβ-fibers, which normally are not excitable by temperature increases, show prolonged erratic responses when heated.

The responses evoked at regenerating nerve sprouts usually show erratic behavior, i.e. the discharges are irregular and not well reproducible with repeated stimulation. If longitudinal growth of regenerating nerve fibers is enabled, however, coding characteristics of the impulse generator usually redevelop, i.e. reproducible discharge frequencies increasing with stimulus intensity, both in heat-responsive C-fibers (Dickhaus et al., 1976) and mechano-sensitive (tactile) Aβ-fibers (Sanders and Zimmermann, 1986). The erratic type discharges presumably elicit abnormal dysesthetic sensations regularly reported by neuropathic patients such as tingling, itching or electrifying.

3.1. Ionic channels in injured axons

Accumulation and increased membrane density of Na^+ channels have been detected histochemically in injured DRG axons, at sites proximal to the nerve transsection (Devor, 1994; England et al., 1996). These findings fit in with the increased excitability and spontaneous activity of DRG neurons following axonal injury, since Na^+ channels are the key elements of electrogenesis in the nervous system. Na^+ channels which can be blocked by tetrodotoxin (TTX) subserve impulse conduction along nerve axons, whereas the TTX-resistant subtypes are related to graded depolarizations in sensory receptors, i.e. the receptor potentials. The observation that systemic lidocaine silences ectopic neuroma and DRG discharges without blocking nerve conduction (Devor et al., 1992) suggests that TTX-resistant Na^+ channels can be blocked selectively by a local anesthetic at low concentration. Thus, the neuropathic pain inducing discharges of peripheral origin may well respond therapeutically to systemic local anesthetics.

At least six novel subtypes of Na^+ channels have recently been identified by mRNA analysis in DRG neurons (reviewed by Cummins et al., 2000). Part of these Na^+ channels is sensory neuron specific (SNS) and has not been found in other parts of the nervous system. The SNS-type Na^+ channels are mostly TTX-resistant. Subtypes SNS/PN3 and SNS/NaN, although downregulated following nerve injury, were found to be accumulated at sites of nerve injury in neuropathic humans and animals (Coward et al., 2000). On the other hand, the expression of subtype α-III Na^+ channels is increased following axotomy. This channel, not detectable in normal DRG neurons, shows faster recovery following inactivation and this can facilitate repetitive firing of injured neurons at low-threshold, which has been supposed as a mechanism of ectopic and erratic impulse generation. Knocking down SNS/PN3 but not NaN channel, gene expression with antisense oligodeoxynucleotides prevented hyperalgesia and allodynia following nerve injury in rats (Porecca et al., 1999). These Na^+ channel subtypes with their particular distribution to the peripheral somatosensory system are now considered targets for the development of novel analgesic drugs for neuropathic pain.

From growth cone research, it is known that Ca^{2+} entry into the nerve ending through Ca^{2+} channels regulates many functions including growth-related proteins. It is conceivable that Ca^{2+} channels develop also on regenerative nerve sprouts. Our research provides evidence that release of substance P from rat nerve neuroma depends on calcium (White and Zimmermann, 1988), and recently N- and L-type Ca^{2+} channels have been found to contribute to calcitonin gene-related peptide (CGRP) release from rat skin non-neuropathic nerve endings in vitro (Kress et al., 2001). However, no work has been devoted to the possible functional role of peripheral nerve Ca^{2+} channels or intra-/extracellular calcium concentration in neuropathic pain. Neither has the possible functional role of K^+ channels at the nerve sprouts been investigated, although K^+ channel opening may result in hyperpolarization or stabilization of membrane potential, and therefore, counteract the generation of ectopic discharges which induce neuropathic pain.

3.2. Chemosensitivity of nerve sprouts

Regenerating axonal sprouts develop chemosensitivity within days of the nerve injury. In the experiment shown in Fig. 2, a desheathed neuroma was placed in a chamber for superfusion with solutions containing various neuromediator substances (Zimmermann, 1985). Superfusion of the neuroma with bradykinin resulted in long-lasting impulse discharges in 26% of C-fibers, but not in A-fibers. The discharge frequency increased with the bradykinin concentration, the threshold being about 1 μM/l. In regenerating C-fibers, there is an early development of chemosensitivity to various substances, including bradykinin, histamine, serotonin, capsaicin and many other chemicals that are known to excite also normal nociceptors in skin or muscle (Leah et al., 1988; Hartung et al., 1989; Welk et al., 1990; Rivera et al., 2000).

When adrenaline was given to the superfusion fluid, no change in discharge rate occurred in these studies. However, the response to subsequently administered bradykinin was greatly enhanced (Fig. 2), up to 10 times the response without adrenaline (Zimmermann and Koschorke, 1987). Thus, in the regenerating C-fiber, an enormous sensitization of a chemosensory response occurs when adrenaline is present. This finding suggests that bradykinin receptors develop at the regenerating nerve terminals with either an adrenergic modulator site or an interaction with a closely associated adrenoceptor. Sensitization of the bradykinin response by adrenaline or sympathetic stimulation is not seen in normal C-fiber nociceptors, and adrenaline does not have excitatory effects on normal nociceptors, neither alone nor in combination with other chemicals.

3.3. Sympathetically maintained pain—concepts, controversies and confusions

As the neuroma contains both afferent C-fibers and efferent post-ganglionic sympathetic C-fibers which release noradrenaline and adrenaline, it seems that an enhanced sensitivity of the regenerating sprout towards the detection of nociceptive substances can be activated in situations of increased levels of sympathetic activity (Jänig, 1988). Interestingly enough, excitatory interactions of the

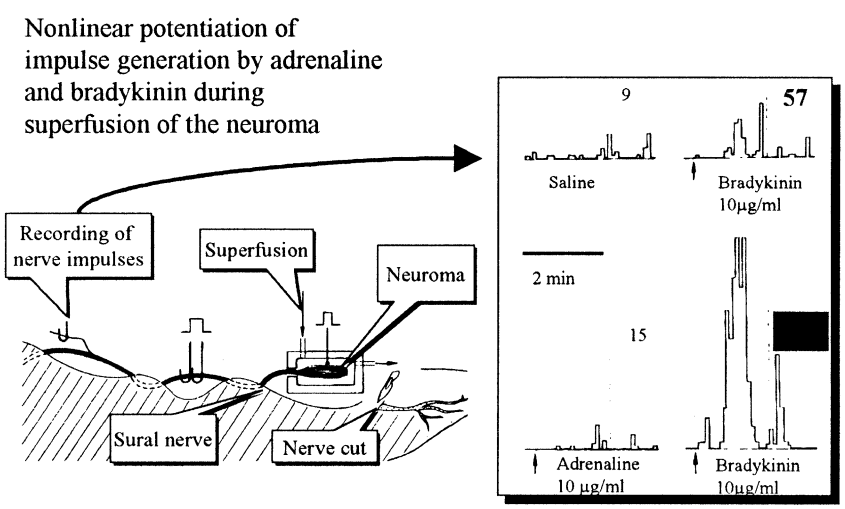


Fig. 2. Chemical excitation and sensitization of C-fiber sprouts in an experimental neuroma of the cat. As is shown in the schematic diagram (left), an experimental neuroma of the sural nerve was desheathed and placed in a perspex chamber for continuous superfusion. The neuroma was induced by ligation and transection of the nerve 2 weeks before. The nerve was dissected-free at two more proximal sites for electrical stimulation and recording from small filaments. Single-unit action potentials of A- and C-fibers could be identified in the electrical records from the filaments. The animal was deeply anesthesized for the nerve transection and for the superfusion experiment. Four records of a multi-unit discharge from a filament are displayed (right). The integrated discharge of all responding units are shown during superfusion with saline, bradykinin or adrenaline. Numbers given at each record give the total number of impulses recorded for 2 min up to the broken vertical line. Arrows mark the start of superfusion with the given substance.

sympathetic nervous system with afferent nociceptive neurons occur also in the dorsal root ganglion (Jänig and Schmidt, 1992; Bossut and Perl, 1995; Xie et al., 1995). This responsiveness to sympathetic transmitters may contribute to causalgia, the chronic pain of burning character seen in patients with trauma of the main nerve of a limb. Mitchell (1872) was the first to recognize the deleterious excitatory contribution of the sympathetic nervous system to causalgia.

There are other pathophysiological conditions, however, with which the sympathetic nervous system produces excitatory effects on nociceptive afferents (Bossut et al., 1996). The painful disorders originally subsumed under the terms "sympathetic reflex dystrophy" or "sympathetically maintained pain" have now been renamed as "complex regional pain syndrome—CRPS type I, II", because the involvement of mechanisms additional to the sympathetic nervous system has since been recognized, such as the hypophysical–adrenocortical system, neuro-immune interactions, neuropeptides, chronic inflammation and psychosomatically mediated mental and emotional influences. Years of research, controversies and the activities of a "special interest group" of the International Association for the Study of Pain (IASP) have ended up with the needs to reconsider and redefine an area of pain research and therapy, which has mushroomed much beyond the original contention of sympathico-afferent interactions (Harden, 2000; Stanton-Hicks, 2000).

3.4. Cytokines in neuropathic pain

Recent studies focussed on the role of cytokines in the pathophysiology of neuropathic pain, and the present evidence suggests that interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) both may be involved. Epineurial application of TNF-α elicited acute mechanical hyperalgesia in the awake rat (Sorkin and Doom, 2000), and neutralizing antibodies to TNF-α-receptor-1, not TNF-α-receptor-2, administered at the site of nerve injury reduced pain behavior in mice (Sommer et al., 1998). Etanercept, a recombinant TNF-α-receptor (p75)-Fc fusion protein successfully used for anti-inflammatory treatment of patients with rheumatoid arthritis, also reduced pain behavior in neuropathic mice, both with systemic and local administration to the nerve injury site (Sommer et al., 1999). Combined epineurial therapy with neutralizing antibodies to TNF-α and IL-1 receptors had an additive effect in reducing neuropathic pain in mice (Schäfers et al., in press). Nerve biopsies from patients with neuropathies revealed higher TNF-α immunoreactivities (IR) in myelinating Schwann cells when the neuropathy was painful, and serum soluble TNF-α-receptor 1 levels were higher in patients with a centrally mediated mechanical allodynia (Empl et al., 2001). Thus, central mechanisms of (brain derived?) TNF-α certainly is involved in the perception of neuropathic pain (Ignatowski et al., 1999).

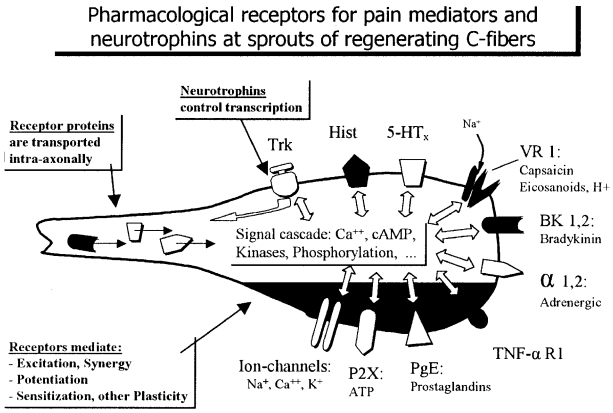


Fig. 3. Schematic diagram of a regenerating axonal sprout with those pharmacological receptors which are known to be expressed in the membrane of the axonal sprout. The receptor types are indicated by arbitrary symbols. Trk: tyrosine kinase receptor; Hist: histamine receptor; 5-HT_x: 5-hydroxytryptanine (serotonin)-receptor, *x* indicating the receptor variants; VR 1: vanilloid receptor-1; BK 1,2: bradykinin receptors 1 and 2; α 1,2: adrenoceptors α1, α2; TNF-α R1: tumor necroses factor-α receptor 1; PgE: prostaglandin receptor; P2X: purinergic 2 X-receptor. Double arrows indicate bidirectional interactions between receptors and second messenger systems, which mediate, e.g. phosphorylation of receptor and channel proteins, intracellular calcium release, receptor internalization and other mechanisms modulating receptor activity. The inset texts explain the system components and events/reactions, which develop within days to months following axonal injury.

These data suggest an involvement of TNF-α in neuropathic pain, with mechanisms in the peripheral and central nervous system. This cytokine plays a major role in many forms of nervous system damage and its contribution to pain may be a sequela of such damage, or a direct excitatory effect on the nociceptive system.

Fig. 3 summarizes the surface receptors and membrane channels which have been directly or indirectly identified at regenerative peripheral nerve sprouts following nerve injury. Basically, they are the same as those that occur in normal C-fiber terminals. The abnormal sensations they mediate and the widespread dysfunction they entail might depend on their lower density and immaturity. However, it is likely that intracellular processes such as 2nd messenger signal cascades, protein kinase-mediated receptor activation or transformation contribute to modifications of receptors, inducing sensitization and other abnormal functions at the axonal sprouts. Research is needed into these intracellular processes to understand what is going wrong and how the spreading out of neuropathic mechanisms can be prevented at their origin.

4. Distant spread of pathophysiology into peripheral and central nervous system following nerve injury

Recent research has uncovered cascades of events at the systemic, cellular and molecular levels following peripheral nerve injury which result in a slow but continual

propagation of a condition of pathophysiology into all parts of the injured neuron and the associated central nervous system. This change may reach the cortex where the somatosensory map related to the injured nerve's peripheral territory may become reorganized (Flor et al., 1995). This review will be restricted to changes in the primary neuron and the spinal cord. I will describe the sequence of pathobiological changes in short, with reference to Fig. 4, starting at the right hand side.

As was detailed above, sprouts of C-fibers develop an abnormal and erratic excitability. In particular, several pain mediators (e.g. bradykinin, prostaglandins, cytokines) and sympathetic nervous system transmitters are involved in these ectopic discharges. Normal nerve terminals take up signal substances (e.g. Nerve Growth Factor (NGF) and other growth factors) from their target cells, which are transmitted by axonal transport to the DRG cell body and modify gene transcription and protein synthesis. After nerve transection, sprouts can no longer take up the signal molecules from the previous target of this nerve cell. Deprivation of target-derived factors probably is part of the signal for a profound nerve cell body response that has been described long ago as a response to a distant axotomy (Cragg, 1970). At the level of transcription control in the DRG nucleus, we observe an induction of the c-*jun* gene about 1 day after axotomy. We know now that c-*jun* is an immediate early gene (IEG) and that its encoded protein, c-Jun, is part of the Activated Protein-1 (AP-1) promoter of transcription of many downstream genes (reviewed by Herdegen and Leah, 1998). Usually, the AP-1 gene induction is operated by a dimer of c-Fos and c-Jun, the proteins encoded by c-*fos* and c-*jun* genes. However, nerve transection does not induce c-*fos* gene expression in axotomized DRG neurons, which is a remarkable deviation from the common association of these genes in the nervous system. C-*jun* without c-*fos* is now considered a specific marker for an axonal lesion, and it is the starting event of a long-lasting cascade of transcriptional processes resulting either in axonal regeneration or in cell death.

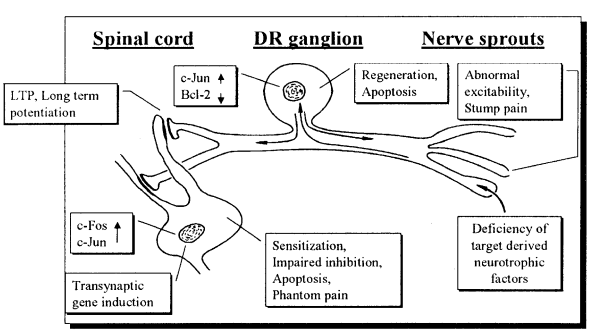


Fig. 4. Spreading cellular pathobiology involved in cell death, regeneration and pain following peripheral nerve transection. Viewing the sequence of events should start at sprouts of neuroma (right side) and then move to dorsal root (DR) ganglion neuron to postsynaptic spinal cord neuron. Important sequelae of the nerve transection are contained in inset texts. Composite diagram from data by Herdegen, Gillardon and Zimmermann 1989–1998.

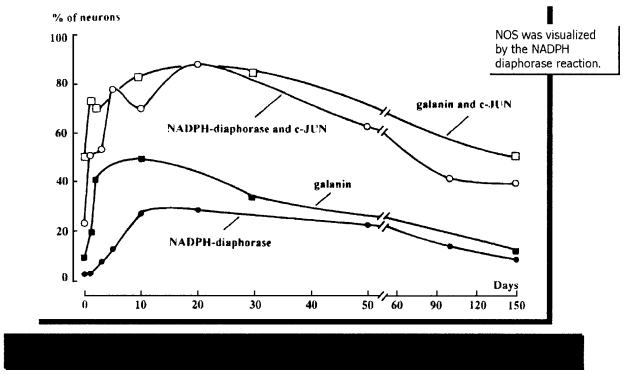


Fig. 5. Time course of galanin expression and NADPH-diaphorase enzyme reaction, and colocalization with nuclear c-Jun, in dorsal root ganglion neurons following sciatic nerve transection in rats. Lower curves (filled symbols) show percentage of neurons in L4 and L5 dorsal root ganglia labelled by galanin-immunoreactivity or NADPH-diaphorase, which is associated with nitric oxide synthase (NOS) content in the neuron. Upper curves (open symbols) show the percentages of Galanin or NADPH-diaphorase-labelled neurons also displaying nuclear c-Jun immunoreactivity (i.e. colocalization). Data from Fiallos-Estrada et al. (1993) and Herdegen et al. (1993).

It is now well established that c-Jun expression in the DRG neuron after nerve transection is associated with changes in neuropeptide contents: substance P and CGRP decrease, whereas galanin and NOS increase dramatically during the weeks and months following axotomy. NOS and galanin are highly colocalized with c-Jun in the same DRG neurons (Fig. 5) and this suggests that c-Jun is a transcription-controlling protein for the NOS and galanin genes. As NO is mostly an excitatory neuromediator, its increased production and release at the intraspinal presynaptic terminal may facilitate afferent synaptic transmission to the dorsal horn neurons, thus contributing to spinal neuronal sensitization and hyperalgesia. In addition, it is conceivable that the enhanced NO release into the spinal cord represents an injury signal by which the pathophysiological processes following nerve injury are carried over from the peripheral to the central nervous system. Galanin has inhibitory effects, its functional meaning in the nerve injury responses will be dealt with by Dr. Wiesenfeld-Hallin in this volume.

Electrophysiological studies provide direct evidence for enhanced synaptic transmission to dorsal horn neurons following nerve injury, with long-term potentiation (LTP) being one of the mechanisms involved (see below).

A neuropathic signal pattern, probably consisting of multiple abnormal electrical and biochemical components, induces expression of immediate early genes (IEGs) c-*fos*, c-*jun*, krox-24 in dorsal horn neurons with subsequent transcriptional alterations of downstream genes, as well as other processes of neuroplasticity. Particular functional sequelae of these cellular operations are neuronal sensitiza-

tion, apoptosis of dorsal horn neurons, impairment of spinal inhibitory, all of which contribute to neuropathic pain and dysesthesia.

5. Spinal plasticity and sensitization following peripheral nerve injury

There is ample evidence for sensitization of spinal dorsal horn cells and facilitation of spinal reflexes initiated by a repetitive or prolonged noxious afferent input. In particular, enhanced synaptic transmission is becoming evident by wind-up phenomena (Davies and Lodge, 1987) or long-term potentiation (LTP) (Liu and Sandkühler, 1995) following a short train of afferent stimulation at C-fiber strength. The latter authors recorded field potentials, i.e. compound postsynaptic potentials of dorsal horn neurons, in response to single volleys in sciatic C-fibers. A few seconds of tetanic electrical stimulation of the sciatic nerve at C-fiber strength resulted in a considerable increase of postsynaptic field potentials in responses to single C-fiber stimuli, reaching typically 200% of the control values before tetanic stimulation. This LTP persisted throughout the duration of the experiment under anesthesia for up to 8 h. The authors found that glutamate and tachykinin receptors were involved in the transition to LTP, and LTP could thus be prevented by administration of, e.g. NMDA and/or neurokinin 1 (NK 1) receptor antagonists.

Apart from electrical C-fiber stimulation, natural physiological and pathophysiological pain stimuli and prolonged pain conditions also resulted in LTP. In particular, LTP was observed following peripheral nerve lesions (Sandkühler and Liu, 1998). Such LTP mechanisms might be involved in the behavioral hyperalgesia observed in rats following nerve lesions.

Microelectrode studies have revealed functional details of spinal dorsal horn neurons of rats with a chronic constriction injury of the sciatic nerve (Laird and Bennett, 1993). Recordings were made about 10 days after inducing the neuropathy. Many neurons showed abnormal characteristics; these included responses to very gentle mechanical stimulation of the nerve injury site, absence of peripheral receptive fields and very high spontaneous activity. In some cases, the responses to mechanical stimulation at the nerve injury site lasted very long, e.g. 10 min. The authors conclude that dorsal horn neuronal sensitization and other mechanisms make a considerable contribution to the abnormal behavior of rats with a peripheral neuropathy.

5.1. Pain and nerve injury-induced transcriptional processes initiate and maintain long-term plasticity, a cellular pain memory

From the available body of evidence, it is conceivable that virtually every long-term change in nervous system function implies modified gene expression in nerve cells. At the DNA level, processes of transcription control can be assessed by studying the expression of immediate early genes (IEGs), the master switches of the transcription machinery (Fig. 6). Hunt et al. (1987) were the first to show by immunocytochemistry that expression of c-Fos protein occurs in the nuclei of dorsal horn neurons after noxious stimulation. This finding has repeatedly been confirmed and much extended since. More recently, it was shown that nociceptive input to spinal neurons also results in the expression of other IEGs, such as krox-24, c-*jun*, junB, junD, and fosB. The pronounced expression of a multitude of IEGs after noxious events suggests that induced gene transcription may be a mechanism involved in the long-term plasticity reported in the preceding and following sections.

What are the mediators in conditions of pain and neuropathy which have a potential to induce transcriptional processes? The best known candidate is glutamate, the universal excitatory transmitter in the central nervous system, and NMDA-receptors are the best to investigate to trigger intracellular signals that induce long-lasting effects at the transcriptional level. The coincidental activation of NMDA and neurokinin 1 (NK 1) receptors on dorsal horn neurons is particularly powerful, and NO also has a high potential to induce lasting changes in pre- and postsynaptic excitability. Prostaglandins, which have a newly discovered role in the transmission of nociceptive messages in the central nervous system, many cytokines released from inflammatory cells in the nervous system and some growth

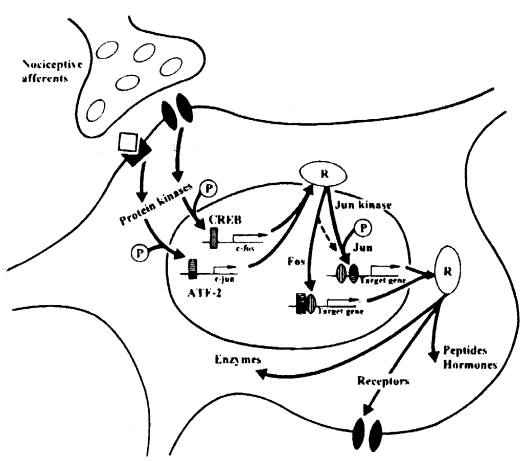


Fig. 6. Induced transcription of immediate-early genes c-*fos* and c-*jun* in spinal neurons following nociceptive stimulation or afferent nerve injury. An intracellular signal cascade is activated by postsynaptic receptors (e.g. NMDA and NK 1) that induces phosphorylation of transcription proteins CREB and/or ATF-2. These bind to promoter elements of the c-*fos* and c-*jun* genes. c-Fos and c-Jun proteins dimerize and bind to promoter or enhancer elements of other genes ("target genes"), which may include genes for various peptides, homones, receptors or enzymes. The transactivation of target genes by the inducible transcription factor proteins c-Fos and c-Jun involves phosphorylation, e.g. via Jun kinase. From Zimmermann and Herdegen (1996).

factors as well can contribute to the concerto of cellular activation in the pain pathway.

The intracellular signal cascade that finally reaches the nucleus include protein kinases (Fig. 6), in particular, protein kinase C (PKC) (Malmberg, 2000). Its catalytic domain interacts with a wide range of membrane proteins, including neurotransmitter subunits and transcription-controlling proteins such as CREB, conveying these into a phosphorylated state. Another essential signal molecule is the calcium ion released from intracellular stores as well as entering the cell by the NMDA-receptor-coupled ionophore and some voltage sensitive Ca channels. Constitutional transcription proteins CREB and ATF-2 become phosphorylated and bind to the promoters of the c-*fos* and c-*jun* genes, resulting in the synthesis of their gene products, the nuclear proteins c-Fos and c-Jun (the capital letters indicating proteins). These proteins dimerize to form the AP-1 complex that activates many downstream genes. Here, again, phosphorylation processes induced by kinases like MAP kinase and jun kinase (JNK) seem to determine the general direction of the gene activation. The functional ambivalence of c-*jun* activation has been mentioned above, with alternative potentials to support regeneration of a primary afferent neuron or to initiate apoptosis and kill the neuron. As far as we know, the decision between the two pathways depends to a large extent on whether or not, where and when phosphorylation by JNK takes place.

The protein products of IEGs induced by noxious afferent stimulation have also been found in the brain. The distribution pattern of these immunoreactivities (IR) in the CNS was mostly homologous to what is generally considered as the central pain system (cf. Fig. 2 in Zimmermann and Herdegen, 1994). In contrast, the dorsal column–medial lemniscal system, which represents the neuronal basis of tactile discrimination and proprioception does not normally express IEGs. After noxious stimulation, IEGs are also visible in nuclei of the limbic system, hypothalamic areas and hippocampus (Aloisi et al., 1997). It is conspicuous that basal IEG expression can be seen in many areas that show elevated levels of IEG expression following noxious peripheral stimulation. Thus, IEG-encoded proteins are not just markers for neuronal activity, they specifically indicate that processes have been activated at the nuclear level in selected neuronal populations which, according to our view, are meaningful in relation to long-term plasticity in the pain system.

Peripheral nerve injury can deeply change the inducibility of c-*fos* by afferent stimulation (Molander et al., 1992). These authors used electrical stimulation of the sciatic nerve before and after a distal transection of this nerve. When stimulation was confined to the large myelinated (Aαβ-)fibers in the sciatic nerve, no c-Fos expression was seen in the dorsal horn and gracile nucleus. In contrast, sciatic nerve stimulation at C-fiber strength resulted in c-Fos expression in dorsal horn neurons, as has been repeatedly shown by other authors. Thus, spinal neuronal c-Fos expression is a very common and general phenomenon following almost all types of noxious stimulation. Non-noxious stimulation, including electrical nerve stimulation at a Aα/β intensity, does not, however, result in c-Fos expression. This pattern is changed following a sciatic nerve lesion: strong c-Fos expression was observed in the dorsal horn and in the gracile nucleus following electrical stimulation at Aαβ intensity, which certainly is not noxious or painful normally (Molander et al., 1992).

These results may be related to the observation of allodynia in neuropathic animal models and in patients. Allodynia denotes pain sensations elicited by low-threshold mechanical stimuli, which are not painful in normal skin. Experimental and clinical evidence suggests that pain sensations in allodynia are due to impulses in large myelinated fibers (Aβ-fibers in a cutaneous nerve), which normally are not perceived as painful.

A straightforward interpretation of these observations is that Aβ-fibers have made new synaptic connections with neurons having been functionally connected predominantly with C-fibers before the nerve injury. Appropriate sprouting of Aβ-fibers into the superficial dorsal horn layers following peripheral nerve injury has been reported (Woolf et al., 1992). However, this explanation does not apply to the dorsal column neurons as these do not receive an appreciable C-fiber input. An alternative explanation can be found in the hypothesis that new discharge patterns emerge in response to Aβ-fiber stimulation in multi-receptive neurons of the spinal cord and at other levels of the somatosensory system. Such discharge patterns to Aβ-fiber input should thus mimic C-fiber-evoked responses, which typically consist of prolonged discharges to single electrical afferent stimuli and summation or wind-up phenomena during repeated low-threshold stimuli. This hypothesis should be directly tested in electrophysiological experiments on dorsal horn and dorsal column neurons in animals with a nerve transection. A conspicuously related clinical phenomenon has been observed long ago in patients with trigeminal neuralgia (Lindblom and Kugelberg, 1959).

6. Deficits in central inhibitory mechanisms following nerve injury

There is evidence that part of neuropathic pain and hyperalgesia is due to inefficiency of endogenous inhibitory systems. Here, we show that both the descending inhibition and the opioid system may become deficient at the spinal level.

The spinal pain transmission system is under continuous inhibitory control from brainstem centers, e.g. the periaqueductal gray and the locus coeruleus. The efficacy of such descending inhibitory controls can be assessed in animals by a reversible cold block of the descending pathway in the thoracic spinal cord: spinal dorsal horn

neuronal discharges go up to a maximum when released from tonic inhibition, the increase of a standard nociceptive response being a measure for the degree of inhibition. In this way, we have compared the degree of descending inhibition in anesthetized animals with and without a neuropathic lesion induced 2 weeks before (Zimmermann, 1991). We found that the descending inhibition was nearly 50% lower than normal in the neuropathic animals. Thus, although descending inhibitory controls in neuropathic conditions are still functioning, the inhibitory effect might have become weaker.

In other experiments, authors found an increase of tonic descending facilitation of dorsal horn neuronal transmission following a peripheral nerve lesion, the facilitatory descending neurons being located in the ventromedial medulla (Porreca et al., 2001).

6.1. The opioid system fails in neuropathic animals

A decreased efficacy of the spinal opioid system has been shown following peripheral nerve section. Terenius (1979) has reported that endorphin-like immunoreactivity in cerebrospinal fluid of pain patients was lower with neuropathic than with other pain mechanisms. In rats, the incidence of autotomy after sciatic nerve section was correlated with a decreased β-endorphin content in brain and spinal cord (Panerai et al., 1987). Opioid receptor binding was reduced in the spinal cord of the rat after a dorsal rhizotomy (Zajac et al., 1989). These biochemical results indicate that the endogenous opioid system shows a long-lasting decrease in efficacy in responses to peripheral nerve lesions.

These findings were corroborated by functional studies. Lombard and Besson (1989) found that in arthritic rats, the spontaneous activity of dorsal horn cells was suppressed to 46% of control by systemic morphine (2 mg/kg i.v.). In contrast, in rats deafferented by a multiple dorsal rhizotomy, the ongoing discharges of a sample of dorsal horn neurons were not inhibited by the same dose of morphine and an additional morphine dose of 4 mg/kg produced a small inhibitory effect only. These results suggest that the opioid inhibitory system was less sensitive in the deafferented animals compared to the arthritic animals. Similarly, the threshold dose of intrathecal morphine to inhibit a spinal nociceptive reflex was increased in animals showing autotomy after sciatic nerve section in rats (Xu et al., 1991).

A dramatic NMDA-receptor-dependent decrease in efficacy of spinal opioid analgesia was observed following nerve injury in rats (Mao et al., 1995). Before and after the transection of a sciatic nerve, the responsiveness of the spinal opioid system was assessed by measuring the paw-withdrawal-latency with respect to a noxious heat-stimulus (Fig. 6) and the analgesic effect of intrathecal administration of varying doses of morphine was determined. The effective dose ranged between 3 and 20 μg of morphine to obtain antinociception. When a nerve lesion was produced 8 days before the morphine-test, the dose–effect curve of morphine was shifted to higher doses by a factor of 6 (Fig. 6), indicating that the opioid receptors or other determinants of the spinal opioid system were greatly attenuated by the nerve lesion. It is, indeed, most interesting that this shift of the effective dose range could be prevented by pretreatment of the animals with MK-801, an NMDA-receptor antagonist. The authors explain these findings by uncoupling the μ-opioid-receptor from a G-protein and/or changing opioid receptor-gated ion channel activity, both of which are processes that require phosphorylation at various intracellular sites including an NMDA-receptor (Mayer et al., 1995).

7. Apoptosis of peripheral and spinal neurons in neuropathies

While the induction of c-*jun* with subsequent transcriptional activities has been associated with the propensity of axotomized neurons to survive after successful axonal regeneration (Herdegen and Leah, 1998), there is now evidence that c-*jun* may as well open the scene for programmed cell death such as apoptosis. From in vitro studies on sympathetic neurons, the induction of c-*jun* is closely associated with neuronal cell death induced by NGF starvation. Further insight into the mechanisms of cell death has come from studies on the bcl-2 gene family. Overexpression of bcl-2 protects neurons from cell death following axotomy and various other adverse stimuli indicating that Bcl-2 may block a late event in the cell death cycle, whereas Bax protein promotes cell death.

Therefore, we have studied the association of c-Jun expression following nerve transection with Bcl-2 and Bax. We found a decreased ratio of Bcl-2 to Bax proteins preferentially in small-sized DRG neurons expressing c-Jun (Gillardon et al., 1996). This finding indicates that c-Jun may initiate apoptosis of DRG neurons as well as regeneration. We know now that the differential for c-Jun to kill or regenerate injured neurons is related to the level of phosporylation by jun kinase (JNK): from work on brain ischemia, we learned essentially that phosphorylation of c-Jun will facilitate apoptosis, while non-phosphorylated c-Jun rather may support neuronal regeneration (Herdegen and Waetzig, 2001). Thus, part of DRG neurons will die after axonal injury, and this is of great relevance to the deafferentation of postsynaptic spinal neurons (see below).

Following peripheral nerve section, apoptosis was also discovered in dorsal horn neurons (Azkue et al., 1998). We used the TUNEL reaction to assess DNA fragmentation, a sign of apoptotic cell death (Fig. 7). Seven days after sciatic nerve transection, many TUNEL-labeled neurons were found in the L5 spinal segment which is the main input area of the sciatic nerve. DNA fragmentation in the

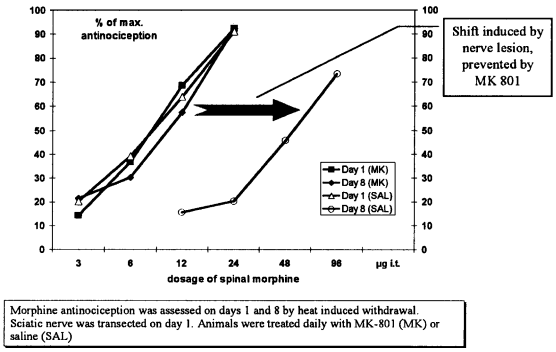


Fig. 7. Attenuation of antinociception by spinal morphine administration in rats before (Day 1) or after (Day 8) transection of the sciatic nerve. Antinociception by intrathecal morphine administration at varying dosages (abscissa) was assessed by the morphine's effect on a heat induced withdrawal reaction. On the ordinate, 0% = no suppression of heat induced withdrawal, 100% = full suppression. MK = data from animals daily injected with the NMDA receptor antagonist MK-801, SAL = daily injections with saline. Redrawn from Mao et al. (1995).

dorsal horn could be prevented by pretreatment of the animals with MK-801, a competitive NMDA-receptor antagonist. The prevention of cell death was virtually 100% when an infusion of MK-801 was performed throughout the time following the nerve lesion. The total number of TUNEL positive neurons in the L5 spinal segment was estimated at 6000, which is a considerable loss for a rat's spinal cord. We hypothesize that spinal neurons dying transsynaptically had inhibitory functions, which corroborates with the attenuation of morphine efficacy after sciatic nerve transection (see Fig. 8; Mao et al., 1995).

8. Mechanisms of postherpetic neuralgia

Postherpetic neuralgia (PHN) is a persistent chronic burning pain often associated with allodynia and/or hypoesthesia that may occur as a sequela of reactivation of latent varicella-zoster virus in dorsal root ganglia. While the disease is invariably painful in its acute phase during the skin rush, the incidence of PHN over months or years after the disease depends on several risk factors, including the age of the patient, the intensity of shingle eruption as well as the area of the affected skin (Zaal et al., 2000). It is clear now that many of the DRG neurons affected by the virus degenerate, most probably by the mechanism of apoptosis, as has been discussed for virtually all virus diseases involving the nervous system (Fazakerley and Allsopp, 2001). Although infection and latency of varicella virus have been studied in animals (Wroblewska et al., 1993), there is no animal model for PHN so far, but some findings are available from research on patients and animals which throw some light onto the pathophysiological mechanisms of this painful disorder.

Biopsy and pathological studies of human skin innervation following shingles have shown considerable to severe nerve degenerations and losses of neurons in related dorsal root ganglia. Some of these ganglia were cystic or contained inflammatory cells one year after the acute herpes, and the spinal dorsal horns were shrunken (Smith, 1978; Watson et al., 1991), indicating considerable degeneration in the spinal cord.

A recent investigation on biopsies from patient skin after shingles provided quantitative data on epidermal in-

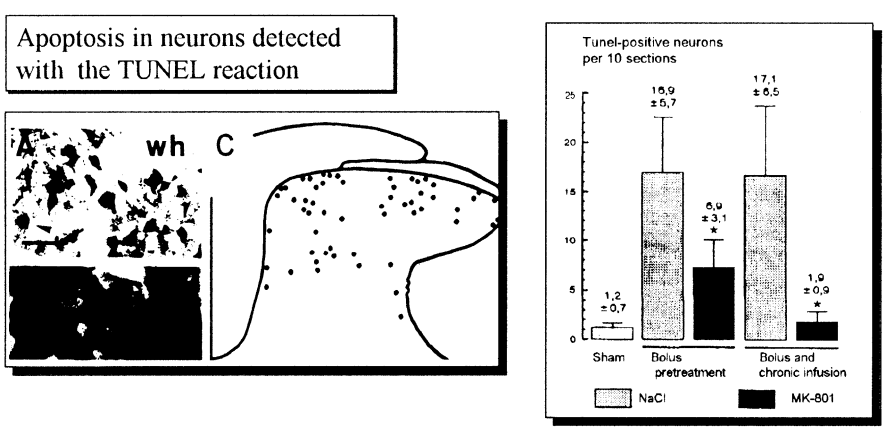


Fig. 8. Apoptosis of spinal dorsal horn neurons in L4 and L5 segments of rats following sciatic nerve transection. A and B sample dorsal horn histology sections with neurons labelled by the TUNEL reaction which specifically indicates DNA breaks which are characteristic for apoptosis, 7 days following transection of sciatic nerve. C, cumulative plot locating TUNEL labelled neurons in the dorsal horn ipsilateral to the sciatic nerve transection. Right-hand diagram shows numbers of TUNEL labelled dorsal horn neurons per 10 histological sections of spinal cord. Animals received either a bolus treatment with the NMDA antagonist MK-801 (1 mg/kg i.p.) 15 min prior to the sciatic nerve transection, or a bolus plus a continuous infusion (0.5 μg/kg h, implanted Alzet miniosmotic pump) of MK-801 for 1 week. Control animals received isotonic saline (NaCl). Sham = data of rats with surgery to expose the nerve but without nerve transection. From Azkue et al. (1998).

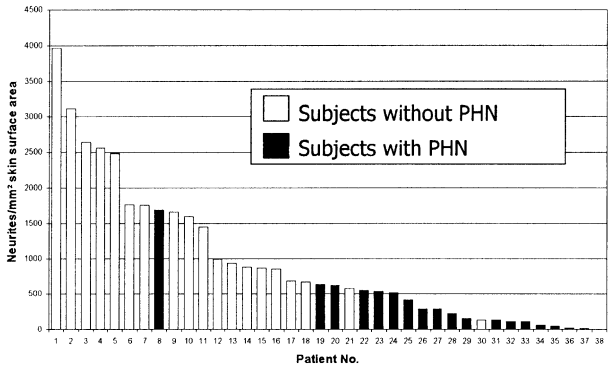


Fig. 9. Epidermal nerve density in human patients following herpes zoster disease. Skin biopses were taken from zoster affected skin and nerve fibers were counted and plotted per square millimeter skin surface area. White columns denote results from patients with no postherpetic neuralgia (PHN), black columns from patients with PHN. From Oaklander (2001).

nervation (Oaklander, 2001). The density of nerve endings in biopsies from the epidermis ranged between 2 and nearly 4000 neurites/mm^2 skin surface (Fig. 9), but there was a clear segregation between patients with and without PHN: the majority of subjects without PHN had more than 670 neurites/mm^2 skin surface area, whereas the majority of patients with PHN had less than 670 neurites/mm^2. These histological findings are in accordance with the clinical observation of diminished tactile sensitivity (hypo-aesthesia) in postherpetic skin: a more pronounced hypo-aesthesia is associated with a higher incidence of ongoing postherpetic neuralgia (Zaal et al., 2000).

As most of the nerve terminals in the epidermis are from non-myelinated nociceptive fibers, the loss of C-neurons is probably included in the nerve degeneration and this has been considered crucial for the development of PHN. That the decrease in epidermal innervation density indicates loss of C-fibers in PHN patients has been indirectly shown by measuring the axon reflex or neurogenic inflammation with laser doppler flowmetry (Baron and Saguer, 1993). These authors found an inverse correlation between the intensity of PHN (rated by the patients on a visual analog scale) and the degree of neurogenic inflammation elicited by a standard iontophoretic administration of histamine to induce substance P release from C-fiber terminals in the skin. Thus, degeneration of afferent C-fibers in skin as assessed by an absent or decreased neurogenic vasodilatation is highly predictive of PHN.

In order to explain allodynia, which is a common feature of PHN, it has been hypothesized that low-threshold Aβ-fibers sprout in the dorsal horn and form new synapses with neurons in the superficial dorsal horn previously connected with afferent C-fibers that have died from the virus attack (Baron, 2000). While such replacement sprouting has not been directly shown in PHN, other experimental nerve injury has been reported to trigger sprouting of myelinated afferents within the dorsal horn (Woolf et al., 1992).

Thus, it can be expected that any treatment that protects afferent neurons form cell death will prevent the occurrence of postherpetic neuralgia and allodynia. Clinical observations have repeatedly shown that aggressive early antiviral treatment decreases the risk of postherpetic neuralgia. In younger patients, a more efficient immune system has a better antiviral potential and, therefore, the risks of PHN pain are lower than with elderly patients.

9. Conclusions

Research over the past 15 years has resulted in a better understanding of neuropathic pain mechanisms. Modern concepts focus on the possibility that central sensitization is involved in virtually all types of neuropathy. Since much of the disease is due to delayed secondary pathobiology, early treatment is aimed at preventing progressive impairment and pain severity. Apoptosis of peripheral and central neurons following any primary nervous system damage is a novel mechanism, suggesting the development of neuroprotective strategies as a therapeutic principle to prevent chronic neuropathic pain.

10. Uncited references

Baron and Saguer, 1995
Ji and Woolf, 2001
Kress and Fickenscher, 2001
Wall and Gutnick, 1974

References

Abad, F., Feria, M., Boada, J., 1989. Chronic amitriptyline decreases autotomy following dorsal rhizotomy in rats. Neurosci. Lett. 99, 187–190.

Albe-Fessard, D., Lombard, M.C., 1983. Use of an animal model to evaluate the origin of and protection against deafferentation pain. In: Bonica, J.J., Lindblom, U., Iggo, A. (Eds.), 1983. Advances in Pain Research and Therapy, vol. 5. Raven Press, New York, pp. 691–700.

Aloisi, A.M., Zimmermann, M., Herdegen, T., 1997. Sex-dependent effects of formalin and restraint on c-*fos* expression in the septum and hippocampus on the rat. Neuroscience 81, 951–958.

Azkue, J.J., Zimmermann, M., Hsieh, T.F., Herdegen, T., 1998. Peripheral nerve insult induces NMDA-receptor-mediated, delayed degeneration in spinal neurons. Eur. J. Neurosci. 10, 2204–2206.

Baron, R., 2000. Peripheral neuropathic pain: from mechanisms to symptoms. Clin. J. Pain 16 (Suppl.), S12–S20, Review.

Baron, R., Saguer, M., 1993. Postherpetic neuralgia: are C-nociceptors involved in signalling and maintenance of tactile allodynia? Brain 116, 1477–1496.

Baron, R., Saguer, M., 1995. Mechanical allodynia in postherpetic neuralgia: evidence for central mechanisms depending on nociceptive C-fiber degeneration. Neurology 45, S63–S65.

Bennett, G.J., 1994a. Animal models of neuropathic pain. Proceedings of the 7th World Congress on Pain. In: Gebhart, G.F., Hammond, D.L., Jensen, D.S. (Eds.), 1994a. Progress in Pain Research and Management, vol. 2. IASP Press, Seattle, pp. 495–510.

Bennett, G.J., 1994b. Neuropathic pain. In: Wall, P.D., Melzack, R. (Eds.), Textbook of Pain. 3rd edn. Churchill Livingstone, Edinburgh, pp. 201–224.

Bennett, G.J., Xie, Y.-K., 1988. A peripheral mononeuropathie in rat that produces disorders of pain sensation like those seen in man. Pain 33, 87–107.

Berman, D., Rodin, B.E., 1982. The influence of housing condition on autotomy following dorsal rhizotomy in rats. Pain 13, 307–311.

Brinkhus, H.B., Zimmermann, M., 1983. Characteristics of spinal dorsal horn neurons after partial chronic deafferentation by dorsal root transection. Pain 15, 221–236.

Bossut, D.F., Perl, E.R., 1995. Effects of nerve injury on sympathetic excitation of A delta mechanical nociceptors. J. Neurophysiol. 73, 1721–1723.

Bossut, D.F., Shea, V.K., Perl, E.R., 1996. Sympathectomy induces adrenergic excitability of cutaneous C-fiber nociceptors. J. Neurophysiol. 75, 514–517.

Burton, A.W., Lee, D.H., Saab, C., Chung, J.M., 1999. Preemptive intrathecal ketamine injection produces a long-lasting decrease in neuropathic pain behaviors in a rat model. Reg. Anesth. Pain Med. 24, 208–213.

Carlton, S.M., Lekan, H.A., Kim, S.H., Chung, J.M., 1994. Behavioral manifestations of an experimental model for peripheral neuropathy produced by spinal nerve ligation in the primate. Pain 56, 155–166.

Christensen, D., Gautron, M., Guilbaud, G., Kayser, V., 2001. Effect of gabapentin and lamotrigine on mechanical allodynia-like behaviour in a rat model of trigeminal neuropathic pain. Pain 93, 147–153.

Chul Han, H., Hyun Lee, D., Mo Chung, J., 2000. Characteristics of ectopic discharges in a rat neuropathic pain model. Pain 84, 253–261.

Coderre, T.J., Grimes, R.W., Melzack, R., 1986. Deafferentation and chronic pain in animals: an evaluation of evidence suggesting autotomy is related to pain. Pain 26, 61–84.

Coward, K., Plumpton, C., Facer, P., Birch, R., Carlstedt, T., Tate, S., Bountra, C., Anand, P., 2000. Immunolocalization of SNS/PN3 and NaN/SNS2 sodium channels in human pain states. Pain 85, 41–50.

Cragg, B.G., 1970. What is the signal for chromatolysis? Brain Res. 23, 1–21.

Culp, W.J., Ochoa, J. (Eds.), Abnormal Nerves and Muscles as Impulse Generators. Oxford Univ. Press, Oxford.

Cummins, T.R., Dib-Hajj, S.D., Black, J.A., Waxman, S.G., 2000. Sodium channels and the molecular pathophysiology of pain. In: Sandkühler, J., Bromm, B., Gebhart, G.F. (Eds.), 2000. Progress in Brain Research, vol. 129. Elsevier, Amsterdam, pp. 3–19.

Davies, S.N., Lodge, D., 1987. Evidence for involvement of *N*-methyl-D-aspartate receptors in 'wind-up' of class 2 neurones in the dorsal horn of the rat. Brain Res. 424, 402–406.

Devor, M., 1994. The pathophysiology of damaged peripheral nerves. In: Wall, P.D., Melzack, R. (Eds.), Textbook of Pain. 3rd edn. Churchill Livingstone, Edinburgh, pp. 79–101.

Devor, M., Wall, P.D., Catalan, N., 1992. Systemic lidocaine silences ectopic neuroma and DRG discharge without blocking nerve conduction. Pain 48, 261–268.

Dickhaus, H., Zimmermann, M., Zotterman, Y., 1976. The development in regenerating cutaneous nerves of C-fibre receptors responding to noxious heating of the skin. In: Zotterman, Y. (Ed.), Sensory Functions of the Skin in Primates. Pergamon, Oxford, pp. 415–425.

Eaton, M.J., Martinez, M., Karmally, S., Lopez, T., Sagen, J., 2000. Initial characterization of the transplant of immortalized chromaffin cells for the attenuation of chronic neuropathic pain. Cell Transplant. 9, 637–656.

Empl, M., Renaud, S., Erne, B., Fuhr, P., Straube, A., Schaeren-Wiemers, N., Steck, A.J., 2001. TNF-alpha expression in painful and nonpainful neuropathies. Neurology 56, 1371–1377.

England, J.D., Happel, L.T., Kline, D.G., Gamboni, F., Thouron, C.L., Liu, Z.P., Levinson, S.R., 1996. Sodium channel accumulation in humans with painful neuromas. Neurology 47, 272–276.

Fazakerley, J.K., Allsopp, T.E., 2001. Programmed cell death in virus infections of the nervous system. In: Gosztonyi, G. (Ed.), The Mechanisms of Neuronal Damage in Virus Infections of the Nervous System. Current Topics in Microbiology and Immunology, vol. 253. Springer, Berlin, pp. 95–119.

Feria, M., Abad, F., Sanchez, A., Abreu, P., 1993. Magnesium sulphate injected subcutaneously suppresses autotomy in peripherally deafferented rats. Pain 53, 287–293.

Flor, H., Elbert, T., Knecht, S., Wienbruch, C., Pantev, C., Birbaumer, N., Larbig, W., Taub, E., 1995. Phantom-pain as a perceptual correlate of cortical reorganization following arm amputation. Nature 375, 482–484.

Fox, A., Kesingland, A., Gentry, C., McNair, K., Patel, S., Urban, L., James, I., 2001. The role of central and peripheral Cannabinoid1 receptors in the antihyperalgesic activity of cannabinoids in a model of neuropathic pain. Pain 92, 91–100.

Gillardon, F., Klimaschewski, L., Wickert, H., Krajewski, S., Reed, J.C., Zimmermann, M., 1996. Expression pattern of candidate cell death effector proteins Bax, Bcl-2, Bcl-X, and c-Jun in sensory and motor neurons following sciatic nerve transection in the rat. Brain Res. 739, 244–250.

Ginzburg, R., Seltzer, Z., 1990. Subarachnoid spinal cord transplantation of adrenal medulla suppresses chronic neuropathic pain behavior in rats. Brain Res. 523, 147–150.

Harden, R.N., 2000. A clinical approach to complex regional pain syndrome. Clin. J. Pain 16 (Suppl.), S26–S32, Review.

Hartung, M., Leah, J., Zimmermann, M., 1989. The excitation of cutaneous nerve endings in a neuroma by capsaicin. Brain Res. 499, 363–366.

Herdegen, T., Leah, J.D., 1998. Inducible and constitutive transcription factors in the mammalian nervous system: control of gene expression by Jun, Fos and Krox, and CREB/ATF proteins. Brain Res. Rev. 28, 370–490.

Herdegen, T., Waetzig, V., 2001. AP-1 proteins in the adult brain: facts and fiction about effectors of neuroprotection and neurodegeneration. Oncogene 20, 2424–2437.

Howe, J.F., Loeser, J.D., Calvin, W.H., 1977. Mechanosensitivity of dorsal root ganglia and chronically injured axons: a physiological basis for the radicular pain of nerve root compression. Pain 3, 25–41.

Ignatowski, T.A., Covey, W.C., Knight, P.R., Severin, C.M., Nickola, T.J., Spengler, R.N., 1999. Brain-derived TNFalpha mediates neuropathic pain. Brain Res. 841, 70–77.

Inbal, R., Devor, M., Tuchendler, O., Lieblich, I., 1980. Autotomy following nerve injury: genetic factors in the development of chronic pain. Pain 9, 327–337.

Jänig, W., 1988. Pathophysiology of nerve following mechanical injury. In: Dubner, R., Gebhart, G.F., Bond, M.R. (Eds.), Proc. Vth World Congr. Pain. Elsevier, Amsterdam, pp. 89–108.

Jänig, W., Schmidt, R.F. (Eds.), Reflex Sympathetic Dystrophy: Pathophysiological Mechanisms and Clinical Implications. VCH Verlagsgesellschaft, Weinheim.

Ji, R.-R., Woolf, C.J., 2001. Neuronal plasticity and signal transduction in nociceptive neurons: implications for the initiation and maintenance of pathological pain. Neurobiol. Dis. 8, 1–10.

Kauppila, T., 1998. Correlation between autotomy-behavior and current theories of neuropathic pain. Neurosci. Biobehav. Rev. 23, 111–129.

Kim, S.H., Chung, J.M., 1992. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. Pain 50, 355–363.

Kress, M., Fickenscher, H., 2001. Infection by human varicella-zoster virus confers norepinephrine sensitivity to sensory neurons from rat dorsal root ganglia. FASEB J. 15, 1037–1043.

Kress, M., Izydorczyk, I., Kuhn, A., 2001. N- and L- but not P/Q-type calcium channels contribute to neuropeptide release from rat skin in vitro. NeuroReport 12, 867–870.

Laird, J.M., Bennett, G.J., 1993. An electrophysiological study of dorsal

horn neurons in the spinal cord of rats with an experimental peripheral neuropathy. J. Neurophysiol. 69, 2072–2085.

Leah, J., Koschorke, G.-M., Welk, E., Zimmermann, M., 1988. The expression of sensory receptors on regenerating and regenerated cutaneous C fibres. In: Hamann, W., Iggo, A. (Eds.), Transduction and Cellular Mechanisms in Sensory Receptors. Progress in Brain Research, vol. 74. Elsevier, Amsterdam, pp. 341–348.

Lindblom, U., Kugelberg, 1959.

Liu, X.-G., Sandkühler, J., 1995. Long-term potentiation of C-fiber-evoked potentials in the rat spinal dorsal horn is prevented by spinal *N*-methyl-D-aspartic acid receptor blockage. Neurosci. Lett. 191, 43–46.

Lombard, M.-C., Besson, J.-M., 1989. Attempts to gauge the relative importance of pre- and postsynaptic effects of morphine on the transmission of noxious messages in the dorsal horn of the rat spinal cord. Pain 37, 335–345.

Lombard, M.-C., Nashold Jr., B.S., Albe-Fessard, D., Salman, N., Sakr, C., 1979. Deafferentation hypersensitivity in the rat after dorsal rhizotomy: a possible animal model of chronic pain. Pain 6, 163–174.

Malmberg, A.B., 2000. Protein kinase subtypes involved in injury-induced nociception. In: Sandkühler, J., Bromm, B., Gebhart, G.F. (Eds.), 2000. Progress in Brain Research, vol. 129. Elsevier, Amsterdam, pp. 51–59.

Mao, J., Price, C.D., Mayer, D.J., 1995. Experimental mononeuropathy reduces the antinociceptive effects of morphine: implications for common intracellular mechanisms involved in morphine tolerance and neuropathic pain. Pain 61, 353–364.

Mayer, D.J., Mao, J., Price, D.D., 1995. The development of morphine tolerance and dependence is associated with translocation of protein kinase C. Pain 61, 365–374.

Mitchell, S.W., 1872. Injuries to Nerves and their Consequences. Lippincott, Philadelphia.

Molander, C., Hongpaisan, J., Grant, G., 1992. Changing pattern of c-Fos expression in spinal cord neurons after electrical stimulation of the chronically injured sciatic nerve in the rat. Neuroscience 50, 223–236.

Oaklander, A.L., 2001. The density of remaining nerve endings in human skin with and without postherpetic neuralgia after shingles. Pain 92, 139–145.

Ossipov, M.H., Jerussi, T.P., Ren, K., Sun, H., Porreca, F., 2000. Differential effects of spinal (*R*)-ketoprofen and (*S*)-ketoprofen against signs of neuropathic pain and tonic nociception: evidence for a novel mechanism of action of (*R*)-ketoprofen against tactile allodynia. Pain 87, 193–199.

Panerai, A.E., Sacerdote, P., Brini, A., Bianchi, M., Mantegazza, P., 1987. Autotomy and central nervous system neuropeptides after section of the sciatic nerve in rats of different strains. Pharmacol., Biochem. Behav. 28, 385–388.

Porreca, F., Burgess, S.E., Gardell, L.R., Vanderah, T.W., Malan Jr., T.P., Ossipov, M.H., Lappi, D.A., Lai, J., 2001. Inhibition of neuropathic pain by selective ablation of brainstem medullary cells expressing the μ-opioid receptor. J. Neurosci. 15, 5281–5288.

Puke, M.J.C., Wiesenfeld-Hallin, Z., 1993. The differential effects of morphine and the alpha2-adrenoceptor agonists clonidine and dexmedetomidine on the prevention and treatment of experimental neuropathic pain. Anesth. Analg. 77, 104–109.

Rivera, L., Gallar, J., Pozo, M.A., Belmonte, C., 2000. Responses of nerve fibres of the rat saphenous nerve neuroma to mechanical and chemical stimulation: an in vitro study. J. Physiol. 527, 305–313.

Rodin, B.E., Kruger, L., 1984. Deafferentation in animals as a model for the study of pain: an alternative hypothesis. Brain Res. 319, 213–228.

Sanders, K.H., Zimmermann, M., 1986. Mechanoreceptors in rat glabrous skin: redevelopment of function after nerve crush. J. Neurophysiol. 55, 644–659.

Sandkühler, J., Liu, X.-G., 1998. Induction of long-term potentiation at spinal synapses by noxious stimulation or nerve injury. Eur. J. Neurosci. 10, 2476–2480.

Sato, J., Morimae, H., Takanari, K., Seino, Y., Okada, T., Suzuki, M., Mizumura, K., 2000. Effects of lowering ambient temperature on pain-related behaviors in a rat model of neuropathic pain. Exp. Brain Res. 133, 442–449.

Schäfers, M., Brinkhoff, J., Neukirchen, S., Marziniak, M., Sommer, C., 2001. Combined epineurial therapy with neutralizing antibodies to tumor necrosis factor-alpha and interleukin-1 receptor has an additive effect in reducing neuropathic pain in mice. Neurosci. Lett. (in press).

Seltzer, Z., Rapoport, Z.H., Zagzag, D., 1985. A chronically implanted delivery system of drugs to a nerve-end neuroma: effects on a behavioural chronic pain model. J. Neurosci. Methods 13, 223–229.

Seltzer, Z., Tal, M., Sharav, Y., 1989. Autotomy behavior in rats following peripheral deafferentation is suppressed by daily injections of amitriptyline, diazepam and saline. Pain 37, 245–250.

Seltzer, Z., Dubner, R., Shir, Y., 1990. A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury. Pain 43, 205–218.

Seltzer, Z., Beilin, B.Z., Ginzburg, R., Paran, Y., Shimko, T., 1991. The role of injury discharge in the induction of neuropathic pain behavior in rats. Pain 46, 327–336.

Seltzer, Z., Wu, T., Max, M.B., Diehl, S.R., 2001. Mapping a gene for neuropathic pain-related behavior following peripheral neurectomy in the mouse. Pain 93, 101–106.

Shir, Y., Sheth, R., Campbell, J.N., Raja, S.N., Seltzer, Z., 2001a. Soy-containing diet suppresses chronic neuropathic sensory disorders in rats. Anesth. Analg. 92, 1029–1034.

Shir, Y., Zeltser, R., Vatine, J.J., Carmi, G., Belfer, I., Zangen, A., Overstreet, D., Raber, P., Seltzer, Z., 2001b. Correlation of intact sensibility and neuropathic pain-related behaviors in eight inbred and outbred rat strains and selection lines. Pain 90, 75–82.

Smith, F.P., 1978. Pathological studies of spinal nerve ganglia in relation to intractable intercostal pain. Surg. Neurol. 10, 50–53.

Sommer, C., Schmidt, C., George, A., 1998. Hyperalgesia in experimental neuropathy is dependent on the TNF receptor 1. Exp. Neurol. 151, 138–142.

Sommer, C., Petrausch, S., Lindenlaub, T., Toyka, T.V., 1999. Neutralizing antibodies to interleukin 1-receptor reduce pain associated behavior in mice with experimental neuropathy. Neurosci. Lett. 270, 25–28.

Sorkin, L.S., Doom, C.M., 2000. Epineurial application of TNF elicits an acute mechanical hyperalgesia in the awake rat. J. Peripher. Nerv. Syst. 5, 96–100.

Stanton-Hicks, M., 2000. Complex regional pain syndrome (type I, RSD; type II, causalgia): controversies. Clin. J. Pain 16 (Suppl.), S33–S40, Review.

Suzuki, R., Matthews, E.A., Dickenson, A.H., 2001. Comparison of the effects of MK-801, ketamine and memantine on responses of spinal dorsal horn neurones in a rat model of mononeuropathy. Pain 91, 101–109.

Terenius, L., 1979. Endorphins in chronic pain. In: Bonica, J.J., Liebeskind, J.C., Albe-Fessard, D.G. (Eds.), Proceedings of the Second World Congress on Pain. Adv. Pain Res. Ther., vol. 3. Raven Press, New York, pp. 459–471.

Thomas, D.A., Ren, K., Besse, D., Ruda, M.A., Dubner, R., 1996. Application of nitric oxide synthase inhibitor, N^{ω}-nitro-L-arginine methyl ester, on injured nerve attenuates neuropathy-induced thermal hyperalgesia in rats. Neurosci. Lett. 210, 1–3.

Vatine, J.J., Devor, M., Belfer, I., Raber, P., Zeltser, R., Dolina, S., Seltzer, Z., 2000. Preoperative open field behavior predicts levels of neuropathic pain-related behavior in mice. Neurosci. Lett. 279, 141–144.

Wall, P.D., Gutnick, M., 1974. Properties of afferent nerve impulses originating from a neuroma. Nature 248, 740–743.

Wall, P.D., Devor, M., Inbal, R., Scadding, J.W., Schonfeld, D., Seltzer, Z., Tomkiewicz, M.M., 1979. Autotomy following peripheral nerve lesions: experimental anesthesia dolorosa. Pain 7, 103–111.

Watson, C.P.N., Deck, J.H., Morshead, C., van der Kooy, D., Evans, R.J., 1991. Postherpetic neuralgia: further post-mortem studies of cases with and without pain. Pain 44, 105–117.

Welk, E., Leah, J.D., Zimmermann, M., 1990. Characteristics of A- and C-fibers ending in a sensory nerve neuroma in the rat. J. Neurophysiol. 63, 759–766.

White, D.M., Zimmermann, M., 1988. The bradykinin-induced release of substance P from nerve fibre endings in the rat saphenous nerve neuroma is not related to electrophysiological excitation. Neurosci. Lett. 92, 108–113.

Wiesenfeld, Z., Hallin, R.G., 1980. Stress-related pain behaviour in rats with peripheral nerve injuries. Pain 8, 279–284.

Woolf, C.J., Shortland, P., Coggeshall, R.E., 1992. Peripheral nerve injury triggers central sprouting of myelinated afferents. Nature 355, 75–78.

Wroblewska, Z., Valyi-Nagy, T., Otte, J., Dillner, A., Jackson, A., Sole, D.P., Fraser, N.W., 1993. A mouse model for varicella-zoster latency. Microb. Pathog. 15, 141–151.

Xie, Y., Zhang, J., Petersen, M., LaMotte, R.H., 1995. Functional changes in dorsal root ganglion cells after chronic nerve constriction in the rat. J. Neurophysiol. 73, 1811–1820.

Xu, X.-J., Wiesenfeld-Hallin, Z., 1991. The threshold for the depressive effect of intrathecal morphine on the spinal nociceptive flexor reflex is increased during autotomy after sciatic nerve section in rats. Pain 46, 223–229.

Yu, W., Haoa, J.X., Xu, X.J., Saydoff, J., Sherman, S., Eriksson, A., Haegerstrand, A., Wiesenfeld-Hallin, Z., 1998. Immunoisolating encapsulation of intrathecally implanted bovine chromaffin cells prolongs their survival and produces anti-allodynic effect in spinally injured rats. Eur. J. Pain 2, 143–151.

Zaal, M.J.W., Völker-Dieben, H.J., D'Amaro, J., 2000. Risk and prognostic factors of postherpetic neuralgia and focal sensory denervation: a prospective evaluation in acute herpes zoster ophthalmicus. Clin. J. Pain 16, 345–351.

Zajac, J.M., Lombard, M.C., Peschanski, M., Besson, J.M., Roques, B.P., 1989. Autoradiographic study of mu and delta opioid binding sites and neutral endopeptidase-24.11 in rat after dorsal root rhizotomy. Brain Res. 477, 400–403.

Zimmermann, M., 1985. Functional characteristics of sensory fibres in regenerating cutaneous nerves. In: Delwaide, P.J., Gorio, A. (Eds.), Clinical Neurophysiology in Peripheral Neuropathies. Elsevier, Amsterdam, pp. 41–56.

Zimmermann, M., 1991. Central nervous mechanisms modulating pain-related information: do they become deficient after lesions of the peripheral or central nervous system? In: Casey, K.L. (Ed.), Pain and Central Nervous System Disease: The Central Pain Syndromes. Raven Press, New York, pp. 183–199, Review.

Zimmermann, M., Herdegen, T., 1994. Control of gene transcription by Jun and Fos proteins in the nervous system. Am. Pain Soc. J. 3, 33–48.

Zimmermann, M., Herdegen, T., 1996. Plasticity of the nervous system at the systemic, cellular and molecular levels: a mechanism of chronic pain and hyperalgesia. In: Carli, G., Zimmermann, M. (Eds.), Towards the Neurobiology of Chronic Pain. Progress in Brain Research, vol. 110. Elsevier, Amsterdam, pp. 233–259.

Zimmermann, M., Koschorke, G.M., 1987. Chemosensitivity of nerve sprouts in experimental neuroma of cutaneous nerves of the cat. In: Schmidt, R.F., Schaible, H.-G., Vahle-Hinz, C. (Eds.), Fine Afferent Nerve Fibers and Pain. VCH Verlagsgesellschaft, Weinheim, pp. 105–113.