Neurotrophins in spinal cord nociceptive pathways

Neurotrophins in spinal cord nociceptive pathways

Adalberto Merighi
Department of Veterinary Morphophysiology and Rita Levi-Montalcini Center for Brain Repair, Via Leonardo da Vinci 44, 10095 Grugliasco, Torino, Italy EU
Phone: ++390116709118; Fax: ++390112369118; e-mail: adalberto.merighi@unito.it

Giorgio Carmignoto
CNR Institute of Neuroscience and Department of Experimental Biomedical Sciences, Viale G. Colombo 3, 35121 Padova, Italy, EU
Phone: ++390498276075; Fax: ++390498276049; e-mail: gcarmi@bio.unipd.it

Sara Gobbo
Department of Experimental Biomedical Sciences, Viale G. Colombo 3, 35121 Padova, Italy, EU
Phone: ++390498276066; Fax: ++390498276049; e-mail: gcarmi@bio.unipd.it

Laura Lossi
Department of Veterinary Morphophysiology, Via Leonardo da Vinci 44, 10095 Grugliasco, Torino, Italy EU
Phone: ++390116709116; Fax: ++390112369138; e-mail: laura.lossi@unito.it

Chiara Salio
Department of Veterinary Morphophysiology, Via Leonardo da Vinci 44, 10095 Grugliasco, Torino, Italy EU
Phone: ++390116709114 Fax: ++390112369138; e-mail: chiara.salio@unito.it

Angela Maria Vergnano
Department of Veterinary Morphophysiology, Via Leonardo da Vinci 44, 10095 Grugliasco, Torino, Italy EU
Phone: ++390116709114 Fax: ++390112369138; e-mail: angela.vergnano@unito.it

Michaela Zonta
Department of Experimental Biomedical Sciences, Viale G. Colombo 3, 35121 Padova, Italy, EU
Phone: ++390498276066; Fax: ++390498276049; e-mail: gcarmi@bio.unipd.it

Abstract

Neurotrophins are a well-known family of growth factors for the central and peripheral nervous systems. In the course of the last years, several lines of evidence converged to indicate that some members of the family, particularly NGF and BDNF, also participate in structural and functional plasticity of nociceptive pathways within the dorsal root ganglia and spinal cord. A subpopulation of small-sized dorsal root ganglion neurons is sensitive to NGF and responds to peripheral NGF stimulation with up-regulation of BDNF synthesis and increased anterograde transport to the dorsal horn. In the latter, release of BDNF appears to modulate or even mediate nociceptive sensory inputs and pain hypersensitivity. We summarize here the status of the art on the role of neurotrophins in nociceptive pathways, with special emphasis on short-term synaptic and intracellular events that are mediated by this novel class of neuromessengers in the dorsal horn. Under this perspective we review the findings obtained through an array of techniques in naive and transgenic animals that provide insight into the modulatory mechanisms of BDNF at central synapses. We also report on the results obtained after immunocytochemistry, in situ hybridization, and monitoring intracellular calcium levels by confocal microscopy, that led to hypothesize that also NGF might have a direct central effect in pain modulation. Although it is unclear whether or not NGF may be released at dorsal horn endings of certain nociceptors in vivo, we believe that these findings offer a clue for further studies aiming to elucid ate the putative central effects of NGF and other neurotrophins in nociceptive pathways.

Pain is the perception of an aversive or unpleasant sensation originating from a given region of the body and/or viscera. The International Association for the Study of Pain has given the following definition: *Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage*. Therefore, pain is highly subjective and comprises affective (emotional) and sensory discriminative components, involving integration and elaboration of peripheral inputs conveyed to different areas of the central nervous system (CNS) along to the so called nociceptive pathways. These latter are initially activated by a specific subset of peripheral sensory receptors, the nociceptors, that provide information about tissue damage from somatic or visceral structures. Noxious stimuli encoded by nociceptors are first conveyed to the dorsal horn (DH) of the spinal cord or the main spinal nucleus of the trigeminal nerve, then they travel to supraspinal relay centers, such as the thalamus, and finally reach the corticolimbic structures where they are perceived as pain.

We will give here an account of the current knowledge about the role of neurotrophins (NTs) in the primary processing of noxious stimuli in the spinal cord DH. Before doing so we will briefly mention some general features of the anatomical, physiological, and neurochemical organization of primary afferent pathways, to put things under the right perspective.

Organization of the primary afferent pathways in the spinal cord

The organization and physiological properties of primary afferent pathways in the spinal cord have been extensively and authoritatively reviewed elsewhere (Ruda et al., 1986; Willis, Jr. and Coggeshall, 1991; Todd and Spike, 1993; Ribeiro-Da-

Silva, 1994; Schadrack and Zieglgansberger, 1998; Millan, 1999; Alvares and Fitzgerald, 1999; Dubner and Ren, 1999; Petersen-Zeitz and Basbaum, 1999; McHugh and McHugh, 2000; Burnstock, 2000; Herrero et al., 2000; Gerber et al., 2000a; Willis, 2001; Hunt and Mantyh, 2001; Ji and Woolf, 2001; Morisset et al., 2001; Mendell and Arvanian, 2002; Willis, 2002), and cutaneous primary afferent fibers (PAFs) have been studied in deeper detail. Therefore, the following short description will be mainly based upon the data available for this type of fibers.

General properties of PAFs

According to their diameter, structure, and functional properties, cutaneous PAFs can be grouped into three categories: *i.* C fibers, that are thin, unmyelinated and display slow-conducting velocity; *ii.* Ad fibers, that are of intermediate diameter, scarcely myelinated and have intermediate conduction velocity; and *iii.* Aß fibers, that are of large size, myelinated and display fast conduction velocity. In the skin these three categories of fibers are typically present in proportions of about 70, 10 and 20% respectively, although some variations may occur. All types of cutaneous afferents can trans mit non –nociceptive information, but, under normal circumstances, only C and Ad fibers transmit nociceptive information. C and Ad fibers originate from small size type B neurons in the dorsal root ganglia (DRGs) and reach the spinal cord via the dorsal roots, although unmyelinated fibers originating from the DRGs are present in the ventral root as well.

In general terms, upon exposure of the skin to a noxious stimulus, Ad fibers elicit a first rapid phase of *sharp* pain, whereas C fibers evoke a second wave of *dull* pain (Treede et al., 1992; Millan, 1999). There are multiple classes of both C and Ad fibers, and their characterization is somewhat complicated by the existence of interspecies differences, heterogeneity in the properties of these fibers in different

tissues, and terminological ambiguities. We will try to simplify this issue as follows. C fibers comprise: *i.* non nociceptive thermoreceptors; *ii.* low threshold mechanoceptors responsive to pressure, and *iii.* nociceptors (Willis, 1991; Treede et al., 1992). The C fiber nociceptors represent a heterogeneous group of receptors among which are comprised chemoreceptors, high threshold thermoreceptors and mechanoreceptors. Some of these may be specifically activated by chemical irritants, heath or mechanical stimuli, but others may respond to different types of stimuli and are thus referred to as polymodal or CMH (chemical, mechanical, heath) nociceptors. Polymodal nociresponsive C fibers have a crucial role in the sensitization of spinal neurons, a process underlying chronic painful states.

Ad fibers comprise two classes of mechanoreceptors: one is normally activated by high intensity mechanical stimuli above the noxious threshold, while the other displays a lower threshold (Treede et al., 1992; Beydoun et al., 1996, 1997). Members of the fist class are usually referred to as Type I mechanoreceptors. These high threshold rapidly conducting receptors are, under normal conditions, weakly responsive to high intensity heat cold and chemical stimuli. However, upon repetitive thermal stimulation these fiber may become sensitized, and in the presence of tissue damage, they respond to heat with sustained responses of long duration and slow latency.

The Type II Ad mechanoreceptors have been primarily described in the primate hairy skin. They display a lower threshold to noxious heat stimuli than the corresponding Type I fibers (Treede et al., 1992; Beydoun et al., 1996, 1997). Finally there are some Ad fibers more responsive to cooling than mechanical stimuli (Simone and Kajander, 1996, 1997).

Aß cutaneous fibers originate from large-sized type A DRG neurons and they also reach the spinal cord via the dorsal roots. In the absence of tissue or nerve injury Aß fibers respond only to touch, vibration, pressure and other modes on non-noxious, low intensity mechanical stimulation of the skin (Willis and Coggeshall, 1991; Mense, 1993; Woolf et al., 1994b).

Cutaneous nociceptors can encode the intensity of no xious thermal and mechanical stimuli and the spatial localization of noxious stimuli (Treede et al., 1992; Dubner and Ren, 1999). This indicates the existence of central mechanisms of spatial and temporal summation of pain signaling (Vierck, et al., 1997). However, the prompt response of nociceptors to noxious stimuli underlies the so called *alerting role of acute pain*, and very likely occurs with little modulation at peripheral or central levels (Handwerker and Kobal, 1993). Thus, in the absence of tissue damage, repetitive noxious stimulation may be associated with a decreased response of some polymodal C fibers, a form of receptor adaptation observed in other sensory modalities. However, when tissue damage occurs nociceptor adaptation is largely outweighed by central and peripheral mechanisms of sensitization.

Pain originating in organs other than the skin displays some distinguishing features with respect to those outlined above: first, the distinction of a sharp and dull phase of pain perception is not obvious; second, deep somatic and visceral pain is often associated with a pronounced autonomic component in which hypotension, nausea, sweating and other clinical signs related to stimulation of sympathetic and parasympathetic pathways may become apparent; and third, deep and visceral pain is often diffuse, i.e. it may be difficult to exactly localize its source, and it is frequently subjectively referred to other intact tissues.

Nociceptive fibers in muscles are free nerve endings distributed within the connective tissue between individual muscle fibers and investing fascial envelopes. They are small- sized group III myelinated fibers (equivalent to cutaneous Ad fibers) and group IV unmyelinated fibers (equivalent to C fibers). The first are activated by mechanical stimuli and respond to muscle stretch and contraction, besides to non-noxious pressure. However, about one third of them are nociceptors, typically being activated by hypoxia and/or ishaemia, and localized noxious rise in pressure (Hoheisel et al., 1993; Mense, 1993; Marchettini et al., 1996, 2000). Group III fibers can be sensitized by thermal and chemical stimuli. Group IV fibers share several characteristics of group III and about 50% of them are nociceptive.

Nociceptive fibers in joints mainly consist of group III and IV free nerve endings and a small subset of large corpuscolar myelinated Ad-like fibers. These nociceptors respond to anomalous mechanical solicitation of joint beyond the normal range of movement.

Nociceptive information from the thoracic and upper abdominal viscera reaches the CNS via the sympathetic chains, whereas that from the lower gut and bladder follows the route of pelvic parasympathetic nerves. There is evidence for the existence of polymodal C and Ad fibers in the hart and gut (Cervero and Connell, 1984; Meller and Gebhart, 1994; Cervero, 1995; Gebhart, 1995; Blackshaw and Gebhart, 2002). Genuine Aß fibers are absent in viscera, suggesting that non-noxious information in viscera must be transmitted by Ad and C fibers. Therefore, according to the pattern theory, visceral pain is intensity-encoded by non-specific fibers likewise responsive to innocuous stimulation (Handwerker and Kobal, 1993; Cervero, 1995).

Termination of PAFs in the spinal gray matter

In thick sections, the gray matter of the spinal cord has a cytoarchitectonic laminar organization in which 10 different laminae have been recognized (Rexed, 1954). Lamina II, the substantia gelatinosa Rolandi, is further subdivided into an outer (II_o) and an inner (II_i) portion.

The pattern of termination of PAF in the cord is mainly ipsilateral, although the presence of contralateral projections has been reported by several investigators, and tract-tracing studies have demonstrated the existence of preferential laminar terminations for different types of PAFs. Moreover, in the dorsal root entry zone close to the surface of the spinal cord, PAFs originating from different peripheral territories become segregated into medial and lateral streams. The medial stream comprises the large-sized Aß fibers which divide within the dorsal funiculus into ascending and descending branches. These latter swing into the gray matter and make synapses in laminae II-VI of the DH and lamina X, the region surrounding the central canal. The largest ascending branches run all the way to the dorsal column nuclei in the medulla oblongata. The lateral stream comprises Ad and C fibers, which, upon entry, divide into short ascending and descending branches within the dorsolateral tract of Lissauer. The longitudinal projections of Ad and C fibers extend along several consecutive segments of the cord (Willis and Coggeshall, 1991). Non-nociceptive Ad fibers terminate primarily in laminae II_i and III, whereas nociceptive Ad fibers terminate in laminae I, II_o, V and X.

C fibers terminate in laminae I-II and X.

In the superficial DH three main types of PAF terminal configurations have been defined. In two of these, a PAF terminal forms the core of a multisynaptic

complex commonly referred to as a synaptic glomerulus. The third type of synaptic configuration made by PAF is a conventional axo-dendritic synapse, which is however particularly enriched in dense-cored, large granular vesicles (LGVs) which are known to contain one or more neuropeptides, and are thus also referred to as P-type vesicles (Merighi, 2002). Synaptic glomeruli are mostly peculiar to lamina II. There are two different types of glomeruli (type I and type II) with different and characteristic ultrastructural and neurochemical features. It is now clearly demonstrated that the central terminal of type I glomeruli originates from a C fiber, whereas the terminals of Ad fibers are engaged in type II glomeruli (Valtschanoff et al., 1994; Ribeiro-Da-Silva, 1994).

Neurochemistry of nociceptive PAFs

The issue of the neurochemical characterization of nociceptive PAFs is a complex one since differences are expected to derive from: *i.* the existence of heterogeneous functional types of PAF (as outlined above), i.e. C fibers compared to Ad fibers; *ii.* the different tissue targets of innervation, although this point needs further clarification; and *iii.* the phenotypic switch that occurs between the normal intact state versus inflammation and/or tissue/nerve injury.

In general terms, PAF contain and release a cocktail of biologically active molecules rather than a single neuromessenger. This seems to be a general rule rather than an exception since the costorage and coexistence of multiple messengers in neurons is a widespread phenomenon throughout the CNS and PNS (Lundberg, 1996; Hökfelt et al., 2000; Merighi, 2002). DRG cells are unipolar neurons giving rise to a common stem axon that immediately bifurcates into a centrifugal process reaching the peripheral tissues, and a centripetal process that

enters the spinal cord. Although some caution should be exercised (Merighi, 2002), one expects that the same combination of messengers is found at central and peripheral endings of these neurons. A combination of excitatory amino acids (EAAs) and peptides is usually found in small- to medium-sized DRG neurons that give rise to nociceptive C and Ad fibers (Hökfelt, 1991; Lundberg, 1996; Hökfelt et al., 2000; Merighi, 2002).

Glutamate was one of the first neurotransmitter proposed for PAFs (Johnson, 1972; Hutchinson et al., 1978), and nowadays the role of this amino acid as a major excitatory neurotransmitter in the spinal cord (and throughout the CNS) is fully established. The availability of antisera directed against amino acids fixed in tissue sections has made possible the visualization of glutamate (and other amino acids) in specific neurons and pathways that use them as neurotransmitters (Ottersen and Störm-Mathisen, 1984; Ottersen and Störm-Mathisen, 1987). Light immunocytochemical studies revealed that glutamate is detected in both type A (large) and B (medium and small) DRG neurons and in a rich plexus of fibers in the superficial laminae of the DH (Battaglia and Rustioni, 1988; Rustioni and Weinberg, 1989). At the ultrastructural level, glutamate immunoreactivity was localized in terminals which formed the core of type I and II glomeruli or were engaged in simple axo-dendritic synapses (De Biasi and Rustioni, 1988; Maxwell et al., 1990; Merighi et al., 1991; Valtschanoff et al., 1994). At the subcellular level staining was selectively localized to small, clear, round synaptic vesicles (Merighi et al., 1991; Valtschanoff et al., 1994). Aspartate has also been implicated as a putative neurotransmitter of PAFs (Rustioni and Weinberg, 1989). This is of relevance since aspartate has been demonstrated to be a selective agonist of the N-methyl-D-aspartate (NMDA) glutamate receptor (Curras and

Dingledine, 1992). Glutamate and aspartate activate both metabotropic (mGlu) receptors coupled via G-proteins to soluble second messengers, and ionotropic receptors directly coupled to cation-permeable ion channels. All these receptors show a complex pattern of localization in different neuronal types of the DH. Three major groups of mGlu receptors have been recognized (Conn and Pin, 1997; Valerio et al., 1997): group I receptors ($mGlu_1$ and $mGlu_5$) are localized to the superficial laminae of the DH and positively coupled to phospholipase C (PLC), and possibly nitric oxide synthase (NOS), whereas group II ($mGlu_2$ and $mGlu_3$) and III ($mGlu_4$, $mGlu_6$ and $mGlu_8$) are negatively coupled to adenyl cyclase (AC). Specific receptor types differentially alter intracellular calcium concentration ($[Ca^{2+}]_i$) and transduction mechanisms, thereby modulating neuronal excitability by, for example, ion channel/receptor phosphorylation and modulation of gene transcription.

DL-a-NH_2-2,3,-dihydro-5-methyl-3-oxo-4-isoxazolepropanoic acid (AMPA) receptor isoforms are made of several subunits, among which the Glu R2 plays a major role in controlling Ca^{2+} permeability and conductance. The different isoforms have specific patterns of distribution in the spinal cord (Popratiloff et al., 1998; Albuquerque et al., 1999; Engelman et al., 1999; Akesson et al., 2000; Lu et al., 2002). The conductance of AMPA receptors localized on DH nociresponsive neurons is predominantly to Na^+, and these receptors show low affinity for glutamate (compared to NMDA), low voltage-dependence, rapid kinetics and desensitization upon selective stimulation (Stanfa and Dickenson, 1999; Li et al., 1999a; Szekely et al., 2002). NMDA receptors are composed of heteromultimeric subunits and can be classified into several different types differentially distributed throughout the CNS. In the DH the NMDA R_1 is the

predominant form (Tölle et al., 1993; Bardoni et al., 2000; Bardoni, 2001). All classes of NMDA receptors display slow channel kinetics, a variable degree of voltage-dependent Mg^{++} block, and marked permeability to Ca^{2++}.

Neuropeptides are highly concentrated in the superficial DH and lamina X. An impressive number of immunocytochemical studies in the last decades provided a detailed mapping of their distribution. For the purpose of the present work we will restrict our discussion to the tackykinins and the calcitonin gene-related peptide (CGRP) that coexist with EAAs in at least a subset of PAF terminals (Merighi, 2002).

The tackykinins are a family of structurally related peptides derived from two precursor proteins (Helke et al., 1990). The most widely known member of the family is substance P (SP) which has been implicated in nociceptive neurotransmission since the early 1950s (Lembeck, 1953). Other members of the family include neurokinin A (NKA), neurokinin B (NKB), and neuropeptide K (Helke et al., 1990). Light and electron microscopy distribution studies have demonstrated that: *i.* SP and NKA are widely distributed in PAF terminals in laminae I-III, and X of the spinal cord (Ruda et al., 1986), although the existence of intrinsic spinal neurons containing SP is also widely recognized (Rustioni and Weinberg, 1989; Todd and Spike, 1993; Ribeiro-Da-Silva, 1994); *ii.* they coexist with EAAs in single PAF terminals where they are segregated in LGVs; and *iii.* they may be costored together and/or with other peptides, namely CGRP, in individual LGVs (Merighi, 2002).

Three different G protein –coupled receptors na med NK_1, NK_2 and NK_3 have been so far described in mammals with maximal affinity for SP, NKA, and NKB respectively (Regoli et al., 1994). Nonetheless, NKA may also activate NK_1

receptors, and SP NK_2 sites. Both NK_1 and NK_2 receptors are positively coupled with PLC (Bentley and Gent, 1995; Mantyh et al., 1997). NK_1 receptor and its mRNA are widely distributed in the DH (Nakaya et al., 1994; Bleazard et al., 1994; Liu et al., 1994a; Brown et al., 1995) where they are present in numerous cell bodies mainly localized in laminae I and III, but only a few in lamina II. The pronociceptive peptide CGRP is also particularly abundant in sensory pathways, where the predominant form is aCGRP (Amara et al., 1985). As mentioned above, in PAF terminals CGRP is often costored with SP (or NKA) within individual LGVs. CGRP acts on at least two different types of receptors named $CGRP_1$ and $CGRP_2$ which are both coupled to AC (Wimalawansa et al., 1993; Wimalawansa, 1996). The mechanism of action of CGRP at central PAF endings in the DH remains to be elucidated.

Functional interactions of EAAs, SP and CGRP

The cooperative effects of glutamate, SP and CGRP at central and peripheral endings of nociceptors have been the object of extensive studies.

At central endings, glutamate regulates nociceptive neurotransmission by both pre and postsynaptic mechanisms. NMDA and different types of mGlu receptors have been localized to DH neurons (i.e. postsynaptically to PAFs) as well as to PAFs (i.e. presynaptically). Activation of presynaptic NMDA receptors exerts a feedback positive action on the release of EAAs and SP from PAF terminals, thereby enhancing synaptic transmission (Liu et al., 1994d; Liu and Sandkuhler, 1998; Boyce et al., 1999; Lu et al., 2003). On the other hand the role of individual mGlu receptor subtypes presynaptically localized to PAF terminals remains to be elucidated, since they might either increase or decrease synaptic release in relation

to their intracellular signal transduction mechanisms. At present, evidence for an inhibitory role of group II and III mGlu receptors has been provided (Zhong et al., 2000; Gerber et al., 2000b; Zhou et al., 2001).

High- intensity stimulation of PAFs produces a fast, AMPA/kainate-receptor-mediated EPSC in the superficial laminae of the DH. Activation of low-threshold afferent fibres generates typical AMPA-receptor- mediated excitatory postsynaptic spontaneous potentials (EPSCs) only, indicating that kainate receptors may be restricted to synapses formed by high-threshold nociceptive and thermoreceptive PAFs (Li et al., 1999b). Although the histological localization of kainate receptors remains uncertain, it seems likely that at least part of them is located on PAF terminals (Kerchner et al., 2002), and that their desensitization reduces the mechanical allodynia and thermal hyperalgesia that follows PAF injury (Zhou et al., 1997; Kerchner et al., 2001b). Presynaptic kainate receptors are also linked to changes in GABA/glycine release from spinal cord interneurons (Kerchner et al., 2001a).

Similarly to glutamate, SP is likely to act both pre and postsynaptically onto NK_1 receptors at synapses between PAF terminals and DH neurons (Routh and Helke, 1995; Heppenstall and Fleetwood-Walker, 1997; Todd, 2002).

The cooperative action of glutamate, tackykinins and CGRP on DH neurons follows a well established temporal pattern, and their respective roles depend upon the nature and duration of noxious stimulation. Initial triggering is provided by AMPA receptors that display extremely rapid kinetics, and their activation mediates a rapid depolarization of DH neurons over a few msec. Slower and more sustained EPSPs lasting up to 10 sec are consequent to activation of NMDA, group I mGlu, and NK_1 (NK_2 and CGRP) receptors.

At peripheral endings, antidromic action potentials (related to the efferent function of nociceptive PAFs) may be triggered in collateral fibers giving rise to the so called *axon reflex*. This leads to release of EAAs, SP, CGRP that, together with other mediators, enhance nociceptive transmission by a feedback action on PAF terminals, and by acting onto the surrounding tissues thereby increasing inflammation and pain (Wood and Docherty, 1997). The peripheral terminals of small caliber PAFs express AMPA, NMDA and kainate receptors. Local administration of ketamine, a NMDA channel blocker, inhibits primary and secondary hyperalgesia in humans. The algogenic action of glutamate at periphery is likely to be also linked to its capability to stimulate release of SP from PAFs or sympathetic terminals. In keeping with this findings, activation of peripheral NMDA receptors on PAFs triggers the release of SP at their central endings (Liu et al., 1994b). This process may be mediated by NO (Sorkin, 1993; Aimar et al., 1998).

Finally it is of interest to note that large caliber Aß fibers also display AMPA and NMDA receptors at both their central and peripheral endings (Wood and Docherty, 1997). This raises the possibility that this category of fibers is also modulated by EAAs, that can thus play a role in the induction of mechanical allodynia.

Functional and neurochemical characterization of DH neurons involved in nociception

Although there is a variety of morphological neuronal types in the spinal cord, and particularly in the DH, three main classes of neurons can be defined in relation to their response to primary afferent sensory input (Millan, 1999).

The first class is represented by neurons that specifically respond to noxious stimuli. These nociceptive-specific neurons are silent under normal circumstances, become activated only by high intensity noxious stimuli conveyed to the cord by C and Ad fibers, and display a limited capability to encode stimulus intensity. They are primarily located in laminae I and II_o, but are also found in laminae V-VI.

The second class of is represented by the multireceptorial or wide-dynamic range (WDR) neurons. The terms WDR refers to their property of responding to a wide range of stimulus intensities, from innocuous to noxious, with a direct stimulus-response relationship. These cells represent the principal neuronal type capable to encode stimulus intensity. WDR neurons are activated by thermal, mechanical and chemical stimuli conveyed to the cord by either C, Ad and Aß fibers. They are mainly located in laminae IV-VI, but are also detected in laminae I, II_o and X (and in the ventral horn).

The third class is represented by non-nociceptive neurons, that are found primarily in laminae II_i, III and IV, although a few also occur in lamina I.

On the basis of their output destination, DH neurons may be instead classified as projection neurons, that send their axons outside the gray matter to supraspinal centers, and interneurons, whose axonal arborizations are confined within the spinal cord. Both can be activated by nociceptive PAFs, and comprise any of the functional types (nociceptive-specific, WDR and non- nociceptive) described above. Spinal cord interneurons play a crucial role in the modulation and integration of nociceptive stimuli to be relayed to higher centers, and thus are likely implicated in the process of neuronal sensitization and referred pain. Interneurons of lamina II are of particular relevance under this aspect. For the

sake of simplicity, interneurons can be further subdivided into excitatory and inhibitory, according to their modulatory role on nociceptive inputs. However it must be kept in mind that some of them may be able to exert both effects through activation of different receptor types.

Excitatory interneurons have a role in the indirect polysynaptic activation of projection neurons, and also form positive feedback circuits onto PAF terminals. In a similar fashion, inhibitory interneurons exert their roles through pre and postsynaptic mechanisms. It seems likely that the majority of inhibitory interneurons directly target projection neurons of different functional types (nociceptive-specific and WDR), as well as PAF terminals . An additional possibility is the existence of a direct circuitry connecting inhibitory and excitatory interneurons (Fields et al., 1991; McHugh and McHugh, 2000; Le Bars, 2002).

The neurotransmitters utilized by excitatory interneurons are not fully characterized, but several lines of evidence indicate EAAs and neuropeptides as likely candidates. On the other hand, inhibitory interneurons have been extensively characterized in terms of their neurotransmitter content. These neurons utilize three main families of neuromessengers (acetylcholine, opioid peptides and inhibitory amino acids) that may show more or less complex patterns of coexistence/costorage. Most pertinent to the present discussion, the inhibitory interneurons that use ?-amino-butyric acid (GABA) and/or glycine as their (principal) neurotransmitter(s) are widely distributed in the superficial laminae of the dorsal horn (Todd et al., 1995, 1996; Kerkut and Bagust, 1995).

GABA acts on two types of receptors named $GABA_A$ and $GABA_B$. $GABA_A$ receptors control a chloride-permeable ion channel, that upon opening usually

leads to cell hyperpolarization. $GABA_B$ receptors are G protein coupled. Upon activation they inhibit AC, decrease Ca^{2+} currents, increase K^+ currents and thus hyperpolarize neurons and reduce transmitter release (Malcangio and Bowery, 1996). Glycine is often coexisting with GABA, and acts on strychnine-sensitive $glycine_A$ receptors (Todd et al., 1996; Laing et al., 1994). Neurons expressing GABA and glycine receptors are often postsynaptic to PAF terminals, but presynaptic GABA receptors have also been localized on these terminals (Xi and Akasu, 1996; Rudomin, 1999).

NTs in nociceptive pathways

During development, sensory modality in DRG neurons and their fibers is linked to specific growth factor requirements. A large percentage of type B DRG neurons and their C fibers is sensitive to capsaicin, the pungent alkaloid of hot peppers and vanilloid. These capsaicin-sensitive fibers can be further subdivided into two different populations (Bennett et al., 1998). The first contains CGRP and SP and depends upon nerve growth factor (NGF) for its initial survival and development (see below). The second, that can be labeled by IB-4 lectin, expresses a subclass of ATP receptors and is dependent upon glial cell-derived neurotrophic factor (GDNF). Cutaneous mechanoceptors and muscle proprioceptive fibers depend upon brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) respectively, but in the absence of neurotrophin-4 (NT-4) function, precursor cells intended to become BDNF-dependent mechanoreceptors instead differentiate into NT-3-dependent proprioceptive neurons (Liebl et al., 2000).

It is now clearly established that the role of NTs in nociceptive pathways is not limited to development. For example, NGF not only functions as a trophic factor for a large population of DRG neurons, but also plays a key role in mediating alterations of the C fiber phenotype in certain conditions of enhanced pain stimulation (such as those following inflammation) leading to hyperalgesia.

In a more general context, NTs also appear to be able to interfere with short-term synaptic events in the mature CNS. Indeed, a growing body of data indicates that NTs are directly involved in the exchange of information between central neurons in several areas of the brain and spinal cord. NGF, BDNF, NT-3 or NT-4 have all been implicated in synaptic potentiation and plasticity (Gottschalk et al., 1998; Pozzo-Miller et al., 1999; Xu et al., 2000; Yang et al., 2001; Kaplan and Cooper, 2001), and long-term potentiation (LTP) and/or long-term depression (LTD) in the hippocampus (Saarelainen et al., 2000; Xie et al., 2000), visual system (Huang et al., 1999; Sermasi et al., 2000; Pesavento et al., 2000), and, most related to the present discussion, spinal cord (Arvanov et al., 2000).

The effects of NTs at peripheral and central endings of PAFs should be considered in an overall organic frame (Bennett, 2001). In general, locally produced NTs are taken up by peripheral endings of nociceptors, exert a series of biological effects at these sites, and are then retrogradely transported to DRGs. An anterograde transport along the dorsal roots has been documented for at least some members of the family, that may be therefore released at central endings of PAFs within the spinal cord.

We will discuss below the evidence available so far for each individual NT for a role in the modulation of nociceptive inputs that are relayed to the spinal cord. Nonetheless, as it will become apparent in the following, there are several types of

interactions among members of this family of neurotrophic factors in nociceptive pathways.

Before discussing these issues, we will briefly mention some general concepts on the induction and regulation of NT synthesis and release from neurons, to put things in the right perspective. However, one should have well in mind that most information regarding this issue has been obtained *in vitro* or in brain areas other than the spinal cord.

Neuronal synthesis and release of NTs

NTs can be released from neurons by an unconventional mechanism that involves an increase in $[Ca^{2+}]_i$ from intracellular stores and is independent from extracellular Ca^{2+}, but rather depends upon extracellular Na^+ influx via voltage gated sodium channels (Blochl and Thoenen, 1995; Blochl and Thoenen, 1996). It seems possible that a rise in cAMP also triggers NT release (Thoenen, 1995). Activation of different types of glutamate/GABA receptors (that, as already mentioned, are widely expressed in nociceptive pathways) may stimulate or inhibit synthesis and/or release of NTs, particularly NGF and BDNF. Although production of these NTs may be regulated by neuronal activity, this does not appear to be the case for NT-3 (Lindholm et al., 1994). Glutamate receptors display different but still not completely understood interactions with NTs *in vitro*. Some authors have reported that NMDA receptors may be involved in BDNF upregulation (Favaron et al., 1993; Kokaia et al., 1993), but this has been denied by others (Zafra et al., 1990; Wetmore et al., 1994; Lauterborn et al., 2000). Moreover, in these studies it was observed that positive modulation of

AMPA or kainate receptors increases NT expression in hippocampal and cortical neurons (Zafra et al., 1990; Lauterborn et al., 2000) .

The effect of GABA receptor activation on NT expression is also far from being clear. Under certain conditions activation of GABA receptors seems to downregulate NT production (Zafra et al., 1991; Thoenen, 1995), whereas an increase in NTs and their mRNAs was observed in other experimental contexts (Heese et al., 2000; Obrietan et al., 2002). This issue is further complicated by the depolarizing effect of GABA during very early stage of development (Berninger et al., 1995; Marty et al., 1996; Obrietan et al., 2002).

Nonetheless, given that transmitter amino acids are likely to influence NT levels in the CNS, in a reciprocal fashion NTs exert a marked influence upon gene expression and synaptic transmission. These actions are not limited to long term plastic changes in synapses, which in the case of the nociceptive pathways are much better characterized and understood, but also comprise a number of trk-mediated rapid effects via multiple intracellular signal transduction pathways. NT signaling through trks not only occurs postsynaptically, but also in a retrograde fashion upon activation of presynaptic receptors at the same synapses from which NTs have been released. The dissection of these trk- mediated mechanisms in nociceptive pathways is still far from being complete.

Postsynaptic actions are linked to phosphorylation of ion channels and transmitter receptors which potentiaties EPSPs, in particular NMDA receptor- mediated currents (Kaplan and Stephens, 1994; Levine et al., 1998). The intracellular mechanism involved likely consists in a rise of $[Ca^{2+}]_i$ with activation of PLC or other protein kinases (Sakai et al., 1997; Yu et al., 1997; Suen et al., 1997a).

An alternative or additional mechanism has been proposed for BDNF that can enhance NMDA receptor- mediated currents by acting at the allosteric glicine$_B$ site via a retrograde presynaptic mechanism (Jarvis et al., 1997).

Presynaptic actions of NTs have been shown to consist in an increase of synaptic currents, again linked to a rise of $[Ca^{2+}]_i$ that is responsible for an augmented release of transmitters, among which glutamate (Berninger and Poo, 1996). Rise in $[Ca^{2+}]_i$ is dependent upon extracellular Ca^{2+} and likely involves L-type voltage-dependent Ca^{2+} channels, which can be phosphorylated by different protein kinases that may be induced via trk receptors (Berninger and Poo, 1996; Sakai et al., 1997; Lesser et al., 1997; Sherwood et al., 1997; Baldelli et al., 1999; Baldelli et al., 2002).

NGF

NGF was initially characterized for its growing effects on sensory neurons (Levi-Montalcini and Angeletti, 1968). These effects are exerted upon binding to high-affinity trkA receptors which are expressed in approximately 40% of rat adult DRG neurons (Richardson et al., 1986; Verge et al., 1989; Smeyne et al., 1994). During development NGF appears to be necessary for survival of small diameter DRG neurons giving rise to nociceptive C fibers projecting to laminae I-II of the DH (Otten et al., 1980; Otten et al., 1982; Ruit et al., 1992). NGF also influences the phenotypic development of cutaneous high threshold mechanoreceptors of the Ad type (Ritter et al., 1991; Ritter and Mendell, 1992; Lewin et al., 1992). From development to adulthood, there is a shift in the role of NGF that initially acts as a survival factor (in particular for outgrowth and proliferation in sensory axons),

and becomes then indispensable for the maintenance of a differentiated phenotype of DRG neurons (Carroll et al., 1992; Ruit et al., 1992; Lewin et al., 1992).

Functional role of NGF in adult DRG neurons

In general terms, reduction of the NGF supply to DRGs that follows a peripheral nerve damage in adult animals leads to a diminution of SP (and other peptides) production in *neuropathic pain*, whereas an accumulation of NGF results in increased SP (and other peptides) synthesis in *inflammatory pain*. A remarkable indication of this role of NGF on plasticity of primary sensory neurons expressing SP comes from the results of the surgical re-routing of muscle sensory fibers to skin (Lewin and McMahon, 1991). It is well known that the levels of endogenous NGF are by far higher in skin than in muscle, and, accordingly, re-oriented PAFs display an increased contend of SP.

NGF counteracts the loss of CGRP and SP expressing DRG neurons following injury (Fitzgerald et al., 1985; Inaishi et al., 1992), and regulates the expression of these two neuropeptides *in vitro* (Lindsay et al., 1989; Lindsay and Harmar, 1989). Other studies in which a synthetic protein, trkA-IgG, was used to sequester endogenous NGF and block the survival effects of NGF on cultured sensory neurons, showed that administration of trkA-IgG produces a sustained thermal and chemical hypoalgesia and leads to a downregulation of the sensory neuropeptide CGRP (McMahon et al., 1995).

Experiments *in vivo* confirmed and extended these observations. Upon basal conditions lumbar DRG neurons express high levels of CGRP, SP and somatostatin, while there are only limited amounts of vasoactive intestinal polypeptide (VIP), cholecystokinin (CCK), neuropeptide Y (NPY) and galanin.

Following sciatic nerve transection there is a *phenotypic shift* in peptide expression with substantial decrease in expression of CGRP, SP, somatostatin and their mRNAs in parallel with increased VIP, CCK and galanin (Munglani et al., 1996; Ramer et al., 1998; Hökfelt et al., 2000). In parallel, peripheral axotomy causes Aß fibers to sprout into lamina II, a region from which they are normally excluded (Bennett et al., 1996). Intrathecal NGF administration counteracts the decrease in expression of CGRP and SP, but not somatostatin, and reduces the numbers of VIP-, CCK-, NPY- and galanin-expressing neurons after injury (Verge et al., 1995). The action of NGF on SP may be explained by the presence of a NGF-responsive element controlling SP mRNA transcription in the promoter region of the preprotachykinin gene (Heumann, 1994). In addition, the lack of NGF effects on somatostatin producing DRG cells is consistent with the absence of trkA receptor protein and mRNA in these neurons (Verge et al., 1995). Moreover, NGF (but not NT-3 or BDNF) prevents both the axotomy- induced reduction in CGRP staining within lamina II and the sprouting of Aß fibers into this region. It is likely that the prevention of Aß fiber sprouting is a secondary consequence of NGF rescuing small fibers originating from trkA-expressing DRG neurons (Bennett et al., 1996). Similarly, expression of NGF by recombinant adenovirus is capable to induce a robust axonal regeneration into normal as well as ectopic locations within the DH after dorsal root injury (Romero et al., 2001). Behavioral studies, in which rats treated with NGF (and GDNF but not BDNF) recover sensitivity to noxious heat and pressure following axotomy, confirm the importance of NGF in maintenance of adult sensory neurons. Moreover, they confirmed that NGF and BDNF have different effects *in vivo,* on the ground of the

their well known actions on distinct subpopulations of DRG neurons (Ramer et al., 2000).

NGF, hyperalgesia and inflammation

Administration of excess NGF to neonatal or mature animals can lead to a profound behavioral hyperalgesia (Lewin et al., 1993). Neonatal NGF treatment results in a marked mechanical hyperalgesia that persists until maturity. This hyperalgesia is explained by an NGF- mediated sensitization of Ad nociceptive PAFs to mechanical stimuli. Treatment of juvenile animals leads to a very similar behavioral hyperalgesia, but there is not sensitization of Ad nociceptors to mechanical stimuli. Adult animals also develop mechanical hyperalgesia upon NGF treatment, but without sensitization of Ad nociceptive afferents. In addition, adult animals develop heat hyperalgesia. Therefore, it appears that the NGF-induced mechanical hyperalgesia is brought about by different mechanisms in neonatal and adult rats. Furthermore, NGF-induced mechanical hyperalgesia in adult animals may be due to central changes, whereas heat hyperalgesia is likely to result from sensitization of peripheral receptors to heat. In keeping, adult rats that received intraplantar injections of NGF develop thermal hyperalgesia linked to reduction of thermal nociceptive threshold in capsaicin sensitive PAFs (Amann et al., 1995). Similar effects are observed in humans after intradermal injection of recombinant NGF (Dyck et al., 1997).

Numerous experiments with transgenic mice lacking or overexpressing NGF clearly confirmed the occurrence of perturbations in nociceptive transmission in the presence of anomalous levels of this NT. When the basal levels of skin NGF are reduced by producing transgenic animals that expressed a fusion gene construct containing an antisense NGF cDNA linked to the K14 keratin promoter,

mice display a profound hypoalgesia to noxious and mechanical stimuli (Davis et al., 1993). Similar results are obtained after mis-expression and deletion studies of the *ngf* gene (Akopian et al., 1996). In keeping, mice with null mutation of the trkA gene exhibit a severe deficit in nociception following thermal and mechanical stimuli (Smeyne et al., 1994). On the other hand, increase of NGF basal levels in the skin (Davis et al., 1993) or the spinal white matter (Ribeiro-Da-Silva et al., 2000) in transgenic animals leads to hyperalgesia and/or allodynia. Excess target-derived NGF does not alter physiological response properties or the types of neurons containing CGRP, but rather induces an increase in the relative proportions of myelinated nociceptors (Ritter et al., 2000). Therefore, all these data clearly demonstrate the capability of experimentally administered NGF to modulate the phenotype of DRG neurons, and that endogenous production of NGF regulates the sensitivity of nociceptive systems (McMahon, 1996). Although the aforementioned data are mainly referred to cutaneous afferents, visceral nociceptive afferents also express trkA receptors and respond to NGF in a similar fashion (Dmitrieva and McMahon, 1996; Dmitrieva et al., 1997).

Peripheral inflammation leads to profoundly increased pain sensitivity: noxious stimuli generate a greater response, and stimuli that are normally innocuous elicit pain, as a consequence of an increased local sensitivity of peripheral terminals of Ad and C fibers. Since the endogenous levels of NGF raise substantially in inflamed tissues (Weskamp and Otten, 1987; Donnerer et al., 1992; Woolf et al., 1994a; McMahon, 1996), it was not surprising that NGF turned out to be a cardinal link between inflammation and hyperalgesia. NGF produced in peripheral tissues (or administered locally) acts on cells expressing trkA receptors such as

inflammatory cells, sympathetic neurons, and PAF terminals originating from NGF-sensitive DRG neurons.

In general, hyperalgesia associated with experimental inflammation is blocked by the pharmacological *antagonism* of NGF in several animal models (McMahon, 1996). Experimental inflammation results in local sensory hypersensitivity and up-regulates SP and CGRP in DRG neurons innervating the inflamed tissue (Woolf et al., 1994a; Amann et al., 1995). Systemic administration of anti-NGF neutralizing antibodies prevents behavioral changes (McMahon et al., 1995), neuropeptide up-regulation, and the inflammation- induced expression of the immediate early gene *c-fos* in DH neurons (Woolf et al., 1994a). Following carrageenan inflammation, which raises the endogenous levels of NGF in inflamed tissues, a marked increase in the proportion of active Ad and C nociceptors is observed (Koltzenburg et al., 1999). Spontaneously active fibers are sensitized to heat, but the mechanical threshold of nociceptive PAFs remains unchanged. When the NGF-neutralizing molecule trkA-IgG is administered together with carrageenan, primary afferent nociceptors do not sensitize and display essentially normal response properties.

Under physiological conditions, NGF in inflamed tissue derives from different sources including PAF terminals, Schwann cells, sympathetic neurons, mast cells, macrophages, fibroblast, and skin keratinocytes (Brown et al., 1991). When the relative contribution of these different cell types to the hyperalgesic action of NGF in inflammation was examined, it was observed that sympathetic neurons transiently contribute to inflammatory hyperalgesia. However, mast cells (which are a potential source of NGF) and sensory neurons are important for the sustained action of NGF in increased nociceptive transmission (Woolf et al.,

1996). The hyperalgesic actions of NGF are in part consequence of the sensitization of the peripheral terminals of high threshold nociceptors, either as a result of a direct action of NGF on trkA expressing PAFs, or indirectly, via the release of sensitizing mediators from trkA expressing inflammatory cells and postganglionic sympathetic neurons (Amann et al., 1995). Indeed chronic degranulation of mast cells significantly impairs the rise of NGF levels in inflamed tissues (Woolf et al., 1996), possibly as a consequence of an impairment in synthesis, storage or release of NGF from these inflammatory cells, or a reduction in the release of tumor necrosis factor a or other cytokines from mast cells, which, in turn, stimulate other cell types to produce NGF.

In animals subjected to Freund's adjuvant-induced arthritis (AIA), a model of long- lasting inflammatory pain, there is an increased immunoreactivity for the NGF trkA receptor and the low affinity $p75^{NTR}$ in NGF-sensitive, but not GDNF-sensitive, DRG neurons. The rise of $p75^{NTR}$ occurs immediately upon Freund's adjuvant administration, and is followed by increase of trkA immunoreactivity linked to the development of a long-lasting inflammatory response. In parallel, the two receptors are up-regulated at central endings of NGF-sensitive nociceptors (Pezet et al., 2001). Moreover, blockade of NGF bioactivity using an antagonist of trkA and $p75^{NTR}$ results in suppression of inflammatory pain (Owolabi et al., 1999). These results implicate NGF in long-term mechanisms accompanying chronic inflammatory pain, via the regulation of its high affinity receptor trkA $p75^{NTR}$.

As previously mentioned, a growing body of evidence suggests that chronic pathologic pain results from long-term plasticity of central nociceptive pathways (Woolf and Costigan, 1999). Diverse peripheral neuropathies are characterized by

a loss or reduction of NGF supply to PAFs and DRGs (Woolf, 1996). Several types of PAF lesions and diabetic neuropathy are characterized by decrease of the mRNA levels for trkA and $p75^{NTR}$ in DRG neurons with a further reduction of NGF availability to these cells (Verge et al., 1995; Delcroix et al., 1997; Delcroix et al., 1998). Reduction of NGF availability could be a cause of cisplatin-induced peripheral neuropathies, and exogenous administration of NGF may be helpful to prevent or reduce cisplatin neurotoxicity in cancer patients (Aloe et al., 2000). Also, NGF levels are reduced in the spinal cord during experimental allergic encephalomyelitis (Calzà et al., 1997, 2002), and the clinical course of the disease ameliorates following NGF administration (Villoslada et al., 2000). Very recently, precursors of NGF have been shown to be the predominant forms of this NT in brain and peripheral tissues, and evidence has been provided that proNGF preferentially binds $p75^{NTR}$ with high affinity. In human and rat skin and nerve extracts, a 53 kDa band was detected by Western blot using antibodies against rhNGF or preproNGF, that could correspond to a previously described modified preproNGF-like molecule (Yiangou et al., 2002). Expression of these molecules is markedly reduced in skin extracts from patients with subclinical diabetic neuropathy, but is increased in extracts of inflamed colon from patients with Crohn's disease. Antibodies to both rhNGF and preproNGF immunostain basal keratinocytes in tissue sections of normal human and rat skin, and show accumulation of immunoreactive material in nerve fibers distally to sciatic nerve ligation in rats. PreproNGF antibody immunostain rat large/medium sized DRG neurons, whereas only small neurons are stained with antibodies to mature rhNGF. These observation suggest that preproNGF may be preferentially taken up and transported by $p75^{NTR}$. On these bases, the different molecular forms derived

from preproNGF are likely to be of importance in sensory mechanisms, and deserve further investigation (Yiangou et al., 2002).

As repeatedly mentioned before, PAFs not only express the high affinty trkA receptor for NGF, but also the low affinity $p75^{NTR}$ (Henry et al., 1994; Kitzman et al., 1998). The level of expression of $p75^{NTR}$ in DRG neurons appears to be modulated by NGF level in target tissues (Kitzman et al., 1998). The interrelationships of $p75^{NTR}$ and NGF (and other NTs) in determining the biological functions of the molecule(s) are still poorly understood. Directly related to the present discussion, $p75^{NTR}$ enhances the retrograde flow of NGF from PAF terminals to DRGs. In fact, NGF is retrogradely transported in sensory neurons within DRGs, where it alters transcription of a number of proteins and peptides. NGF-mediated modification of gene expression in DRG neurons during inflammation is central to the pathophysiology of persistent pain. The phenotype changes produced by NGF during inflammation include the aforementioned downregulation/upregulation of several neuropeptides, which may amplify sensory input signals in the spinal cord and augment neurogenic inflammation in the periphery, but also upregulation of receptors, ion channels and growth related molecules which may lead to a hyperinnervation of injured tissue by promoting terminal sprouting.

Both the endosome-assisted dispatch of activated trkA receptors to DRGs as well as transport of a NGF-trkA complex may be responsible for these NGF-mediated actions at the DRG level (Ehlers et al., 1995; Grimes et al., 1996; Riccio et al., 1997). By these mechanisms, NGF can enhance the production of several nociception-related molecules in C fiber terminals besides to SP and CGRP. Among these, certain types of Na+ channels, proton –activated ion channels and

vanilloid receptors (Guo et al., 2001) are of particular interest. Also, it was recently shown that intratechal NGF administration induces novel purinergic receptor P2X(3) expression in DRGs and spinal gray matter, with intense immunoreactivity in axons projecting to laminae I, II_o and X (Ramer et al., 2001). In DRG neurons the purinergic receptor P2X(3) is found predominantly in GDNF-sensitive nociceptors. Therefore, *de novo* expression of P2X(3) in NGF-sensitive nociceptors may contribute to chronic inflammatory pain (Ramer et al., 2001). Moreover, peripheral production of NGF during inflammation and its retrograde transport to DRGs induces p38 MAPK activation in the soma of C fiber nociceptors. Inflammation also increases protein (but not mRNA) levels of the heat- gated ion channel TRPV1 (VR1) in these neurons, which is then transported to peripheral but not central C fiber terminals. Inhibiting p38 activation in DRGs reduces the increase in TRPV1 in the DRG and inflamed skin, and diminishes inflammation- induced heat hypersensitivity. Likely, activation of p38 in the DRG following retrograde NGF transport, by increasing TRPV1 levels in nociceptor peripheral terminals in a transcription- independent fashion, contributes to the maintenance of inflammatory heat hypersensitivity (Ji et al., 2002).

Therefore, following peripheral inflammation a complex array of long-term phenotypic modifications of NGF-sensitive nociceptors is likely to be responsible of a series of cellular and molecular events eventually leading the onset of pathologic pain. Under this perspective and of particular relevance to the present discussion, NGF has also been demonstrated to be capable to induce expression of BDNF and its mRNA in trkA bearing DRG neurons after intratechal treatment

(Apfel et al., 1996; Michael et al., 1997; Cho et al., 1997). This issue will be discussed below in the section of this chapter dedicated to BDNF.

As mentioned above, there is evidence that the delay of several hours in the onset of mechanical hyperalgesia evoked by systemic or spinal administration of NGF (Lewin et al., 1994), is related to upregulation of SP, CGRP and NKA synthesis in DRG, and transport to central terminals in the DH. In keeping, antibodies against NGF or trkA fusion proteins, not only block inflammatory hyperalgesia at periphery, but also the increase of sensory neuropeptide levels in DRG neurons and PAF central terminals (Donnerer et al., 1993; Lewin and Mendell, 1993; Lewin et al., 1993; Croll et al., 1994; Woolf et al., 1994a; McMahon et al., 1995; Malcangio et al., 1997). Moreover, NGF- induced hyperalgesia and wind-up of DH neurons are abolished by NK_1 receptor antagonists, in accord with the idea that the longer-term pronociceptive actions of NGF are of an *indirect* type, and mediated through the sensitization of SP-responsive DH neurons (Thompson et al., 1995b; McMahon, 1996; Ma and Bisby, 1998). A further confirmation of this indirect role of NGF as a central mediator of hyperalgesia comes from the observation that its pronociceptive capabilities are limited by the accumulation of N-terminal SP metabolites (Larson and Kitto, 1997).

Nonetheless, trkA and $p75^{NTR}$ are expressed in the spinal gray matter with a laminar pattern corresponding to that of nociceptive C and Ad fibers (Fig. 1A, B, E). By using real time confocal imaging of calcium signal in acute spinal cord slice preparations from young rats, we have obtained preliminary evidence that NGF acutely increases $[Ca^{2+}]_i$ in a subpopulation of lamina II neurons (Merighi et al., 1999; Fig. 2). The possibilty that NGF has central effects in the DH is

confirmed by observation that systemic treatment with NGF induces a significant

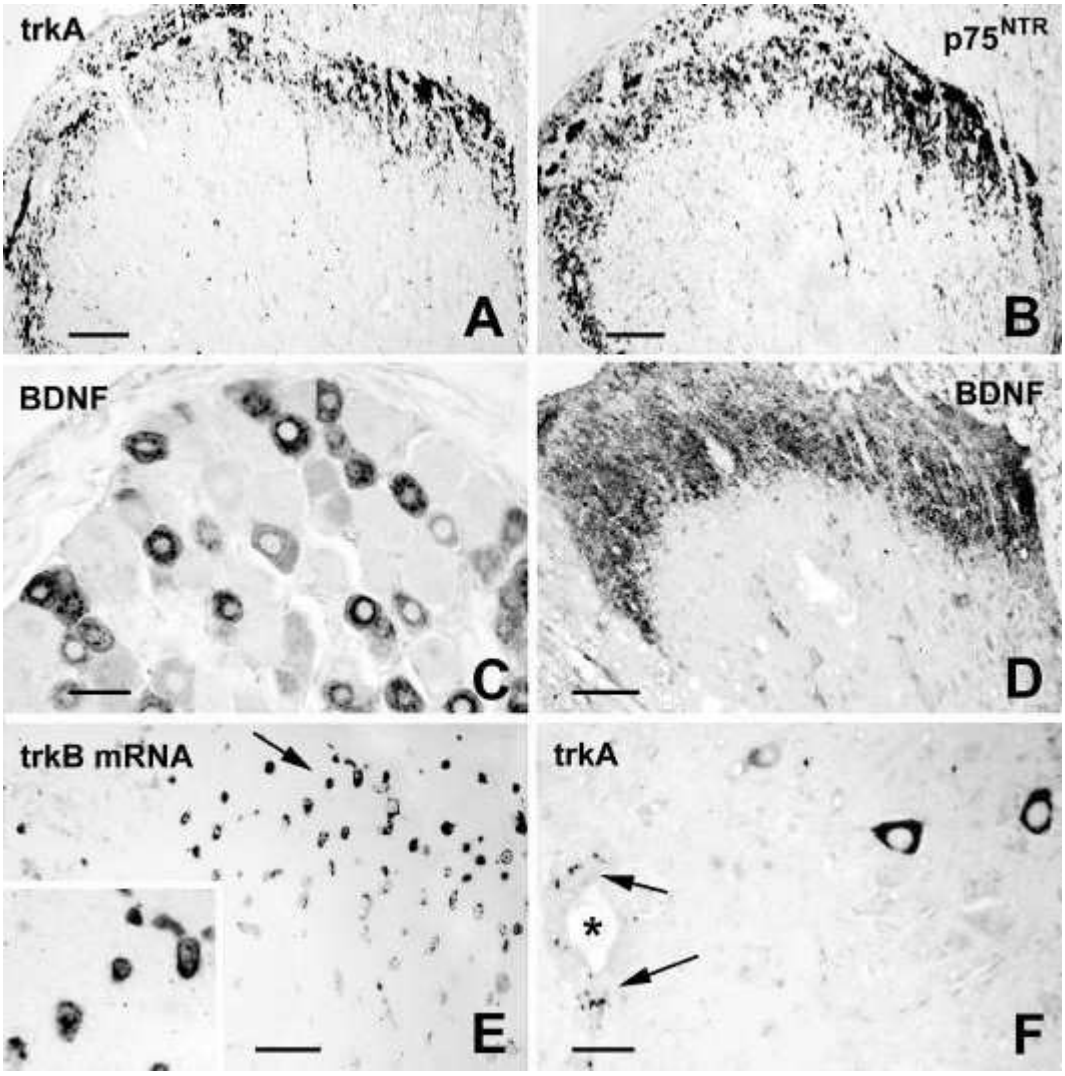

Figure 1: Distribution of NTs and their receptors in nociceptive pathways.
A-B: Immunocytochemical localization of the high-affinity NGF receptor trkA (A) and the low-affinity $p75^{NTR}$ (B) in fibers of the superficial laminae of the DH. C-D: localization of BDNF immunoreactivity in DRG neurons (C) and the superficial dorsal horn (D) . E: In situ hybridization of trkB mRNA in the dorsal horn. Note the presence of numerous positive neurons in lamina II. The arrow indicates the area shown at higher magnification in the insert. F: TrkA -immunoreactive cell bodies and fibers (arrows) in the gray matter (lamina X) surrounding the central canal (asterisk). Bars = 25 µm.

increase in evoked release of substance P in an isolated spinal cord preparation (Malcangio et al., 1996). However, acute superfusion of NGF through a naive rat spinal cord preparation does not alter basal or electrically evoked release of SP-like immunoreactivity (Malcangio et al., 1997).

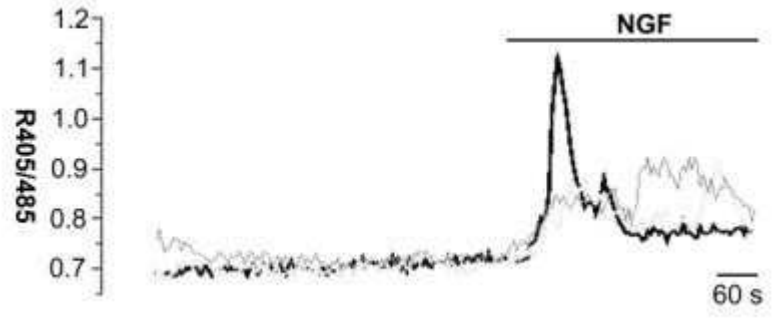

Figure 2: Kinetics of the $[Ca^{2+}]_i$ change in three neurons from the P8 rat substantia gelatinosa following acute NGF (500 ng/ml) administration, as expressed by the ration between Indo-1 emission wavelength at 405 and 485 nm. A total of nine cells were recordered during this experiment. We have recordered a total of 36 cells in three different experiments, and 14 of them were NGF-responsive (mean +/-SEM: 42.7 +/-11.3).

Anterograde transport of NGF to the DH has not been demonstrated (Anand et al., 1995), and thus the *in vivo* relevance of these findings remains to be established.

BDNF

It is well known that phenotype alterations in C fibers may be paralleled by the so-called *phenotypic switch* in Aß fibers, which, under inflammatory conditions, begin to synthesize and release SP (Woolf et al., 1992; Neumann et al., 1996). In initial experiments, NGF was hypothesized to have a role in this phenotypic switch, since it was shown to affect the central terminals of PAFs by switching the sensory modalities of Aß fibers and modifying the pattern of their responses to electrophysiological stimulation (Lewin and Mendell, 1994; Thompson et al., 1995a). Also, these NGF-induced modifications were reduced following treatment with the NK_1 receptor antagonist RP675880 thus reinforcing the idea of a close link between NGF and SP in mediating changes in nociceptive transmission (Thompson et al., 1995a).

However, large diameter Aß fibers do not express trkA receptors (McMahon et al., 1994) and thus NGF could not be *directly* involved in the phenotypic shift. On

the other hand, trkB receptors for BDNF are found in DRG neurons of different diameters, including a population of mechanical nociceptors (Wright and Snider, 1995; Lewin and Barde, 1996; Koltzenburg, 1999). In contrast to cutaneous afferents, about 90% of visceral afferents coexpress trkB and trkA receptors (McMahon et al., 1994), and BDNF has been demonstrated to enhance the sensitivity to capsaicin of vagal PAFs containing SP (Winter, 1998). Therefore it seems possible that BDNF is peripherally upregulated in visceral PAFs upon inflammation, and might be able to modulate the phenotype of these fibers in visceral pain (McMahon et al., 1994; Lewin and Barde, 1996).

Modulation of nociceptor central synapses by BDNF

Unlike NGF, that appears to be primarily involved in the modulation of nociceptive PAFs at periphery, BDNF has a direct role in the modulation of central synapses of nociceptors (Pezet et al., 2002c; Malcangio and Lessmann, 2003). Indeed, there is now compelling evidence that BDNF is capable to affect central neuronal activity at least in the hippocampus, visual system, and, related to the present discussion, spinal cord. As we will discuss below, BDNF intervenes in central sensitization, that, as repeatedly mentioned, is responsible of several types of hyperalgesia and can be considered a particular form of synaptic plasticity similar LTP or LTD. In sensory pathways, BDNF and its mRNA are, under normal conditions, localized in several different DRG neuron subtypes (Fig. 1C), the majority of which corresponds to small- to medium- sized cells expressing trkA and CGRP (Cho et al., 1996; Apfel et al., 1996; Michael et al., 1997). This close relationship between BDNF and trkA/CGRP expressing DRG neurons is mirrored at their central endings in the spinal cord (Fig. 1D), where BDNF-immunoreactivity is restricted to CGRP-containing PAFs. BDNF

immunoreactivity is not detected in IB4- or trkC- labeled DRG neurons, although a few of these cells may express the BDNF mRNA (Michael et al., 1997). Consistently with these observations, BDNF immunoreactivity is absent from the termination zones of large caliber and IB4- labeled PAFs in the spinal cord (Michael et al., 1997). The fact that about one third of DRG neurons constitutively express BDNF in normal adult animals raises the question of which is the biological role of this NT in primary afferent pathways. *In vitro*, a BDNF autocrine loop has been shown to act on survival of a subpopulation of adult DRG neurons (Acheson et al., 1995). However this seems unlikely to take place under normal circumstances *in vivo*, since only 1% of cells expressing BDNF mRNA also express trkB, and only 2% of trkB cells contained BDNF mRNA (Michael et al., 1997). The low degree of coexistence between BDNF immunoreactivity and trkB labeling in DRG neurons is somewhat surprising, considering that target-derived BDNF is believed to be retrogradely transported to the soma of trkA-expressing neurons (Distefano et al., 1992). On the other hand, it appears that there is little retrograde BDNF transport from peripheral terminals of trkB-expressing PAFs, and target-derived BDNF might only represent a small fraction of the total BDNF protein within DRGs (Michael et al., 1997). As previously mentioned in the introduction to this review, there is a complex pattern of messenger coexistence/costorage in PAF central terminals (Merighi, 2002). Therefore, it seems obvious that BDNF may modulate EAA and/or SP actions in the DH.

In PAF terminals of the naïve spinal cord, BDNF immunoreactivity appears to be localized to LGVs, where it might be costored with SP (Michael et al., 1997), although no direct morphological evidence for it has been provided yet, and

BDNF and SP can be released independently after capsaicin administration to the isolated DH (Lever et al., 2001).

Since SP release form PAF terminals in the DH is under the inhibitory control of endogenous GABAergic and opioid interneurons that antagonize NMDA-evoked facilitation, the effects of bicucullin and naloxone on BDNF release have been evaluated, coming to the conclusion that release of endogenous BDNF is not modulated by the GABAergic inhibitory system (Lever et al., 2001). Conversely, release of endogenous BDNF in the isolated rat DH appears to be mediated by NMDA receptor activation after chemical stimulation by capsaicin or electrical stimulation of dorsal roots at C fiber strength. These experiments clearly demonstrate that endogenous BDNF can be released at central synapses of nociceptors. However the cellular mechanisms that intervene in the regulation of such a release remain, for the most, obscure. Lever and colleagues (2001) have shown that after short bursts of high- frequency stimulation of dorsal roots (300 pulses in 75 trains, 100 Hz) BDNF is released together with glutamate and SP. Interestingly, BDNF release is inhibited by the NMDA receptor antagonist D(-)2-amino-5-phosphonopentanoate (D-AP-5) but not by the AMPA/kainate (non-NMDA) antagonist 6-cyano-7-nitro-quino xaline-,2,3 dione (CNQX), indicating that glutamate stimulates BDNF release by activation of NMDA receptors. Nonetheless tetanic high- frequency stimulation (300 pulses in 3 trains, 100 Hz) does not evoke significant BDNF release, suggesting that glutamate alone is not sufficient to induce release of the NT. Release of endogenous BDNF in the DH is likely to occur mainly, if not exclusively, from the central terminals of nociceptive DRG neurons on at least three bases: *i.* BDNF immunoreactivity in the DH is substantially reduced after capsaicin application (Lever et al., 2001), *ii.* there is no

histological evidence for intrinsic or descending BDNF neurons/pathways in the DH (Apfel et al., 1996; Michael et al., 1997; Heppenstall and Lewin, 2001), and *iii.* results of the aforementioned electrophysiological experiments are consistent with the pattern of NMDA glutamate receptor distribution in the DH. Therefore, it seems possible that release of BDNF from PAF central terminals is modulated, among others, by presynaptic NMDA receptors on these fibers (Liu et al., 1994c; Marvizon et al., 1997; Malcangio et al., 1998) and/or postsynaptic NMDA receptors via a retrograde signaling mechanism involving diffusible messengers such as NO (Merighi et al., 2000).

An obvious question to be raised regards the physiological significance of BDNF release in the naïve DH. In an attempt to answer this question and to better characterize the mechanism(s) of action of this NT in the spinal cord, sequestration of endogenous BDNF (and NT-4) by a trkB fusion protein or analysis of BDNF-deficient mice has been employed. However, after BDNF sequestration no changes in either basal pain sensitivity or mechanical hypersensitivity induced by peripheral capsaicin administration (a measure of C fiber- mediated central sensitization) are observed (Mannion et al., 1999). On the other hand, in BDNF-deficient mice selective deficits in the ventral root potential (VPR) evoked by stimulating nociceptive PAFs are observed, whereas the non-nociceptive portion of the VPR remains unaltered (Heppenstall and Lewin, 2001). Although there may be some concern in the interpretation of these results, since a decrease of about 30% of the total number of DRG neurons was previously observed in BDNF null mutants (Ernfors et al., 1994a), Heppenstall and Lewin have shown no obvious alterations in the number and connectivity of nociceptive neurons in their mutants, and come to conclude that BDNF released from

nociceptive PAFs has a role in modulating or even mediating reflex excitability, and that BDNF is necessary for normal baseline nociceptive responses in the spinal cord.

Whereas all these observations converge to support a pronociceptive effect of endogenous BDNF in the spinal cord, the results obtained after application of pharmacological concentrations of exogenous BDNF in different experimental paradigms, are subject to a more complex array of interpretations. Initial studies in which the NT was infused into the midbrain came to the conclusion that BDNF has an antinociceptive effect (Siuciak et al., 1994; Siuciak et al., 1995). Similarly, after engrafting of genetically engineered BDNF-secreting neurons or recombinant adeno-associated viral- mediated over-expression of BDNF in the spinal cord, attenuation of allodynia and hyperalgesia is observed after sciatic nerve constriction (Cejas et al., 2000; Hains et al., 2001a, b; Eaton et al., 2002). Also, topical application of BDNF to the adult rat isolated DH inhibits the electrically evoked release of SP from sensory neurons, and increases K^+-stimulated release of GABA (Pezet et al., 2002a).

On the other hand, others have provided evidence that, in the isolated spinal cord, exogenous BDNF selectively enhances the C fiber- mediated component of the flexor withdrawal reflex and NMDA-evoked responses recorded from ventral roots (Kerr et al., 1999). In keeping with the idea that BDNF has a pronociceptive effect in the DH, we have recently observed that BDNF alters $[Ca^{2+}]_i$ in lamina II neurons after confocal imaging of acute spinal cord slices (Merighi et al., 1999). After capsaicin administration, we came hypothesize that rise of $[Ca^{2+}]_i$ in these neurons is not directly due a postsynaptic effect of BDNF, but rather mediated by

a presynaptic mechanism involving release of SP from PAF terminals (Carmignoto et al., 2003).

Upregulation of BDNF following inflammation or injury

A correct understanding of the role of BDNF in nociception is further complicated by plasticity of PAFs that occurs under pathophysiological and/or experimental conditions. Indeed, the DRG content of BDNF is markedly upregulated in an NGF-dependent fashion upon different experimental manipulations leading to inflammation and/or injury (Thompson et al., 1999).

After intratechal NGF treatment there is a up to 80-90% increase of BDNF and its mRNA in trkA/CGRP expressing DRG neurons, whereas NGF does not increase BDNF expression in those neurons lacking trkA (Apfel et al., 1996; Michael et al., 1997). Similar results are obtained after systemic NGF treatment, a procedure that mimics peripheral inflammatory states (Kerr et al., 1999). Moreover in a number of experimental models that are known to upregulate peripheral NGF, such as experimental inflammation after intraplantar formalin/carageenan (Kerr et al., 1999; Mannion et al., 1999) or neuritis of the nerve roots after lumbar disc herniation (Obata et al., 2002), the pattern of BDNF expression is modified in a similar fashion. C-fiber electrical activity also increases BDNF expression in DRGs, and both inflammation and activity increase full- length trkB receptor levels in the DH (Mannion et al., 1999).

In addition, peripheral nerve injury leads to the same kinds of plastic changes in DRGs and spinal cord. Following a chronic sciatic nerve lesion or axotomy there is a long- lasting ipsilateral increase (2-4 weeks) in BDNF mRNA and protein in large diameter trkB- and trkC-expressing DRGs, whereas BDNF level is

unchanged or reduced in most trkA expressing neurons (Michael et al., 1999; Zhou et al., 1999; Ha et al., 2001; Fuk uoka et al., 2001). Under these conditions, BDNF-immunoreactive nerve terminals in the ipsilateral spinal cord increase in laminae II-IV and nucleus gracile. These studies show that in the DRGs, small trkA neurons switch off their normal synthesis of BDNF, whereas large trkB/C neurons switch to a BDNF phenotype. Plastic changes affecting sensory pathways are associated to thermal hyperalgesia, a sign of neuropathic pain, and appear to be reversed upon relief of behavioral changes. Namely, disappearance of thermal hyperalgesia is associated with downregulation to baseline levels of BDNF in DRGs and spinal cord (Miletic and Miletic, 2002).

Thus, a growing body of evidence indicates that chronic/neuropathic pain due to inflammation and/or peripheral nerve injury is mediated by BDNF at central synapses in the DH, upon a NGF-dependent phenotypic shift in the DRGs that leads to BDNF upregulation. In keeping, BDNF-induced behavioral changes indicative of neuropathic pain are abolished upon administration of a BDNF sequestering trkB-IgG fusion protein (Kerr et al., 1999; Mannion et al., 1999; Thompson et al., 1999), anti-BDNF antibodies to the spinal cord (Fukuoka et al., 2001), or peripherally applied anti-NGF antibodies (Fukuoka et al., 2001). Despite of the extensive work done on this issue, the mechanism of action of BDNF within the spinal cord remains elusive. In a recent review, four different states of sensory processing have been described in relation to plasticity of the sensory system (Woolf and Costigan, 1999). The first state corresponds to normal nociceptive transmission. Under these conditions the system operates at baseline sensitivity, and only an intense stimulation of C and Ad fibers produces short-lasting pain. The second state occurs in a time scale of minutes and is linked to the

onset of post-translational changes within the DH. These changes lead to central sensitization and modify the basal threshold of sensitivity resulting in hyperalgesia and allodynia. The third state is a consequence of activity-dependent transcriptional changes within DRGs and DH, occurring in a time scale of hours. In this case the system is further potentiated with increased responsiveness to C fiber inputs. The fourth state is a consequence of peripheral inflammation and occurs in a time scale of hours/days. In this case potentiation of the system is paralleled by DRG phenotypic switch, leading to highest central sensitization.

All lines of evidence available so far converge to the conclusion that BDNF physiologically acts starting from stage two onward. At this stage (which is *not* associated to inflammation) relatively brief C fiber inputs can sequentially evoke augmented membrane excitability, windup and central sensitization (Woolf and Wall, 1986). Windup is a consequenc e of the removal of Mg^{2+} block of the NMDA receptor with amplification of each subsequent input (Woolf and Costigan, 1999). BDNF likely plays a role in central sensitization upon release from PAF terminals and subsequent binding to trkB-expressing DH interneurons. The activation of trkB (and NMDA, mGluR, NK_1) receptors gives rise to an increase in $[Ca^{2+}]_i$ (Heath et al., 1994; Carmignoto et al., 2003) following extracellular influx and/or release from internal stores. Indeed, the action of BDNF on $[Ca^{2+}]_i$ has been proposed to be mediated by the trkB-induced activation of PLC? with the consequent production of inositol trisphosphate (IP3) and release of Ca^{2+} from internal Ca^{2+} stores (Zirrgiebel et al., 1995; Li et al., 1998). Increase in $[Ca^{2+}]_i$ leads to activation of other tyrosine kinases such as *src* and PKC (Zirrgiebel et al., 1995). The major targets of these kinases are the NR1 and NR2 subunits of the NMDA receptor (Yu et al., 1997; Suen et al., 1997b). These

posttranslational modifications eventually lead to reduction of the NMDA receptor Mg^{2+} block.

Stages three and four are respectively associated with early and late changes in transcription within DRG and DH neurons. Two hours after C fiber stimulation, BDNF mRNA levels increase in DRGs, and the trkB mRNA (Fig. 1F) augments in the DH (Woolf and Costigan, 1999). Namely, although *in situ* hybridization reveals the existence of a large population of DH neurons with trkB mRNA , immunohistochemistry demonstrates a low number of lamina II neurons expressing the full- length trkB protein in the naive spinal cord (Michael et al., 1999; Mannion et al., 1999), and higher levels were only observed following episodes of high neuronal activity, inflammation and/or axotomy (Michael et al., 1999; Mannion et al., 1999; Thompson et al., 1999). Increase in the amount of BDNF in DRGs and central PAF terminals, together with augmented expression of its high affinity receptor in the DH leads to potentiation of the system and further transcriptional changes. Acute BDNF stimulation induces a robust phosphorylation of the transcription factor cAMP response element-binding protein (CREB) in acute hippocampus and visual cortex slices (Pizzorusso et al., 2000). It is therefore conceivable that at least part of the activity-dependent up-regulation of BDNF within the DRGs is also CREB-dependent. Moreover in hippocampus and visual cortex (Pizzorusso et al., 2000), spinal cord (Thompson et al., 1995a) and DRGs (Kim et al., 2000) increased expression of *c-fos* is observed after BDNF *in vitro* or *in vivo.* Also noxious stimulation induces trkB receptor and downstream ERK phosphorylation in DH (Pezet et al., 2002b). Whereas all the above observation give a rather coherent explanation of the postsynaptic effects of BDNF on DH neurons, a presynaptic action onto trkB-

expressing PAF terminals cannot be completely ruled out from observation of the time course of the BDNF acute effect on substantia gelatinosa neurons after real time confocal imaging of $[Ca^{2+}]_i$. Indeed, in slices preincubated with BDNF, $[Ca^{2+}]_i$ oscillations progressively increase with time both in frequency and amplitude (with a peak of activity 2-3 hours after incubation), and eventually affect an augmented number of cells (Merighi et al., 1999). This observation, suggests that the effect of BDNF is not linked to trkB- mediated formation of IP3. Indeed, activation of PLC?, that may results from activation of trkB receptors by BDNF, rapidly triggers the formation of IP3, and then the release of Ca^{2+} from intracellular compartments. The ensuing $[Ca^{2+}]_i$ elevations strictly depend on the continuous formation of IP3, and they rapidly fade away in the absence of the stimulus that activates the IP3-coupled receptor (Pozzan et al., 1994). The direct activation of trkB receptors on DH neurons can, therefore, hardly account for the long- lasting increase in $[Ca^{2+}]_i$ oscillation frequency and amplitude that are observed in slices subjected to a relatively short pre- incubation with BDNF before recording, rather than continuous superfusion for several hours. Accordingly, this latter type of BDNF action is more compatible with an indirect presynaptic mechanism that requires the recruitment of additional (retrograde ?) messenger(s) that in turn trigger $[Ca^{2+}]_i$ oscillations in lamina II.

The existence of such a presynaptic mechanism needs to be substantiated by an unequivocal localization of trkB receptors upon PAF terminals, localization that is at present still under debate.

NT-3

Neurotrophin-3 (NT-3) has a well documented role as a survival factor during development of muscle spindle afferent (group Ia) fibers. Selective survival of

muscle sensory neurons occurs *in vitro* in the presence of NT-3 (Hory-Lee et al., 1993). Moreover, there is a selective loss of muscle afferents after treatment with anti-NT-3 antibodies before birth (Oakley et al., 1995), and muscle PAFs do not survive in mice with null mutation of NT-3 (Snider, 1994). In keeping, these fibers are rescued after reintroducing a NT-3 gene (Wright et al., 1997), and animals lacking the NT-3/trkC signaling pathway do not develop muscle PAFs (Ernfors et al., 1994b). In addition, several lines of evidence demonstrate a role for NT-3 in the rescue of spindle afferent function after axotomy or chemically induced neuropathies (Mendell et al., 2001).

NT-3 exerts its biological effects via trkC receptors that are primarily localized to large caliber myelinated Aß fibers, although trkC has been localized throughout the spinal cord, including the superficial laminae of the DH (Zhou and Rush, 1994), and descending serotoninergic fibers. Large myelinated muscle mechanosensitive PAFs retrogradely transport NT-3 from periphery to DRG neuronal cell bodies, but a centripetal transport to the spinal cord remains be demonstrated (Distefano et al., 1992). If indeed endogenous NT-3 reaches the naive spinal cord, as it could be inferred from the observation that NT-3 is necessary for development of the monosynaptic stretch reflex after birth (Seebach et al., 1999), the mechanism(s) through which it acts at central synapses has been in part elucidated using the monosynaptic stretch reflex as a model system (Mendell et al., 2001). Application of exogenous NT-3 increases the amplitude of dorsal root-evoked monosynaptic AMPA/kainate receptor-mediated EPSP within minutes, and requires the presence of active NMDA receptors on the motorneuron membrane (Arvanov et al., 2000). The action of NT-3 is specifically exerted on

this type of synapses and not on other inputs that reach the motorneurons, and disappears in animals older that 1 week (Mendell et al., 2001).

Although the physiological role of NT-3 does not seem to be restricted to muscle afferents, since excess NT-3 enhances the separation of dermatomes that occurs postnatally (Ritter et al., 2001), the existence and origin(s) of a putative pool of NT-3 in the DH of mature animals remain unclear, and a precise role of NT-3 in nociception still has to be established.

Initial studies in which NT-3 was infused into the midbrain indicate, on a behavioral basis, that it has antinociceptive effects (Siuciak et al., 1994). This is further confirmed in an isolated spinal cord preparation in which a single systemic injection of NT-3 induces mechanical (but not thermal) hypoalgesia (Malcangio et al., 1997). Moreover, NT-3 inhibits the release of SP, suggesting that this mechanism may be responsible for NT-3- induced antinociception. In this experimental context NT-3, although not modifying SP basal outflow, dose-dependently inhibits the electrically evoked, but not capsaicin- induced, release of the peptide. NT-3-induced inhibition persists even in the presence of pharmacological concentrations of NGF in the perfusion fluid, and is still significant when the evoked release of SP is enhanced by a prolonged *in vivo* treatment with NGF. It seems likely that inhibition of SP release by NT-3 is mediated by activation of $GABA_A$ receptors onto DH interneurons since naloxone, but not CGP 36742 (a $GABA_B$ antagonist), abolishes the NT-3 effect on SP release (Malcangio et al., 1997). In keeping, locally administered NT-3 appears to reduce the peripheral sensitization that follows a chemically- induced neuropathy (Helgren et al., 1997).

More recently however, it was reported that NT-3 increases C-fibre stimulation-evoked SP release, and capsaicin superfusion- induced SP release in the isolated spinal cord. From these observations it was concluded that increased SP-release from the spinal cord is not necessarily associated with behavioral hyperalgesia in NT-3 treated rats (Malcangio et al., 2000). Similarly, a number of other experiments lead to support a pronociceptive role of NT-3. Transganglionic labeling of Aß fibres with choleragenoid- horseradish peroxidase (C-HRP) in animals treated intrathecally with NT-3 for 14 days via an osmotic pump shows that the area of C-HRP label expands into lamina II. These NT-3-treated animals have a significant decrease in mechanical nociceptive threshold (White, 1998). Consistent with this finding, intrathecal administration of NT-3 antisense nucleotides attenuates the density of C-HRP labeling in lamina II in nerve injured animals, and significantly attenuates nerve injury- induced allodynia (White, 2000).

NT-4

In situ hybridization studies demonstrate that NT-4 is synthesized by DRG and DH neurons (Heppenstall and Lewin, 2001). Very little work has been done to characterize the effects, if any, of NT-4 in central nociceptive pathways. In NT-4 null mice there are not obvious deficits in flexion reflex (Heppenstall and Lewin, 2001). However in these mutants the anti- nociceptive effect of morphine appears to be partially mediated by NT-4-induced trkB receptor activation (Lucas et al., 2003).

Conclusion

A significant advancement of our understanding of the physiological role of NTs in nociceptive pathways has been achieved in the last years. Nonetheless a number of questions are still unresolved mainly linked to the mechanism(s) through which NTs acutely modulate synaptic transmission at central synapses in the DH. Our possibilities of comprehension of this aspect of NT neurobiology are further hampered by the fact that this family of growth factors exert a wide array of functions during development in the survival and maintenance of nerve cells, and that substantial maturation of the nociceptive pathways still occurs in postnatal life. The recent observation that NTs can be sorted into either the constitutive or regulated secretory pathways (Mowla et al., 1999), explains how NTs can act both as survival factors (when entering the constitutive pathway) and neuromessenger (when entering the regulated pathway). Similarly to what happened with NGF, that was initially considered as the prototype of neurotrophic factors, BDNF is now emerging as the prototype of a novel class of neuromessengers in several areas of the CNS. In keeping, BDNF is processed in the regulated secretory pathway within brain neurons and secreted in an activity-dependent manner (Mowla et al., 1999), and in BDNF knockout mice there is an impairment of synaptic vesicle docking (Pozzo-Miller et al., 1999), confirming that it is released at synapses and influences neuronal activity. In the nociceptive pathways plastic changes mediated by NTs, particularly NGF, have been extensively documented, and the cellular and molecular mechanisms that underlie these changes have been basically unraveled. Nonetheless the acute modulatory effects of NTs at central synapses in the DH are still far to be understood, since some effects of BDNF (and perhaps other members of the family) are hardly explained only on the basis of post-translational and/or activity-dependent

transcriptional modifications of the nociceptors and/or their postsynaptic target neurons in the DH.

Clearly much work still needs to be done to clarify the subcellular site of storage of NTs at central synapses, their pattern of coexistence with EAAs and/or neuropeptides, and functiona l interactions these nociceptive messengers. This information, together with an in-depth ultrastructural and functional analysis of receptor distribution in the DH, will be fundamental to fully understand fast synaptic events that are mediated by NTs in nociceptive pathways.

Acknowledgements

The original work described in this paper has been funded by grants of the Italian MIUR (Fondi FIRB 2001 and COFIN 2002 to AM and GC) and by local grants from the University of Torino.

Abbreviations

AIA, adjuvant-induced arthritis; AMPA, DL-a-NH_2-2,3,-dihydro-5- methyl-3-oxo-4-isoxazolepropanoic acid; AC, adenyl cyclase; $[Ca^{2+}]_i$, intracellular calcium concentration; BDNF, brain-derived neurotrophic fator; CCK, cholecystokinin; CGRP, calcitonin gene-related peptide; C-HRP, choleragenoid- horseradish peroxidase; CNQX, 6-cyano-7-nitro-quinoxaline-,2,3 dione; CNS, central nervous system; CREB, cAMP response element-binding protein; D-AP-5, D(-)2-amino-5-phosphonopentanoate; DH, dorsal horn; DRG, dorsal root ganglion; EAA, excitatory amino acid; EPSP, excitatory postsynaptic spontaneous potential; GABA, ?-amino-butyric acid; GDNF, glial cell-derived neurotrophic factor; IP3, inositol trisphosphate; LTD, long term depression; LTP, long term potentiation; NGF, nerve growth factor; NK, neurokinin; NKA, neurokinin A; NKB, neurokinin B; NMDA, N-methyl-D-aspartate; NO, nitric oxide; NOS, nitric oxide synthase; NPY, neuropeptide Y; NT, neurotrophin; NT-3, neurotrophin-3; NT-4, neurotrophin-4; PAF, primary afferent fiber; PLC, phospholipase C; SP, substance P; VIP, vasoactive intestinal polypeptide; VPR, ve ntral root potential; WDR, wide dynamic range.

References

Acheson, A., Conover, J. C., Fandl, J. P., DeChiara, T. M., Russell, M., Thadani, A., Squinto, S. P., Yancopoulos, G. D., and Lindsay, R. M. (1995) A BDNF autocrine loop in adult sensory neurons prevents cell death, *Nature*, 374: 450-453.

Aimar, P., Pasti, L., Carmignoto, G., and Merighi, A. (1998) Nitric oxide-producing islet cells modulate the release of sensory neuropeptides in the rat substantia gelatinosa, *J.Neurosci.*, 18: 10375-10388.

Akesson, E., Kjaeldgaard, A., Samuelsson, E. B., Seiger, A., and Sundstrom, E. (2000) Ionotropic glutamate receptor expression in human spinal cord during first trimester development, *Brain Res.Dev.Brain Res.*, 119: 55-63.

Akopian, A. N., Abson, N. C., and Wood, J. N. (1996) Molecular genetic approaches to nociceptor development and function, *Trends Neurosci.*, 19: 240-246.

Albuquerque, C., Lee, C. J., Jackson, A. C., and MacDermott, A. B. (1999) Subpopulations of GABAergic and non-GABAergic rat dorsal horn neurons express Ca2+-permeable AMPA receptors, *Eur.J.Neurosci.*, 11: 2758-2766.

Aloe, L., Manni, L., Properzi, F., De Santis, S., and Fiore, M. (2000) Evidence that nerve growth factor promotes the recovery of peripheral neuropathy induced in mice by cisplatin: behavioral, structural and biochemical analysis, *Auton.Neurosci.*, 86: 84-93.

Alvares, D. and Fitzgerald, M. (1999) Building blocks of pain: the regulation of key molecules in spinal sensory neurones during development and following peripheral axotomy, *Pain*, Suppl 6: S71-S85.

Amann, R., Schuligoi, R., Herzeg, G., and Donnerer, J. (1995) Intraplantar injection of nerve growth factor into the rat hind paw: local edema and effects on thermal nociceptive threshold, *Pain*, 64: 323-329.

Amara, S. G., Arriza, J. L., Leff, S. E., Swanson, L. W., Evans, R. M., and Rosenfeld, M. G. (1985) Expression in brain of a messenger RNA encoding a novel neuropeptide homologous to calcitonin gene-related peptide, *Science*, 229: 1094-1097.

Anand, P., Parrett, A., Martin, J., Zeman, S., Foley, P., Swash, M., Leigh, P. N., Cedarbaum, J. M., Lindsay, R. M., Williams-Chestnut, R. E., and Sinicropi, D. V. (1995) Regional changes of ciliary neurotrophic factor and nerve growth factor levels in post mortem spinal cord and cerebral cortex from patients with motor disease, *Nature Med.*, 1: 168-172.

Apfel, S. C., Wright, D. E., Wiideman, A. M., Dormia, C., Snider, W. D., and Kessler, J. A. (1996) Nerve growth factor regulates the expression of brain-derived neurotrophic factor mRNA in the peripheral nervous system, *Mol.Cell Neurosci.*, 7: 134-142.

Arvanov, V. L., Seebach, B. S., and Mendell, L. M. (2000) NT-3 evokes an LTP-like facilitation of AMPA/kainate receptor- mediated synaptic transmission in the neonatal rat spinal cord., *J.Neurophysiol.*, 84: 752-758.

Baldelli, P., Magnelli, V., and Carbone, E. (1999) Selective up-regulation of P- and R-type Ca2+ channels in rat embryo motoneurons by BDNF., *Eur.J.Neurosci.*, 11: 1127-1133.

Baldelli, P., Novara, M., Carabelli, V., Hernandez-Guijo, J. M., and Carbone, E. (2002) BDNF up-regulates evoked GABAergic transmission in developing hippocampus by potentiating presynaptic N- and P/Q-type Ca2+ channels signalling, *Eur.J.Neurosci.*, 16: 2297-2310.

Bardoni, R. (2001) Excitatory synaptic transmission in neonatal dorsal horn: NMDA and ATP receptors, *News Physiol.Sci.*, 16: 95-100.

Bardoni, R., Magherini, P. C., and MacDermott, A. B. (2000) Activation of NMDA receptors drives action potentials in superficial dorsal horn from neonatal rats, *Neuroreport*, 11: 1721-1727.

Battaglia, G. and Rustioni, A. (1988) Coexistence of glutamate and substance P in dorsal root ganglion neurons of the rat and monkey, *J.Comp.Neurol.*, 277: 302-312.

Bennett, D. L. (2001) Neurotrophic factors: important regulators of nociceptive function, *Neuroscientist*, 7: 13-17.

Bennett, D. L., French, J., Priestley, J. V., and McMahon, S. B. (1996) NGF but not NT-3 or BDNF prevents the A fiber sprouting into lamina II of the spinal cord that occurs following axotomy, *Mol.Cell Neurosci.*, 8: 211-220.

Bennett, D. L., Michael, G. J., Ramachandran, N., Munson, J. B., Averill, S., Yan, Q., McMahon, S. B., and Priestley, J. V. (1998) A distinct subgroup of small DRG cells express GDNF receptor components and GDNF is protective for these neurons after nerve injury, *J.Neurosci.*, 18: 3059-3072.

Bentley, G. N. and Gent, J. P. (1995) Neurokinin actions on substantia gelatinosa neurones in an adult longitudinal spinal cord preparation, *Brain Res.*, 673: 101-111.

Berninger, B., Marty, S., Zafra, F., da Penha, B. M., Thoenen, H., and Lindholm, D. (1995) GABAergic stimulation switches from enhancing to repressing BDNF expression in rat hippocampal neurons during maturation in vitro, *Development*, 121: 2327-2335.

Berninger, B. and Poo, M. (1996) Fast actions of neurotrophic factors, *Curr.Opin.Neurobiol.*, 6: 324-330.

Beydoun, A., Dyke, D. B., Morrow, T. J., and Casey, K. L. (1996) Topical capsaicin selectively attenuates heat pain and A delta fiber- mediated laser-evoked potentials, *Pain*, 65: 189-196.

Beydoun, A., Morrow, T. J., and Casey, K. L. (1997) Pain-related laser-evoked potentials in awake monkeys: identification of components, behavioral correlates and drug effects, *Pain*, 72: 319-324.

Blackshaw, L. A. and Gebhart, G. F. (2002) The pharmacology of gastrointestinal nociceptive pathways, *Curr.Opin.Pharmacol.*, 2: 642-649.

Bleazard, L., Hill, R. G., and Morris, R. (1994) The correlation between the distribution of the NK_1 receptor and the actions of tachykinin agonists in the dorsal horn of the rat indicates that substance P does not have a functional role on substantia gelatinosa (lamina II) neurons, *J.Neurosci.*, 14: 7655-7664.

Blochl, A. and Thoenen, H. (1995) Characterization of nerve growth factor (NGF) release from hippocampal neurons: evidence for a constitutive and an unconventional sodium-dependent regulated pathway, *Eur.J.Neurosci.*, 7: 1220-1228.

Blochl, A. and Thoenen, H. (1996) Localization of cellular storage compartments and sites of constitutive and activity-dependent release of nerve growth factor (NGF) in primary cultures of hippocampal neurons, *Mol.Cell Neurosci.*, 7: 173-190.

Boyce, S., Wyatt, A., Webb, J. K., O'Donnell, R., Mason, G., Rigby, M., Sirinathsinghji, D., Hill, R. G., and Rupniak, N. M. (1999) Selective NMDA NR2B antagonists induce antinociception without motor dysfunction: correlation with restricted localisation of NR2B subunit in dorsal horn, *Neuropharmacology*, 38: 611-623.

Brown, J. L., Liu, H., Maggio, J. E., Vigna, S. R., Mantyh, P. W., and Basbaum, A. I. (1995) Morphological characterization of substance P receptor-immunoreactive neurons in the rat spinal cord and trigeminal nucleus caudalis, *J.Comp.Neurol.*, 356: 327-344.

Brown, M. C., Perry, V. H., Lunn, E. R., Gordon, S., and Heumann, R. (1991) Macrophage dependence of peripheral sensory nerve regeneration: possible involvement of nerve growth factor, *Neuron*, 6: 359-370.

Burnstock, G. (2000) P2X receptors in sensory neurones, *Br.J.Anaesth.*, 84: 476-488.

Calzà, L., Fernandez, M., Giuliani, A., Aloe, L., and Giardino, L. (2002) Thyroid hormone activates oligodendrocyte precursors and increases a myelin- forming protein and NGF content in the spinal cord during experimental allergic encephalomyelitis, *Proc.Natl.Acad.Sci.U.S.A.*, 99: 3258-3263.

Calzà, L., Giardino, L., Pozza, M., Micera, A., and Aloe, L. (1997) Time-course changes of nerve- growth factor, corticotropin-releasing hormone, and nitric oxide synthase isoforms and their possible role in the development of inflammatory response in experimental allergic encephalomyelitis., *Proc.Natl.Acad.Sci.USA*, 94: 3368-3373.

Carmignoto, G., Zonta, M., Gobbo, S., Lossi, L., Gustincich, S., Salio, C., and Merighi, A. (2003) Pre-synaptic modulatory effect of brain-derived neurotrophic factor (BDNF) on the release of sensory neuromessengers in rat spinal cord slices, *Pain*, submitted.

Carroll, S. L., Silos-Santiago, I., Frese, S. E., Ruit, K. G., Milbrandt, J., and Snider, W. D. (1992) Dorsal root ganglion neurons expressing trk are selectively sensitive to NGF deprivation in utero, *Neuron*, 9: 779-788.

Cejas, P. J., Martinez, M., Karmally, S., McKillop, M., McKillop, J., Plunkett, J. A., Oudega, M., and Eaton, M. J. (2000) Lumbar transplant of neurons genetically modified to secrete brain-derived neurotrophic factor attenuates allodynia and hyperalgesia after sciatic nerve constriction, *Pain*, 86: 195-210.

Cervero, F. (1995) Visceral pain: Mechanisms of peripheral and central sensitization, *Ann.Med.*, 27: 235-239.

Cervero, F. and Connell, L. A. (1984) Distribution of somatic and visceral primary afferent fibres within the thoracic spinal cord of the cat, *J.Comp.Neurol.*, 230: 88-98.

Cho, H. J., Kim, J. K., Zhou, X. F., and Rush, R. A. (1997) Increased brain-derived neurotrophic factor immunoreactivity in rat dorsal root ganglia and spinal cord following peripheral inflammation, *Brain Res.*, 764: 269-272.

Cho, H. J., Park, E. H., Bae, M. A., and Kim, J. K. (1996) Expression of mRNAs for preprotachykinin and nerve growth factor receptors in the dorsal root ganglion following peripheal inflammation., *Brain Res.*, 716: 197-201.

Conn, P. J. and Pin, J. P. (1997) Pharmacology and functions of metabotropic glutamate receptors, *Ann.Rev.Pharmacol.*, 37: 205-237.

Croll, S. D., Wiegand, S. J., Anderson, K. D., Lindsay, R. M., and Nawa, H. (1994) Regulation of neuropeptides in adult rat forebrain by the neurotrophins BDNF and NGF, *Eur.J.Neurosci.*, 6: 1343-1353.

Curras, M. C. and Dingledine, R. J. (1992) Selectivity of amino acid transmitters acting at N-methyl D-aspartate and amino-3-hydroxy-5-methyl-4-isoxazolypropionate receptors, *Mol.Pharmacol.*, 41: 520-526.

Davis, B. M., Lewin, G. R., Mendell, L. M., Jones, M. E., and Albers, K. M. (1993) Altered expression of nerve growth factor in the skin of transgenic mice leads to changes in response to mechanical stimuli, *Neuroscience*, 56: 789-792.

De Biasi, S. and Rustioni, A. (1988) Glutamate and substance P coexist in primary afferent terminals in the superficial laminae of the spinal cord, *Proc.Natl.Acad.Sci.USA*, 85: 7820-7824.

Delcroix, J. D., Michael, G. J., Priestley, J. V., Tomlinson, D. R., and Fernyhough, P. (1998) Effect of nerve growth factor treatment on p75NTR gene expression in lumbar dorsal root ganglia of streptozocin- induced diabetic rats, *Diabetes*, 47: 1779-1785.

Delcroix, J. D., Tomlinson, D. R., and Fernyhough, P. (1997) Diabetes and axotomy- induced deficits in retrograde axonal transport of nerve growth factor correlate with decreased levels of p75LNTR protein in lumbar dorsal root ganglia, *Brain Res.Mol.Brain Res.*, 51: 82-90.

Distefano, P. S., Friedman, B., Radziejewski, C., Alexander, C., Boland, P., Schick, C. M., Lindsay, R. M., and Wiegand, S. J. (1992) The neurotrophins BDNF, NT-3, and NGF display distinct patterns of retrograde axonal transport in peripheral and central neurons, *Neuron*, 8: 983-993.

Dmitrieva, N. and McMahon, S. B. (1996) Sensitisation of visceral afferents by nerve growth factor in the adult rat, *Pain*, 66: 87-97.

Dmitrieva, N., Shelton, D., Rice, A. S., and McMahon, S. B. (1997) The role of nerve growth factor in a model of visceral inflammation, *Neuroscience*, 78: 449-459.

Donnerer, J., Schuligoi, R., and Stein, C. (1992) Increased content and transport of substance P and calcitonin gene-related peptide in sensory nerves innervating inflamed tissue: evidence for a regulatory function of nerve growth factor in vivo, *Neuroscience*, 49: 693-698.

Donnerer, J., Schuligoi, R., Stein, C., and Amann, R. (1993) Upregulation, release and axonal transport of substance P and calcitonin gene-related peptide in adjuvant inflammation and regulatory function of nerve growth factor, *Regul.Pept.*, 46: 150-154.

Dubner, R. and Ren, K. (1999) Endogenous mechanisms of sensory modulation, *Pain*, Suppl 6: S45-S53.

Dyck, P. J., Peroutka, S., Rask, C., Burton, E., Baker, M. K., Lehman, K. A., Gillen, D. A., Hokanson, J. L., and O'Brien, P. C. (1997) Intradermal recombinant human nerve growth factor induces pressure allodynia and lowered heat-pain threshold in humans., *Neurology*501-505.

Eaton, M. J., Blits, B., Ruitenberg, M. J., Verhaagen, J., and Oudega, M. (2002) Amelioration of chronic neuropathic pain after partial nerve injury by adeno-associated viral (AAV) vector- mediated over-expression of BDNF in the rat spinal cord, *Gene Ther.*, 9: 1387-1395.

Ehlers, M. D., Kaplan, D. R., Price, D. L., and Koliatsos, V. E. (1995) NGF-stimulated retrograde transport of trkA in the mammalian nervous system, *J.Cell Biol.*, 130: 149-156.

Engelman, H. S., Allen, T. B., and MacDermott, A. B. (1999) The distribution of neurons expressing calcium-permeable AMPA receptors in the superficial laminae of the spinal cord dorsal horn, *J.Neurosci.*, 19: 2081-2089.

Ernfors, P., Lee, K. F., and Jaenisch, R. (1994a) Mice lacking brain-derived neurotrophic factor develop with sensory deficits, *Nature*, 368: 147-150.

Ernfors, P., Lee, K.-F., Kucera, J., and Jaenisch, R. (1994b) Lack of neurotrophin-3 leads to deficiencies in the peripheral nervous system and loss of limb proprioceptive afferents, *Cell*, 77: 503-512.

Favaron, M., Manev, R. M., Rimland, J. M., Candeo, P., Beccaro, M., and Manev, H. (1993) NMDA-stimulated expression of BDNF mRNA in cultured cerebellar granule neurones, *Neuroreport*, 4: 1171-1174.

Fields, H. L., Heinricher, M. M., and Mason, P. (1991) Neurotransmitters in nociceptive modulatory circuits, *Annu.Rev.Neurosci.*, 14: 219-245.

Fitzgerald, M., Wall, P. D., Goedert, M., and Emson, P. C. (1985) Nerve growth factor counteracts the neurophysiological and neurochemical effects of chronic sciatic nerve section, *Brain Res.*, 332: 131-141.

Fukuoka, T., Kondo, E., Dai, Y., Hashimoto, N., and Noguchi, K. (2001) Brain-derived neurotrophic factor increases in the uninjured dorsal root ganglion neurons in selective spinal nerve ligation model, *J.Neurosci.*, 21: 4891-4900.

Gebhart, G. F. (1995) Visceral nociception: consequences, modulation and the future, *Eur.J.Anaesthesiol.Suppl.*, 10: 24-27.

Gerber, G., Youn, D. H., Hsu, C. H., Isaev, D., and Randic, M. (2000a) Spinal dorsal horn synaptic plasticity: involvement of group I metabotropic glutamate receptors, *Prog.Brain Res.*, 129: 115-134.

Gerber, G., Zhong, J., Youn, D., and Randic, M. (2000b) Group II and group III metabotropic glutamate receptor agonists depress synaptic transmission in the rat spinal cord dorsal horn, *Neuroscience*, 100: 393-406.

Gottschalk, W., Pozzo-Miller, L. D., Figurov, A., and Lu, B. (1998) Presynaptic modulation of synaptic transmission and plasticity by brain-derived neurotrophic factor in the developing hippocampus., *J.Neurosci.*, 18: 6830-6839.

Grimes, M. L., Zhou, J., Beattie, E. C., Yuen, E. C., Hall, D. E., Valletta, J. S., Topp, K. S., LaVail, J. H., Bunnett, N. W., and Mobley, W. C. (1996) Endocytosis of activated TrkA: evidence that nerve growth factor induces formation of signaling endosomes, *J.Neurosci.*, 16: 7950-7964.

Guo, A., Simone, D. A., Stone, L. S., Fairbanks, C. A., Wang, J., and Elde, R. (2001) Developmental shift of vanilloid receptor 1 (VR1) terminals into deeper regions of the superficial dorsal horn: correlation with a shift from TrkA to Ret expression by dorsal root ganglion neurons, *Eur.J.Neurosci.*, 14: 293-304.

Ha, S. O., Kim, J. K., Hong, H. S., Kim, D. S., and Cho, H. J. (2001) Expression of brain-derived neurotrophic factor in rat dorsal root ganglia, spinal cord and gracile nuclei in experimental models of neuropathic pain, *Neuroscience*, 107: 301-309.

Hains, B. C., Fullwood, S. D., Eaton, M. J., and Hulsebosch, C. E. (2001a) Subdural engraftment of serotonergic neurons following spinal hemisection

restores spinal serotonin, downregulates serotonin transporter, and increases BDNF tissue content in rat, *Brain Res.*, 913: 35-46.

Hains, B. C., Johnson, K. M., McAdoo, D. J., Eaton, M. J., and Hulsebosch, C. E. (2001b) Engraftment of serotonergic precursors enhances locomotor function and attenuates chronic central pain behavior following spinal hemisection injury in the rat, *Exp.Neurol.*, 171: 361-378.

Handwerker, H. O. and Kobal, G. (1993) Psychophysiology of experimentally induced pain, *Physiol.Rev.*, 73: 639-671.

Heath, M. J. S., Womack, M. D., and MacDermott, A. B. (1994) Substance P elevates intracellular calcium in both neurons and glial cells from the dorsal horn of the spinal cord, *J.Neurophysiol.*, 72: 1192-1198.

Heese, K., Otten, U., Mathivet, P., Raiteri, M., Marescaux, C., and Bernasconi, R. (2000) GABA(B) receptor antagonists elevate both mRNA and protein levels of the neurotrophins nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) but not neurotrophin-3 (NT-3) in brain and spinal cord of rats, *Neuropharmacology*, 39: 449-462.

Helgren, M. E., Cliffer, K. D., Torrento, K., Cavnor, C., Curtis, R., Distefano, P. S., Wiegand, S. J., and Lindsay, R. M. (1997) Neurotrophin-3 administration attenuates deficits of pyridoxine- induced large-fiber sensory neuropathy, *J Neurosci.*, 17: 372-382.

Helke, C. J., Krause, J. E., Mantyh, P. W., and Couture, R. (1990) Diversity of tachykinin peptidergic neurons: multiple peptides, receptors and regulatory mechanisms, *FASEB J.*, 4: 1606-1615.

Henry, M. A., Westrum, L. E., Bothwell, M., and Press, S. (1994) Electron microscopic localization of nerve growth factor receptor (p75)-immunoreactivity in pars caudalis/medullary dorsal horn of the cat, *Brain Res.*, 642: 137-145.

Heppenstall, P. A. and Fleetwood-Walker, S. M. (1997) The glycine site of the NMDA receptor contributes to neurokinin1 receptor agonist facilitation of NMDA receptor agonist-evoked activity in rat dorsal horn neurons, *Brain Res.*, 744: 235-245.

Heppenstall, P. A. and Lewin, G. R. (2001) BDNF but not NT-4 is required for normal flexion reflex plasticity and function., *Proc.Natl.Acad.Sci.USA*, 98: 8107-8112.

Herrero, J. F., Laird, J. M., and Lopez-Garcia, J. A. (2000) Wind- up of spinal cord neurones and pain sensation: much ado about something?, *Prog.Neurobiol.*, 61: 169-203.

Heumann, R. (1994) Neurotrophin signalling, *Curr.Opin.Neurobiol.*, 4: 668-679.

Hoheisel, U., Mense, S., Simons, D. G., and Yu, X.-M. (1993) Appearance of new receptive fields in rat dorsal horn neurons following noxious stimulation of skeletal muscle: A model for referral of muscle pain, *Neurosci.Lett.*, 153: 9-12.

Hökfelt, T. (1991) Neuropeptide in perspective: the last ten years, *Neuron*, 7: 867-879.

Hökfelt, T., Broberger, C., Xu, Z.-Q. D., Sergeyev, V., Ubink, R., and Diez, M. (2000) Neuropeptides - an overview, *Neuropharmacology*, 39: 1337-1356.

Hory-Lee, F., Russell, M., Lindsay, R. M., and Frank, E. (1993) Neurotrophin 3 supports the survival of developing muscle sensory neurons in culture, *Proc.Natl.Acad.Sci.U.S.A*, 90: 2613-2617.

Huang, Z. J., Kirkwood, A., Pizzorusso, T., Porciatti, V., Morales, B., Bear, M. F., Maffei, L., and Tonegawa, S. (1999) BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex., *Cell*, 98: 739-755.

Hunt, S. P. and Mantyh, P. W. (2001) The molecular dynamics of pain control, *Nat.Rev.Neurosci.*, 2: 83-91.

Hutchinson, G. B., McLe nnan, H., and Wheal, H. V. (1978) The responses of Renshaw cells and spinal interneurons of the rat to L- glutamate and L-aspartate, *Brain Res.*, 141: 129-136.

Inaishi, Y., Kashihara, Y., Sakaguchi, M., Nawa, H., and Kuno, M. (1992) Cooperative regulation of calcitonin gene-related peptide levels in rat sensory neurons via their central and peripheral processes, *J.Neurosci.*, 12: 518-524.

Jarvis, C. R., Xiong, Z. G., Plant, J. R., Churchill, D., Lu, W. Y., MacVicar, B. A., and MacDonald, J. F. (1997) Neurotrophin modulation of NMDA receptors in cultured murine and isolated rat neurons, *J Neurophysiol.*, 78: 2363-2371.

Ji, R. R., Samad, T. A., Jin, S. X., Schmoll, R., and Woolf, C. J. (2002) p38 MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia, *Neuron*, 36: 57-68.

Ji, R. R. and 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.

Johnson, J. L. (1972) Glutamic acid as a synaptic transmitter candidate in dorsal sensory neuron - reconsideration, *Life Sci.*, 20: 1637-1644.

Kaplan, D. R. and Cooper, E. (2001) PI-3 kinase and IP3: partners in NT3-induced synaptic transmission., *Nat.Neurosci.*, 4: 5-7.

Kaplan, D. R. and Stephens, R. M. (1994) Neurotrophin signal transduction by the Trk receptor, *J.Neurobiol.*, 25: 1404-1417.

Kerchner, G. A., Wang, G. D., Qiu, C. S., Huettner, J. E., and Zhuo, M. (2001a) Direct presynaptic regulation of GABA/glycine release by kainate receptors in the dorsal horn: an ionotropic mechanism, *Neuron*, 32: 477-488.

Kerchner, G. A., Wilding, T. J., Huettner, J. E., and Zhuo, M. (2002) Kainate receptor subunits underlying presynaptic regulation of transmitter release in the dorsal horn, *J.Neurosci.*, 22: 8010-8017.

Kerchner, G. A., Wilding, T. J., Li, P., Zhuo, M., and Huettner, J. E. (2001b) Presynaptic kainate receptors regulate spinal sensory transmission, *J.Neurosci.*, 21: 59-66.

Kerkut, G. A. and Bagust, J. (1995) The isolated mammalian spinal cord, *Prog.Neurobiol.*, 46: 1-48.

Kerr, B. J., Bradbury, E. J., Bennett, D. L., Trivedi, P. M., Dassan, P., French, J., Shelton, D. B., McMahon, S. B., and Thompson, S. W. (1999) Brain-derived neurotrophic factor modulates nociceptive sensory inputs and NMDA-evoked responses in the rat spinal cord., *J.Neurosci.*, 19: 5138-5148.

Kim, H. S., Lee, S. J., Kim, D. S., and Cho, H. J. (2000) Effects of brain-derived neurotrophic factor and neurotrophin-3 on expression of mRNAs encoding c-Fos, neuropeptides and glutamic acid decarboxylase in cultured spinal neurons, *Neuroreport*, 11: 3873-3876.

Kitzman, P. H., Perrone, T. N., LeMaster, A. M., Davis, B. M., and Albers, K. M. (1998) Level of p75 receptor expression in sensory ganglia is modulated by NGF level in the target tissue, *J.Neurobiol.*, 35: 258-270.

Kokaia, Z., Gido, G., Ringstedt, T., Bengzon, J., Kokaia, M., Siesjo, B. K., Persson, H., and Lindvall, O. (1993) Rapid increase of BDNF mRNA levels in cortical neurons following spreading depression: regulation by glutamatergic mechanisms independent of seizure activity, *Brain Res.Mol.Brain Res.*, 19: 277-286.

Koltzenburg, M. (1999) The changing sensitivity in the life of the nociceptor, *Pain*, Suppl 6: S93-102.

Koltzenburg, M., Bennett, D. L., Shelton, D. L., and McMahon, S. B. (1999) Neutralization of endogenous NGF prevents the sensitization of nociceptors supplying inflamed skin, *Eur.J Neurosci.*, 11: 1698-1704.

Laing, I., Todd, A. J., Heizmann, C. W., and Schmidt, H. H. H. W. (1994) Subpopulations of GABAergic neurons in laminae I-III of rat spinal dorsal horn defined by coexistence with classical transmitters, peptides, nitric oxide synthase or parvalbumin, *Neuroscience*, 61: 123-132.

Larson, A. A. and Kitto, K. F. (1997) Mutual antagonism between nerve growth factor and substance P N-terminal activity on nociceptive activity in mice, *J Pharmacol.Exp.Ther.*, 282: 1345-1350.

Lauterborn, J. C., Lynch, G., Vanderklish, P., Arai, A., and Gall, C. M. (2000) Positive modulation of AMPA receptors increases neurotrophin expression by hippocampal and cortical neurons, *J.Neurosci.*, 20: 8-21.

Le Bars, D. (2002) The whole body receptive field of dorsal horn multireceptive neurones, *Brain Res.Brain Res.Rev.*, 40: 29-44.

Lembeck, F. (1953) Zur Frage den zentralen bertragung afferenter Impulse. Das Vorkommen und die Bedeutung der Substanz P in den dorsalen Wurzeln des Rüchkenmarks, *Naunyn-Schmiedeberg's.Arch.Exp.Pathol.Pharm.*, 219: 197-213.

Lesser, S. S., Sherwood, N. T., and Lo, D. C. (1997) Neurotrophins differentially regulate voltage-gated ion channels, *Mol.Cell Neurosci.*, 10: 173-183.

Lever, I. J., Bradbury, E. J., Cunningham, J. R., Adelson, D. W., Jones, M. G., McMahon, S. B., Marvizon, J. C., and Malcangio, M. (2001) Brain-derived neurotrophic factor is released in the dorsal horn by distinctive patterns of afferent fiber stimulation, *J.Neurosci.*, 21: 4469-4477.

Levi-Montalcini, R. and Angeletti, P. U. (1968) Nerve growth factor, *Physiol.Rev.*, 48: 534-569.

Levine, E. S., Crozier, R. A., Black, I. B., and Plummer, M. R. (1998) Brain-derived neurotrophic factor modulates hippocampal synaptic transmission by increasing N-methyl-D-aspartic acid receptor activity., *Proc.Natl.Acad.Sci.U.S.A.*, 95: 10235-10239.

Lewin, G. R. and Barde, Y.-A. (1996) Physiology of neurotrophins, *Ann.Rev.Neurosci.*, 19: 289-317.

Lewin, G. R. and McMahon, S. B. (1991) Dorsal horn plasticity following re-routeing of peripheral nerves: evidence for tissue-specific neurotrophic influences from the periphery, *Eur.J.Neurosci.*, 3: 1112-1122.

Lewin, G. R. and Mendell, L. M. (1993) Nerve growth factor and nociception, *Trends Neurosci.*, 16: 353-359.

Lewin, G. R. and Mendell, L. M. (1994) Regulation of cutaneous C-fiber heat nociceptors by nerve growth factor in the developing rat, *J.Neurophysiol.*, 71: 941-949.

Lewin, G. R., Ritter, A. M., and Mendell, L. M. (1992) On the role of nerve growth factor in the development of myelinated nociceptors, *J.Neurosci.*, 12: 1896-1905.

Lewin, G. R., Ritter, A. M., and Mendell, L. M. (1993) Nerve growth factor-induced hyperalgesia in the neonatal and adult rat, *J.Neurosci.*, 13: 2136-2148.

Lewin, G. R., Rueff, A., and Mendell, L. M. (1994) Peripheral and central mechanisms of NGF-induced hyperalgesia, *Eur.J.Neurosci.*, 6: 1903-1912.

Li, P., Kerchner, G. A., Sala, C., Wei, F., Huettner, J. E., Sheng, M., and Zhuo, M. (1999a) AMPA receptor-PDZ interactions in facilitation of spinal sensory synapses, *Nat.Neurosci.*, 2: 972-977.

Li, P., Wilding, T. J., Kim, S. J., Calejesan, A. A., Huettner, J. E., and Zhuo, M. (1999b) Kainate-receptor- mediated sensory synaptic transmission in mammalian spinal cord, *Nature*, 397: 161-164.

Li, Y. X., Zhang, Y., Ju, D., Lester, H. A., Shuman, E. M., and Davidson, N. (1998) Expression of a dominant negative TrkB receptor, T1, reveals a requirement for presynaptic signaling in BDNF-induced synaptic potentiation in cultured hippocampal neurons., *Proc.Natl.Acad.Sci.U.S.A*, 95: 10884-10889.

Liebl, D. J., Klesse, L. J., Tessarollo, L., Wohlman, T., and Parada, L. F. (2000) Loss of brain-derived neurotrophic factor-dependent neural crest-derived sensory neurons in neurotrophin-4 mutant mice, *Proc.Natl.Acad.Sci.U.S.A*, 97: 2297-2302.

Lindholm, D., Castren, E., Berzaghi, M., Blochl, A., and Thoenen, H. (1994) Activity-dependent and hormonal regulation of neurotrophin mRNA levels in the brain--implications for neuronal plasticity, *J.Neurobiol.*, 25: 1362-1372.

Lindsay, R. M. and Harmar, A. J. (1989) Nerve growth factor regulates expression of neuropeptide genes in adult sensory neurons, *Letters to Nature*, 337: 362-364.

Lindsay, R. M., Lockett, C., Sternberg, J., and Winter, J. (1989) Neuropeptide expression in cultures of adult sensory neurons: modulation of substance P and calcitonin gene-related peptide levels by nerve growth factor, *Neuroscience*, 33: 53-53.

Liu, H., Brown, J. L., Jasmin, L., Maggio, J. E., Vigna, S. R., Mantyh, P. W., and Basbaum, A. I. (1994a) Synaptic relationship between substance P and the substance P receptor: Light and electron microscopic characterization of the mismatch between neuropeptides and their receptors, *Proc.Natl.Acad.Sci.USA*, 91: 1009-1013.

Liu, H., Brown, J. L., Jasmin, L., Maggio, J. E., Vigna, S. R., Mantyh, P. W., and Basbaum, A. I. (1994b) Synaptic relationship between substance P and the substance P receptor: light and electron microscopic characterization of the mismatch between neuropeptides and their receptors., *Proc.Natl.Acad.Sci.U.S.A.*, 91: 1009-1013.

Liu, H., Wang, H., Sheng, M., Jan, L. Y., Jan, Y. N., and Basbaum, A. I. (1994c) Evidence for presynaptic *N*-methyl-D-aspartate autoreceptors in the spinal cord dorsal horn, *Proc.Natl.Acad.Sci.USA*, 91: 8383-8387.

Liu, H., Wang, H., Sheng, M., Jan, L. Y., Jan, Y. N., and Basbaum, A. I. (1994d) Evidence for presynaptic N-methyl-D-aspartate autoreceptors in the spinal cord dorsal horn, *Proc.Natl.Acad.Sci.U.S.A*, 91: 8383-8387.

Liu, X. G. and Sandkuhler, J. (1998) Activation of spinal N- methyl- D-aspartate or neurokinin receptors induces long-term potentiation of spinal C-fibre-evoked potentials, *Neuroscience*, 86: 1209-1216.

Lu, C. R., Hwang, S. J., Phend, K. D., Rustioni, A., and Valtschanoff, J. G. (2002) Primary afferent terminals in spinal cord express presynaptic AMPA receptors, *J.Neurosci.*, 22: 9522-9529.

Lu, C. R., Hwang, S. J., Phend, K. D., Rustioni, A., and Valtschanoff, J. G. (2003) Primary afferent terminals that express presynaptic NR1 in rats are mainly from myelinated, mechanosensitive fibers, *J.Comp.Neurol.*, 460: 191-202.

Lucas, G., Hendolin, P., Harkany, T., Agerman, K., Paratcha, G., Holmgren, C., Zilberter, Y., Sairanen, M., Minichiello, L., Castren, E., and Ernfors, P. (2003) Neurotrophin-4 mediated TrkB activation reinforces morphine-induced analgesia, *Nat.Neurosci.*, 6: 221-222.

Lundberg, J. M. (1996) Pharmacology of cotransmission in the autonomic nervous system: integrative aspects on amines, neuropeptides, adenosine triphosphate, amino acids and nitric oxide., *Pharmacol.Rev.*, 48: 113-178.

Ma, W. and Bisby, M. A. (1998) Increase of preprotachykinin mRNA and substance P immunoreactivity in spared dorsal root ganglion neurons following partial sciatic nerve injury, *Eur.J Neurosci.*, 10: 2388-2399.

Malcangio, M. and Bowery, N. G. (1996) GABA and its receptors in the spinal cord, *Trends Pharmacol.Sci.*, 17: 457-462.

Malcangio, M., Bowery, N. G., Flower, R. J., and Perretti, M. (1996) Effect of interleukin-1? on the release of substance P from rat isolated spinal cord, *Eur.J.Pharmacol.*, 299: 113-118.

Malcangio, M., Fernandes, K., and Tomlinson, D. R. (1998) NMDA receptor activation modulates evoked release of substance P from rat spinal cord, *Br.J.Pharmacol.*, 125: 1625-1626.

Malcangio, M., Garrett, N. E., Cruwys, S., and Tomlinson, D. R. (1997) Nerve growth factor- and neurotrophin-3- induced changes in nociceptive threshold and the release of substance P from the rat isolated spinal cord, *J.Neurosci.*, 17: 8459-8467.

Malcangio, M. and Lessmann, V. (2003) A common thread for pain and memory synapses? Brain-derived neurotrophic factor and trkB receptors, *Trends Pharmacol.Sci.*, 24: 116-121.

Malcangio, M., Ramer, M. S., Boucher, T. J., and McMahon, S. B. (2000) Intrathecally injected neurotrophins and the release of substance P from the rat isolated spinal cord, *Eur.J.Neurosci.*, 12: 139-144.

Mannion, R. J., Costigan, M., Decosterd, I., Amaya, F., Ma, Q. P., Holstege, J. C., Ji, R. R., Acheson, A., Lindsay, R. M., Wilkinson, G. A., and Woolf, C. J. (1999) Neurotrophins: peripherally and centrally acting modulators of tactile stimulus-induced inflammatory pain hypersensitivity., *Proc.Natl.Acad.Sci.U.S.A.*, 96: 9385-9390.

Mantyh, P. W., Rogers, S. D., Honoré, P., Allen, B. J., Ghilardi, J. R., Li, J., Daughters, R. S., Lappi, D. L., Wiley, R. G., and Simone, D. A. (1997) Inhibition of hyperalgesia by ablation of lamina I spinal neurons expressing substance P receptor, *Science*, 278: 275-279.

Marchettini, P., Lacerenza, M., and Formaglio, F. (2000) Sympathetically maintained pain, *Curr.Rev.Pain*, 4: 99-104.

Marchettini, P., Simone, D. A., Caputi, G., and Ochoa, J. L. (1996) Pain from excitation of identified muscle nociceptors in humans, *Brain Res.*, 740: 109-116.

Marty, S., Berninger, B., Carroll, P., and Thoenen, H. (1996) GABAergic stimulation regulates the phenotype of hippocampal interneurons through the regulation of brain-derived neurotrophic factor, *Neuron*, 16: 565-570.

Marvizon, J. C., Martinez, V., Grady, E. F., Bunnett, N. W., and Mayer, E. A. (1997) Neurokinin 1 receptor internalization in spinal cord slices induced by dorsal root stimulation is mediated by NMDA receptors, *J.Neurosci.*, 17: 8129-8136.

Maxwell, D. J., Christie, W. H., Short, A. D., Störm-Mathisen, J., and Ottersen, O. P. (1990) Central boutons of glomeruli in the spinal cord of the cat are enriched with L-glutamate-like immunoreactivity, *Neuroscience*, 36: 83-104.

McHugh, J. M. and McHugh, W. B. (2000) Pain: neuroanatomy, chemical mediators, and clinical implications, *AACN.Clin.Issues*, 11: 168-178.

McMahon, S. B. (1996) NGF as a mediator of inflammatory pain, *Philos.Trans.R.Soc.Lond B Biol.Sci.*, 351: 431-440.

McMahon, S. B., Armanini, M. P., Ling, L. H., and Phillips, H. S. (1994) Expression and coexpression of Trk receptors in subpopulations of adult primary sensory neurons projecting to identified peripheral targets, *Neuron*, 12: 1161-1171.

McMahon, S. B., Bennett, D. L., Priestley, J. V., and Shelton, D. L. (1995) The biological effects of endogenous nerve growth factor on adult sensory neurons revealed by a trkA-IgG fusion molecule, *Nat.Med.*, 1: 774-780.

Meller, S. T. and Gebhart, G. F. (1994) Spinal mediators of hyperalgesia, *Drugs*, 47: 10-20.

Mendell, L. M. and Arvanian, V. L. (2002) Diversity of neurotrophin action in the postnatal spinal cord, *Brain Res.Brain Res.Rev.*, 40: 230-239.

Mendell, L. M., Munson, J. B., and Arvanian, V. L. (2001) Neurotrophins and synaptic plasticity in the mammalian spinal cord, *J.Physiol.*, 533: 91-97.

Mense, S. (1993) Nociception from skeletal muscle in relation to clinical muscle pain, *Pain*, 54: 241-289.

Merighi, A. (2002) Costorage and coexistence of neuropeptides in the mammalian CNS, *Progr.Neurobiol.*, 66: 161-190.

Merighi, A., Aimar, P., Pasti, L., Lossi, L., and Carmigno to, G. (2000) Neuromodulatory effects of nitric oxide in pain perception, In Poli, G., Cadenas,

E., and Packer, L. (Eds.) *Free radicals in brain pathophysiology,* Marcel Dekker, New York, pp. 17-53.

Merighi, A., Polak, J. M., and Theodosis, D. T. (1991) Ult rastructural visualization of glutamate and aspartate immunoreactivities in the rat dorsal horn with special reference to the co-localization of glutamate, substance P and calcitonin gene-related peptide, *Neuroscience*, 40: 67-80.

Merighi, A., Zonta, M., & Carmignoto, G. (1999) Neurotrophins acutely alter intracellular calcium levels in dorsal horn neurons of the rat spinal cord, *Abstracts of the Society of Neuroscience USA*, 25: 1043-1043.

Michael, G. J., Averill, S., Nitkunan, A., Rattray, M., Bennett, D. L. H., Yan, Q., and Priestley, J. V. (1997) Nerve growth factor treatment increases brain-derived neurotrophic factor selectively in trk-A expressing dorsal root ganglion cells and in their central terminations within the spinal cord, *J.Neurosci.*, 17: 8476-8490.

Michael, G. J., Averill, S., Shortland, P. J., Yan, Q., and Priestley, J. V. (1999) Axotomy results in major changes in BDNF expression by dorsal root ganglion cells: BDNF expression in large trkB and trkC cells, in pericellular baskets, and in projections to deep dorsal horn and dorsal column nuclei., *Eur.J Neurosci.*, 11: 3539-3551.

Miletic, G. and Miletic, V. (2002) Increases in the concentration of brain derived neurotrophic factor in the lumbar spinal dorsal horn are associated with pain behavior following chronic constriction injury in rats, *Neurosci.Lett.*, 319: 137-140.

Millan, M. J. (1999) The induction of pain: an integrative review, *Prog.Neurobiol.*, 57: 1-164.

Morisset, V., Ahluwalia, J., Nagy, I., and Urban, L. (2001) Possible mechanisms of cannabinoid-induced antinociception in the spinal cord, *Eur.J.Pharmacol.*, 429: 93-100.

Mowla, S. J., Pareek, S., Farhadi, H. F., Petrecca, K., Fawcett, J. P., Seidah, N. G., Morris, S. J., Sossin, W. S., and Murphy, R. A. (1999) Differential sorting of nerve growth factor and brain-derived neurotrophic factor in hippocampal neurons., *J.Neurosci.*, 19: 2069-2080.

Munglani, R., Harrison, S. M., Smith, G. D., Bountra, C., Birch, P. J., Elliot, P. J., and Hunt, S. P. (1996) Neuropeptide changes persist in spinal cord despite resolving hyperalgesia in a rat model of mononeuropathy, *Brain Res.*, 743: 102-108.

Nakaya, Y., Kaneko, T., Shigemoto, R., Nakanishi, S., and Mizuno, N. (1994) Immunohistochemical localization of substance P receptor in the central nervous system of the adult rat, *J.Comp.Neurol.*, 347: 249-274.

Neumann, S., Doubell, T. P., Leslie, T., and Woolf, C. J. (1996) Inflammatory pain hypersensitivity mediated by phenotypic switch in myelinated primary sensory neurons, *Nature*, 384: 360-364.

Oakley, R. A., Garner, A. S., Large, T. H., and Frank, E. (1995) Muscle sensory neurons require neurotrophin-3 from peripheral tissues during the period of normal cell death, *Development*, 121: 1341-1350.

Obata, K., Tsujino, H., Yamanaka, H., Yi, D., Fukuoka, T., Hashimo to, N., Yonenobu, K., Yoshikawa, H., and Noguchi, K. (2002) Expression of neurotrophic factors in the dorsal root ganglion in a rat model of lumbar disc herniation, *Pain*, 99: 121-132.

Obrietan, K., Gao, X. B., and van den Pol, A. N. (2002) Excitatory actions of GABA increase BDNF expression via a MAPK-CREB-dependent mechanism--a positive feedback circuit in developing neurons, *J.Neurophysiol.*, 88: 1005-1015.

Otten, U., Goedert, M., Mayer, N., and Lembeck, F. (1980) Requirement of nerve growth factor for development of substance P-containing sensory neurones, *Nature*, 287: 158-159.

Otten, U., Ruegg, U. T., Hill, R. C., Businger, F., and Peck, M. (1982) Correlation between substance P content of primary sensory neurones and pain sensitivity in rats exposed to antibodies to nerve growth factor, *Eur.J.Pharmacol.*, 85: 351-353.

Ottersen, O. P. and Störm-Mathisen, J. (1984) Glutamate- and GABA-containing neurons in the mouse and rat brain as demonstrated with a new immunocytochemical technique, *J.Comp.Neurol.*, 229: 374-392.

Ottersen, O. P. and Störm-Mathisen, J. (1987) Localization of amino acid neurotransmitters by immunocytochemistry, *Trends Neurosci.*, 10: 250-255.

Owolabi, J. B., Rizkalla, G., Tehim, A., Ross, G. M., Riopelle, R. J., Kamboj, R., Ossipov, M., Bian, D., Wegert, S., Porreca, F., and Lee, D. K. (1999) Characterization of antiallodynic actions of ALE-0540, a novel nerve growth factor receptor antagonist, in the rat, *J.Pharmacol.Exp.Ther.*, 289: 1271-1276.

Pesavento, E., Margotti, E., Righi, M., Cattaneo, A., and Domenici, L. (2000) Blocking the NGF-TrkA interaction rescues the developmental loss of LTP in the rat visual cortex: role of the cholinergic system., *Neuron*, 25: 165-175.

Petersen-Zeitz, K. R. and Basbaum, A. I. (1999) Second messengers, the substantia gelatinosa and injury-induced persistent pain, *Pain*, Suppl 6: S5-12.

Pezet, S., Cunningham, J., Patel, J., Grist, J., Gavazzi, I., Lever, I. J., and Malcangio, M. (2002a) BDNF modulates sensory neuron synaptic activity by a facilitation of GABA trans mission in the dorsal horn, *Mol.Cell Neurosci.*, 21: 51-62.

Pezet, S., Malcangio, M., Lever, I. J., Perkinton, M. S., Thompson, S. W., Williams, R. J., and McMahon, S. B. (2002b) Noxious stimulation induces Trk receptor and downstream ERK phosphorylation in spinal dorsal horn, *Mol.Cell Neurosci.*, 21: 684-695.

Pezet, S., Malcangio, M., and McMahon, S. B. (2002c) BDNF: a neuromodulator in nociceptive pathways?, *Brain Res.Brain Res.Rev.*, 40: 240-249.

Pezet, S., Onteniente, B., Jullien, J., Junier, M. P., Grannec, G., Rudkin, B. B., and Calvino, B. (2001) Differential regulation of NGF receptors in primary sensory neurons by adjuvant-induced arthritis in the rat, *Pain*, 90: 113-125.

Pizzorusso, T., Ratto, G. M., Putignano, E., and Maffei, L. (2000) Brain-derived neurotrophic factor causes cAMP response element-binding protein phosphorylation in absence of calcium increases in slices and cultured neurons from rat visual cortex, *J.Neurosci.*, 20: 2809-2816.

Popratiloff, A., Weinberg, R. J., and Rustioni, A. (1998) AMPA receptors at primary afferent synapses in substantia gelatinosa after sciatic nerve section, *Eur.J.Neurosci.*, 10: 3220-3230.

Pozzan, T., Rizzuto, R., Volpe, P., and Meldolesi, J. (1994) Molecular and cellular physiology of intracellular calcium stores, *Physiol.Rev.*, 74: 595-636.

Pozzo-Miller, L. D., Gottschalk, W., Zhang, L., McDermott, K., Du, J., Gopalakrishnan, R., Oho, C., Sheng, Z. H., and Lu, B. (1999) Impairments in high- frequency transmission, synaptic vesicle docking, and synaptic protein distribution in the hippocampus of BDNF knockout mice., *J.Neurosci.*, 19: 4972-4983.

Ramer, M. S., Bradbury, E. J., and McMahon, S. B. (2001) Nerve growth factor induces P2X(3) expression in sensory neurons, *J.Neurochem.*, 77: 864-875.

Ramer, M. S., Ma, W., Murphy, P. G., Richardson, P. M., and Bisby, M. A. (1998) Galanin expression in neuropathic pain: friend or foe?, *Ann.N.Y.Acad.Sci.*, 863: 390-401.

Ramer, M. S., Priestley, J. V., and McMahon, S. B. (2000) Functional regeneration of sensory axons into the adult spinal cord, *Nature*, 403: 312-316.

Regoli, D., Boudon, A., and Fauchére, J.-L. (1994) Receptors and antagonists for substance P and related peptides, *Pharmacol.Rev.*, 46: 551-599.

Rexed, B. (1954) A cytoarchitectonic atlas of the spinal cord in the cat, *J.Comp.Neurol.*, 100: 297-379.

Ribeiro-Da-Silva, A. (1994) Substantia gelatinosa of spinal cord, In Paxinos, G. (Ed.) *The rat nervous system*, Academic Press, New York,

Ribeiro-Da-Silva, A., Cuello, A. C., and Henry, J. L. (2000) NGF over-expression during development leads to permanent alterations in innervation in the spinal cord and in behavioural responses to sensory stimuli, *Neuropeptides*, 34: 281-291.

Riccio, A., Pierchala, B. A., Ciarallo, C. L., and Ginty, D. D. (1997) An NGF-TrkA-mediated retrograde signal to transcription factor CREB in sympathetic neurons, *Science*, 277: 1097-1100.

Richardson, P. M., Issa, V. M., and Riopelle, R. J. (1986) Distribution of neuronal receptors for nerve growth factor in the rat, *J.Neurosci.*, 6: 2312-2321.

Ritter, A. M., Lewin, G. R., Kremer, N. E., and Mendell, L. M. (1991) Requirement for nerve growth factor in the development of myelinated nociceptors in vivo, *Nature*, 350: 500-502.

Ritter, A. M. and Mendell, L. M. (1992) Somal membrane properties of physiologically identified sensory neurons in the rat: effects of nerve growth factor, *J.Neurophysiol.*, 68: 2033-2041.

Ritter, A. M., Woodbury, C. J., Albers, K., Davis, B. M., and Koerber, H. R. (2000) Maturation of cutaneous sensory neurons from normal and NGF-overexpressing mice, *J.Neurophysiol.*, 83: 1722-1732.

Ritter, A. M., Woodbury, C. J., Davis, B. M., Albers, K., and Koerber, H. R. (2001) Excess target-derived neurotrophin-3 alters the segmental innervation of the skin, *Eur.J.Neurosci.*, 14: 411-418.

Romero, M. I., Rangappa, N., Garry, M. G., and Smith, G. M. (2001) Functional regeneration of chronically injured sensory afferents into adult spinal cord after neurotrophin gene therapy, *J.Neurosci.*, 21: 8408-8416.

Routh, V. H. and Helke, C. J. (1995) Tachykinin receptors in the spinal cord, *Prog.Brain Res.*, 104: 93-108.

Ruda, M. A., Bennett, G. J., and Dubner, R. (1986) Neurochemistry and neural circuitry in the dorsal horn, *Progr.Brain Res.*, 66: 219-268.

Rudomin, P. (1999) Presynaptic selection of afferent inflow in the spina l cord, *J Physiol Paris*, 93: 329-347.

Ruit, K. G., Elliott, J. L., Osborne, P. A., Yan, Q., and Snider, W. D. (1992) Selective dependence of mammalian dorsal root ganglion neurons on nerve growth factor during embryonic development, *Neuron*, 8: 573-587.

Rustioni, A. and Weinberg, R. J. (1989) The somatosensory system, In Björklund, A., Hökfelt, T., and Swanson, L. W. (Eds.) *Handbook of chemical neuroanatomy. Vol. 7. Integrated systems of the CNS, part II*, Elsevier, Amsterdam, New York, pp. 219-321.

Saarelainen, T., Pussinen, R., Koponen, E., Alhonen, L., Wong, G., Sirvio, J., and Castren, E. (2000) Transgenic mice overexpressing truncated trkB neurotrophin receptors in neurons have impaired long-term spatial memory but normal hippocampal LTP., *Synapse*, 38: 102-104.

Sakai, N., Yamada, M., Numakawa, T., Ogura, A., and Hatanaka, H. (1997) BDNF potentiates spontaneous Ca^{2+} oscillations in cultured hippocampal neurons, *Brain Res.*, 778: 318-328.

Schadrack, J. and Zieglgansberger, W. (1998) Pharmacology of pain processing systems, *Z.Rheumatol.*, 57 Suppl 2: 1-4.

Seebach, B. S., Arvanov, V., and Mendell, L. M. (1999) Effects of BDNF and NT-3 on development of Ia/motoneuron functional connectivity in neonatal rats, *J.Neurophysiol.*, 81: 2398-2405.

Sermasi, E., Margotti, E., Cattaneo, A., and Domenici, L. (2000) Trk B signalling controls LTP but not LTD expression in the developing rat visual cortex., *Eur.J.Neurosci.*, 12: 1411-1419.

Sherwood, N. T., Lesser, S. S., and Lo, D. C. (1997) Neurotrophin regulation of ionic currents and cell size depends on cell context, *Proc.Natl.Acad.Sci.U.S.A*, 94: 5917-5922.

Simone, D. A. and Kajander, K. C. (1996) Excitation of rat cutaneous nociceptors by noxious cold, *Neurosci.Lett.*, 213: 53-56.

Simone, D. A. and Kajander, K. C. (1997) Responses of cutaneous A- fiber nociceptors to noxious cold, *J.Neurophysiol.*, 77: 2049-2060.

Siuciak, J. A., Altar, C. A., Wiegand, S. J., and Lindsay, R. M. (1994) Antinociceptive effect of brain-derived neurotrophic factor and neurotrophin-3., *Brain Res.*, 633: 326-330.

Siuciak, J. A., Wong, V., Pearsall, D., Wiegand, S. J., and Lindsay, R. M. (1995) BDNF produces analgesia in the formalin test and modifies neuropeptide levels in rat brain and spinal cord areas associated with nociception, *Eur.J.Neurosci.*, 7: 663-670.

Smeyne, R. J., Klein, R., Schnapp, A., Long, L. K., Bryant, S., Lewin, A., Lira, S. A., and Barbacid, M. (1994) Severe sensory and sympathetic neuropathies in mice carrying a disrupted Trk/NGF receptor gene, *Nature*, 368: 246-249.

Snider, W. D. (1994) Functions of the neurotrophins during nervous system development: what the knockouts are teaching us, *Cell*, 77: 627-638.

Sorkin, L. S. (1993) NMDA evokes an L-NAME sensitive spinal release of glutamate and citrulline, *Neuroreport*, 4: 479-482.

Stanfa, L. C. and Dickenson, A. H. (1999) The role of non-N-methyl-D-aspartate ionotropic glutamate receptors in the spinal transmission of nociception in normal animals and animals with carrageenan inflammation, *Neuroscience*, 93: 1391-1398.

Suen, P. C., Wu, K., Levine, E. S., Mount, H. T., Xu, J. L., Lin, S. Y., and Black, I. B. (1997a) Brain-derived neurotrophic factor rapidly enhances phosphorylation of the postsynaptic N-methyl-D-aspartate receptor subunit 1., *Proc.Natl.Acad.Sci.U.S.A.*, 94: 8191-8195.

Suen, P. C., Wu, K., Levine, E. S., Mount, H. T., Xu, J. L., Lin, S. Y., and Black, I. B. (1997b) Brain-derived neurotrophic factor rapidly enhances phosphorylation of the postsynaptic N-methyl-D-aspartate receptor subunit 1., *Proc.Natl.Acad.Sci.U.S.A.*, 94: 8191-8195.

Szekely, J. I., Torok, K., and Mate, G. (2002) The role of ionotropic glutamate receptors in nociception with special regard to the AMPA binding sites, *Curr.Pharm.Des.*, 8: 887-912.

Thoenen, H. (1995) Neurotrophins and neuronal plasticity, *Science*, 270: 593-598.

Thompson, S. W., Bennett, D. L., Kerr, B. J., Bradbury, E. J., and McMahon, S. B. (1999) Brain-derived neurotrophic factor is an endogenous modulator of nociceptive responses in the spinal cord, *Proc.Natl.Acad.Sci.U.S.A*, 96: 7714-7718.

Thompson, S. W., Dray, A., McCarson, K. E., Krause, J. E., and Urban, L. (1995a) Nerve growth factor induces mechanical allodynia associated with novel A fibre-evoked spinal reflex activity and enhanced neurokinin-1 receptor activation in the rat, *Pain*, 62: 219-231.

Tho mpson, S. W. N., Dray, A., McCarson, K. E., Krause, J. E., and Urban, L. (1995b) Nerve growth factor induces mechanical allodynia associated with novel A fibre-evoked spinal reflex activity and enhanced neurokinin-1 receptor activation in the rat, *Pain*, 62: 219-231.

Todd, A. J. (2002) Anatomy of primary afferents and projection neurones in the rat spinal dorsal horn with particular emphasis on substance P and the neurokinin 1 receptor, *Exp.Physiol.*, 87: 245-249.

Todd, A. J. and Spike, R. C. (1993) The localization of classical transmitters and neuropeptides within neurons in laminae I-III of the mammalian spinal dorsal horn, *Prog.Neurobiol.*, 41: 609-638.

Todd, A. J., Spike, R. C., Chong, D., and Neilson, M. (1995) The relationship between glycine and gephyrin in synapses of the rat spinal cord, *Eur.J.Neurosci.*, 7: 1-11.

Todd, A. J., Watt, C., Spike, R. C., and Sieghart, W. (1996) Colocalization of GABA, glycine, and their receptors at synapses in the rat spinal cord, *J.Neurosci.*, 16: 974-982.

Tölle, T. R., Berthele, A., Zieglgänsberger, W., Seeburg, P. H., and Wisden, W. (1993) The differential expression of 16NMDA and non-NMDA receptor subunits in the rat spinal cord and in periaqueductal gray., *J.Neurosci.*, 13: 5009-5028.

Treede, R.-D., Meyer, R. A., Raja, S. N., and Campbell, J. N. (1992) Peripheral and central mechanisms of cutaneous hyperalgesia, *Progr.Neurobiol.*, 58: 397-421.

Valerio, A., Paterlini, M., Boifava, M., Memo, M., and Spano, P. F. (1997) Metabotropic glutamate receptors mRNA expression in rat spinal cord, *Neuroreport*, 8: 2695-2699.

Valtschanoff, J. G., Phend, K. D., Bernardi, P. S., Weinberg, R. J., and Rustioni, A. (1994) Amino acid immunocytochemistry of primary afferent terminals in the rat dorsal horn, *J.Comp.Neurol.*, 346: 237-252.

Verge, V. M., Richardson, P. M., Wiesenfeld-Hallin, Z., and Hokfelt, T. (1995) Differential influence of nerve growth factor on neuropeptide expression in vivo: a novel role in peptide suppression in adult sensory neurons., *J.Neurosci.*, 15: 2081-2096.

Verge, V. M. K., Richardson, P. M., Benoit, R., and Riopelle, R. J. (1989) Histochemical characterization of sensory neurons with high-affinity receptors for nerve growth factor, *J.Neurocytol.*, 18: 583-591.

Vierck, C. J., Jr., Cannon, R. L., Fry, G., Maixner, W., and Whitsel, B. L. (1997) Characteristics of temporal summation of second pain sensations elicited by brief contact of glabrous skin by a preheated thermode, *J.Neurophysiol.*, 78: 992-1002.

Villoslada, P., Hauser, S. L., Bartke, I., Unger, J., Heald, N., Rosenberg, D., Cheung, S. W., Mobley, W. C., Fisher, S., and Genain, C. P. (2000) Human nerve growth factor protects common marmosets against autoimmune encephalomyelitis by switching the balance of T helper cell type 1 and 2 cytokines within the central nervous system, *J.Exp.Med.*, 191: 1799-1806.

Weskamp, G. and Otten, U. (1987) An enzyme- linked immunoassay for nerve growth factor (NGF): a tool for studying regulatory mechanisms involved in NGF production in brain and in peripheral tissues, *J.Neurochem.*, 48: 1779-1786.

Wetmore, C., Olson, L., and Bean, A. J. (1994) Regulation of brain-derived neurotrophic factor (BDNF) expression and release from hippocampal neurons is mediated by non-NMDA type glutamate receptors, *J Neurosci.*, 14: 1688-1700.

White, D. M. (1998) Contribution of neurotrophin-3 to the neuropeptide Y-induced increase in neurite outgrowth of rat dorsal root ganglion cells, *Neuroscience*, 86: 257-263.

White, D. M. (2000) Neurotrophin-3 antisense oligonucleotide attenuates nerve injury-induced Abeta-fibre sprouting, *Brain Res.*, 885: 79-86.

Willis, W. D. (1991) *Sensory mechanisms of the spinal cord*, Oxford University Press, New York.

Willis, W. D. (2001) Role of neurotransmitters in sensitization of pain responses, *Ann.N.Y.Acad.Sci.*, 933: 142-156.

Willis, W. D. (2002) Long-term potentiation in spinothalamic neurons, *Brain Res.Brain Res.Rev.*, 40: 202-214.

Willis, W. D., Jr. and Coggeshall, R. E. (1991) *Sensory mechanisms of the spinal cord*, Plenum Press, New York and London.

Wimalawansa, S. J. (1996) Calcitonin gene-related peptide and its receptors: molecular genetics, physiology, pathophysiology, and therapeutic potentials., *Endocrine Rev.*, 17: 533-585.

Wimalawansa, S. J., Gunasekera, R. D., and Zhang, F. (1993) Isolation, purification, and characterization of calcitonin gene-related peptide receptor., *Peptides*, 14: 691-699.

Winter, J. (1998) Brain derived neurotrophic factor, but not nerve growth factor, regulates capsaicin sensitivity of rat vagal ganglion neurones, *Neurosci.Lett.*, 241: 21-24.

Wood, J. N. and Docherty, R. (1997) Chemical activators of sensory neurons, *Annu.Rev.Physiol.*, 59: 457-482.

Woolf, C. J. (1996) Phenotypic modification of primary sensory neurons: the role of nerve growth factor in the production of persistent pain, *Philos.Trans.R.Soc.Lond B Biol.Sci.*, 351: 441-448.

Woolf, C. J. and Costigan, M. (1999) Transcriptional and posttranslational plasticity and the generation of inflammatory pain, *Proc.Natl.Acad.Sci.U.S.A*, 96: 7723-7730.

Woolf, C. J., Ma, Q.-P., Allchorne, A., and Poole, S. (1996) Periphreal cell types contributing to the hyperalgesic action of nerve growth factor in inflammation., *J.Neurosci.*, 16: 2716-2723.

Woolf, C. J., Safieh-Garabedian, B., Ma, Q. P., Crilly, P., and Winter, J. (1994a) Nerve growth factor contributes to the generation of inflammatory sensory hypersensitivity, *Neuroscience*, 62: 327-331.

Woolf, C. J., Shortland, P., and Coggeshall, R. E. (1992) Peripheral nerve injury triggers central sprouting of myelinated afferents, *Nature*, 355: 75-78.

Woolf, C. J., Shortland, P., and Sivilotti, L. G. (1994b) Sensitization of high mechanothreshold superficial dorsal horn and flexor motor neurones following chemosensitive primary afferent activation, *Pain*, 58: 141-155.

Woolf, C. J. and Wall, P. D. (1986) The effectiveness of C-primary afferent fibres of different origins in evoking a prolonged facilitation of the flexor reflex in the rat, *J.Neurosci.*, 6: 221-232.

Wright, D. E. and Snider, W. D. (1995) Neurotrophin receptor mRNA expression defines distinct populations of neurons in rat dorsal root ganglia, *J.Comp.Neurol.*, 351: 329-338.

Wright, D. E., Zhou, L., Kucera, J., and Snider, W. D. (1997) Introduction of a neurotrophin-3 transgene into muscle selectively rescues proprioceptive neurons in mice lacking endogenous neurotrophin-3, *Neuron*, 19: 503-517.

Xi, Z. X. and Akasu, T. (1996) Presynaptic GABAA receptors in vertebrate synapses, *Kurume Med.J*, 43: 115-122.

Xie, C. W., Sayah, D., Chen, Q. S., Wei, W. Z., Smith, D., and Liu, X. (2000) Deficient long-term memory and long-lasting long-term potentiation in mice with

a targeted deletion of neurotrophin-4 gene., *Proc.Natl.Acad.Sci.U.S.A.*, 97: 8116-8121.

Xu, B., Gottschalk, W., Chow, A., Wilson, R. I., Schnell, E., Zang, K., Wang, D., Nicoll, R. A., Lu, B., and Reichardt, L. F. (2000) The role of brain-derived neurotrophic factor receptors in the mature hippocampus: modulation of long-term potentiation through a presynaptic mechanism involving TrkB., *J.Neurosci.*, 20: 6888-6897.

Yang, F., He, X., Feng, L., Mizuno, K., Liu, X. W., Russell, J., Xiong, W. C., and Lu, B. (2001) PI-3 kinase and IP3 are both necessary and sufficient to mediate NT3-induced synaptic potentiation., *Nat.Neurosci.*, 4: 19-28.

Yiangou, Y., Facer, P., Sinicropi, D. V., Boucher, T. J., Bennett, D. L., McMahon, S. B., and Anand, P. (2002) Molecular forms of NGF in human and rat neuropathic tissues: decreased NGF precursor-like immunoreactivity in human diabetic skin, *J.Peripher.Nerv.Syst.*, 7: 190-197.

Yu, X. M., Askalan, R., Keil, G. J., and Salter, M. W. (1997) NMDA channel regulation by channel-associated protein tyrosine kinase Src., *Science*, 275: 674-678.

Zafra, F., Castren, E., Thoenen, H., and Lindholm, D. (1991) Interplay between glutamate and gamma-aminobutyric acid transmitter systems in the physiological regulation of brain-derived neurotrophic factor and nerve growth factor synthesis in hippocampal neurons, *Proc.Natl.Acad.Sci.U.S.A*, 88: 10037-10041.

Zafra, F., Hengerer, B., Leibrock, J., Thoenen, H., and Lindholm, D. (1990) Activity dependent regulation of BDNF and NGF mRNAs in the rat hippocampus is mediated by non-NMDA glutamate receptors, *EMBO J.*, 9: 3545-3550.

Zhong, J., Gerber, G., Kojic, L., and Randic, M. (2000) Dual modulation of excitatory synaptic transmission by agonists at group I metabotropic glutamate receptors in the rat spinal dorsal horn, *Brain Res.*, 887: 359-377.

Zhou, L. M., Gu, Z. Q., Costa, A. M., Yamada, K. A., Mansson, P. E., Giordano, T., Skolnick, P., and Jones, K. A. (1997) (2S,4R)-4-methylglutamic acid (SYM 2081): a selective, high-affinity ligand for kainate receptors, *J.Pharmacol.Exp.Ther.*, 280: 422-427.

Zhou, S., Komak, S., Du, J., and Carlton, S. M. (2001) Metabotropic glutamate 1alpha receptors on peripheral primary afferent fibers: their role in nociception, *Brain Res.*, 913: 18-26.

Zhou, X. F., Chie, E. T., Deng, Y. S., Zhong, J. H., Xue, Q., Rush, R. A., and Xian, C. J. (1999) Injured primary sensory neurons switch phenotype for brain-derived neurotrophic factor in the rat, *Neuroscience*, 92: 841-853.

Zhou, X. F. and Rush, R. A. (1994) Localization of neurotrophin-3-like immunoreactivity in the rat central nervous system, *Brain Res.*, 643: 162-172.

Zirrgiebel, U., Ohga, Y., Carter, B., Berninger, B., Inagaki, N., Thoenen, H., and Lindholm, D. (1995) Characterization of TrkB receptor- mediated signaling pathways in rat cerebellar granule neurons: involvement of protein kinase C in neuronal survival., *J.Neurochem.*, 65: 2241-2250.