BDNF as a pain modulator

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Abstract

At least some neurotrophins may be powerful modulators of synapses, thereby influencing short- and long-lasting synaptic efficiency. BDNF acts at central synapses in pain pathways both at spinal and supraspinal levels. Neuronal synthesis, subcellular storage/co-storage and release of BDNF at these synapses have been characterized on anatomical and physiological grounds, in parallel with trkB (the high affinity BDNF receptor) distribution. Histological and functional evidence has been provided, mainly from studies on acute slices and intact animals, that BDNF modulates fast excitatory (glutamatergic) and inhibitory (GABAergic/glycinergic) signals, as well as slow peptidergic neurotrasmission in spinal cord. Recent studies have unraveled some of the neuronal circuitries and mechanisms involved, highlighting the key role of synaptic glomeruli in lamina II as the main sites for such a modulation.

Keywords: BDNF, Pain, Spinal cord, Neuropeptides, Glutamate

Abbreviations: BDNF, brain-derived neurotrophic factor; CFA, complete Freund's adjuvant; CGRP, calcitonin gene-derived peptide; CREB, cyclic AMP response element binding protein; DCVs, dense core vesicles; DRG, dorsal root ganglion; DRNi, dorsal raphe nuclei; fl-trkB, full length tropomyosine receptor kinase B isoform; FRAP, fluoride- resistant acid phosphatase; GABA, g-aminobutyric acid; GDNF, glial-derived neurotrophic factor; IB4, isolectin B4; mEPSCs, miniature excitatory post-synaptic currents; NGF, nerve growth factor; NT, neurotrophin; NT-3, neurotrophin 3; NT-4/5, neurotrophin 4-5; PAF, primary afferent fibers; PAG, periaqueductal grey; RMN, raphe magnus nucleus; RVM, rostroventromedial medulla; SNL, spinal nerve ligation; STT, spinothalamic tract; trkA, tropomyosine receptor kinase A; trkB, tropomyosine receptor kinase B; trkC, tropomyosine receptor kinase C; TRPV1, transient receptor potential vanilloid-1; tr-trkB, truncated tropomyosine receptor kinase B isoform; VRPs, ventral root potentials; WDR, wide dynamic range.

1. General concepts

Neurotrophins (NTs) are a well characterized family of growth factors playing important roles in regulating neuronal survival, growth and differentiation (Davies, 1994; Snider, 1994). Besides this, at least some NTs may be powerful modulators of synapses, thereby influencing short- and long-term synaptic efficiency (Berninger and Poo, 1996; Lewin and Barde, 1996; Snider, 1994; Thoenen, 1995).

Among the members of the NT family, the brain-derived neurotrophic factor (BDNF) is a 12.4-kDa basic protein, originally isolated from pig brain (Barde et al., 1982). Transgenic mice revealed a key role of BDNF in promoting the survival of some

sensory neurons during development (Hellard et al., 2004). In particular the mechanoceptors innervating the Meissner and Pacinian corpuscles and the chemoreceptors innervating certain types of taste buds appear to be dependent on BDNF for their survival (Sedy et al., 2004; Uchida et al., 2003).
Most of BDNF cellular actions are mediated by its high-affinity receptor, the tropomyosine receptor kinase B (trkB), which also recognizes NT-4/5 (Kaplan and Miller, 1997; Kaplan and Stephens, 1994). TrkB is abundant during development, but also widely distributed in the CNS of adult animals, suggesting a continuing role for BDNF in the adult nervous system.
One important role for BDNF in adulthood appears to be as a central modulator of pain. As we will review here, this NT is an important modulator of sensory neurotransmission in nociceptive pathways both at spinal and supraspinal levels (Malcangio and Lessmann, 2003; Michael et al., 1997; Pezet et al., 2002c; Pezet and McMahon, 2006), and a key player in the central sensitization that underlies many forms of hyperalgesia (Heppenstall and Lewin, 2001; Lewin and Mendell, 1993).

1.1. Purpose of this review

The role of BDNF in nociceptive pathways has been the subject of several authoritative reviews, which have mainly addressed the issue of the intracellular pathways elicited by receptor activation (Bennett, 2001; Binder and Scharfman, 2004; Chao et al., 2006; Malcangio and Lessmann, 2003; Pezet and Malcangio, 2004; Pezet and McMahon, 2006). Despite this, our knowledge of the role of the NT in the processing of pain information is still largely incomplete, mainly because of the lack of a thorough anatomical and functional description of the neuronal circuitry involved, which, as it appears obvious, can only rely on the punctual localization of BDNF and its receptors at synapses, combined with functional analysis.
We will focus here on the circuits involved in the modulation of synapses by BDNF, in other words on the anatomical and physiological data which can be, at present, brought in support of the role of this NT as a pain-related messenger. Since most work on this issue has been carried out at the spinal cord level, and this is our major area of interest, we will of course concentrate on the synapses between the first and second order sensory neurons in this area of CNS. We have also reviewed the wealth of behavioral observations carried out with the purpose of defining the action of BDNF in pain.

1.2. Nociceptive pathways

The perception of pain implicates the activation of peripheral (cutaneous, muscular, articular or visceral) nociceptors, the generation of a nerve signal and the transmission of this signal to the somatosensory cortex. Nociceptive stimuli conveyed from somatic and visceral organs to higher centers follow different routes. In particular, whereas somatic structures are directly innervated by the trigeminal and spinal nerves, most viscera have two distinct types of sensory innervation that reach their target organs following the sympathetic (via the white communicating branches of the thoracic and lumbar nerves) and parasympathetic (via certain cranial nerves) divisions of the autonomic nervous system.
For simplicity, the following description is centered upon spinal cord somatosensory pathways which are responsible for collecting pain stimuli from the skin, muscles, and joints from the entire body with the exception of the head and the proximal regions of the neck which are innervated by the projections of the trigeminal neurons. These pathways consist of at least three neurons: (i) a first order sensory neuron in the dorsal root ganglia (DRGs); (ii) a second order neuron in the spinal cord dorsal horn; (iii) a third order neuron which is generally located in the ventral

posterolateral nucleus of the thalamus.
The main anatomical aspects of these pathways are shortly described in the following paragraphs to put things in perspective.
It should be kept in mind, however, that most of the data reported here are derived from studies in rodents (rat and mouse), and, albeit they can for the most be of general relevance, a few differences across species are known to occur. Therefore when observations are referred to non-rodents, this will be clearly mentioned.

1.2.1. Sensory neurons, primary afferent fibers, and dorsal horn

1.2.1.1. Sensory neurons and primary afferent fibers. Nociceptive (and non-nociceptive) primary afferent neurons are housed in the DRGs, and the sensory ganglia (trigeminal, jugular, nodose, petrosal and geniculate ganglion) associated with certain cranial nerves. These neurons are referred to as pseudounipolar (Dogiel, 1908) because they give rise to a single main process splitting into a peripheral and a central branch displaying some functional peculiarities common to dendrites and axons. The process of primary sensory neurons is commonly referred to as a primary afferent fiber (PAF). Historically, DRG neurons have been more extensively characterized that the others, and classified following morphological, electrophysiological and neurochemical criteria (for review, see Willis and Coggeshall, 2004). A similar classification also holds for trigeminal neurons, and, likely, the other sensory neurons localized in the other aforementioned ganglia.
As a result of more recent progress in chemical neuroanatomy and immunohistochemistry, Averill et al. (1995) have proposed a division of DRG neurons into three groups, as follows:

- RT97-positive medium- to large-sized light neurons (about 40%): These sensory neurons are immunoreactive to the RT97 antibody that recognizes the phosphorylated form of neurofilament protein NF200 (Lawson et al., 1984) do not contain neuropeptides, and are devoid of binding sites for the Griffonia simplicifolia isolectin B4 (IB4). Most of these cells express trkB and tropomyosine receptor kinase C (trkC) receptors (McMahon et al., 1994; Wright and Snider, 1995) and, therefore, are likely to be regulated for their differentiation by BDNF and the neurotrophin 3 (NT-3), respectively. Under physiological conditions, the cells of this group transmit non-nociceptive information by means of large myelinated fibers (Aβ); however, some RT97-positive cells involved in nociception are supposed to have thin-myelinated fibers (Lawson and Waddell, 1991).
- LA4-positive small dark neurons (about 30%): These sensory neurons are immunoreactive to the LA4 antibody, which reacts with a specific cell membrane oligosaccharide (Dodd and Jessell, 1985), but not to RT97. LA4-positive cells typically do express binding sites for IB4 (Averill et al., 1995; Guo et al., 1999) and contain the enzyme fluoride-resistant acid phosphatase (FRAP) (Alvarez et al., 1991), but not peptides. They are nerve growth factor (NGF)-sensitive only during early CNS development; then, tropomyosine receptor kinase A (trkA) expression is down-regulated and, in adulthood, these neurons become dependent on glial-derived neurotrophic factor (GDNF, Molliver et al., 1995). In keeping with their dependence on GDNF, IB4-positive neurons show high levels of expression of the GDNF receptors Ret, GFRa-1, and GFRa-2 (Bennett et al., 1998). Furthermore, in these neurons GDNF regulates the distribution of a pool of pain-related receptors, such as the purinergic receptor-channels P2X3 (Bradbury et al., 1998), the vanilloid receptor TRPV1 (Guo et al., 2001; Michael and Priestley, 1999), and certain voltage-gated channels (i.e., NaN tedrodotoxin-resistant Na^+-channels) (Fjell et al., 1999).
- Peptidergic small-to medium-sized dark neurons (about 30%): These sensory neurons synthesize many pain-related neuropeptides, such as substance P and

calcitonin gene-related peptide (CGRP). In all mammals studied so far, peptides are distributed on both central (Merighi et al., 1991; Ribeiro-Da-Silva and Cuello, 1995) and peripheral terminals (Dalsgaard et al., 1984; Gibbins et al., 1985), and may be released from central PAF endings following an appropriate orthodromic terminal activation, or from peripheral PAF endings following an antidromic terminal activation (Kessler et al., 1999; Kilo et al., 1997). The DRG neurons belonging to this category have unmyelinated or thin-myelinated axons and can be labeled with the LD2 antibody, directed against a-galactose extended gangliosides (Chou et al., 1989). They are sensitive to NGF and express trkA receptors during the embryogenesis as well as in adulthood (Averill et al., 1995; McMahon et al., 1994; Michael et al., 1997). In fact, NGF is crucial not only for survival of this type of neuron (Crowley et al., 1994), but also for regulation of peptide synthesis (Mulderry, 1994), and sensitivity to noxious agents (i.e., capsaicin) (McMahon et al., 1995; Woolf et al., 1996).

Under physiological conditions, both groups of dark neurons transmit nociceptive information by means of thin-myelinated Aδ or unmyelinated C fibers.

The peripheral branch of DRG neurons terminates innervating different tissues and organs and gives rise to different morphological specializations according to sensory modality. In general, non-nociceptive terminals give rise to encapsulated nerve endings displaying several morphological arrangements (e.g., Meissner's corpuscle, Pacinian corpuscles and Ruffini endings), whereas nociceptive fibers terminate in the form of free nerve endings that give rise to discrete arborizations in target organs.

The classification and organization of peripheral primary afferent fibers has been extensively reviewed by others (Belemonte and Cervero, 1996; Meyer et al., 1994; Millan, 1999; Treede et al., 1992; Willis and Coggeshall, 2004). PAFs are classified on the basis of diameter, presence of a myelin sheet, and conduction velocity. Large myelinated Aαβ fibers (or group I and II in muscles) convey low intensity mechanical, tactile, proprioceptive inputs to the CNS, but not noxious stimuli, although a group of A fibers with intermediate Aαβ/Aδ features has been suggested to participate in nociceptive transmission (Mense, 1993; Woolf et al., 1994). Under physiological conditions, nociceptive information is transmitted only by thin-myelinated Aδ or unmyelinated C fibers (group III and IV, respectively, in muscles).

The central branches of PAFs originating from DRG neurons enter the spinal cord via the dorsal roots, even though some sensory axons have also been observed in the ventral roots irrespectively of species (Bostock, 1981; Mawe et al., 1984). Large myelinated fibers run medially into the dorsal funiculus and give rise either to projection fibers terminating in medullary dorsal column (gracile and cuneate) nuclei or to fibers terminating in dorsal horn deep laminae as well as in ventral horn (Smith, 1983). Most non-nociceptive large myelinated PAFs, innervating the skin, terminate in dorsal horn laminae III-VI (Woolf, 1987). All the same, it has been suggested that, in neuropathic pain states, Aαβ fibers may sprout toward inappropriate superficial dorsal horn targets, making synapse with nociceptive specific neurons (Lekan et al., 1996, 1997; Woolf and Doubell, 1994).

Aδ and C fibers approach the dorsal horn via the dorso-lateral funiculus (Lissauer's tract) and, after splitting into an ascending and a descending branch, primarily terminate into the superficial dorsal horn laminae.

High-threshold cutaneous mechanoreceptor Aδ fibers appear to terminate preferentially in lamina I and outer lamina II (II_o, Nagy and Hunt, 1983), but some thin-myelinated fibers are likely to reach deeper localizations, i.e., lamina V, at least in cat (Cruz et al., 1991).

Initial studies in cat showed that C fibers end in laminae I and II (Gobel et al., 1981; Snyder, 1982), and this was subsequently confirmed in other species including rat, mouse, and monkey. The anatomical distribution of C fiber terminals within the superficial DH is related to their neurochemical features. In fact, whereas peptidergic

nociceptors mostly terminate in lamina I and in lamina II_o of the superficial dorsal horn, IB4-positive terminals are concentrated within inner lamina II (II_i), suggesting the existence of two different pathways for pain processing (Molliver et al., 1995; Snider and McMahon, 1998).
Nociceptive PAFs originating from sensory neurons of encephalic ganglia terminate within the trigeminal spinal nucleus, which has a cytoarchitectonic organization analogous to that of the spinal cord dorsal horn. The pattern of termination has been mostly described for trigeminal afferents.

1.2.1.2. Dorsal horn (lamina II). For a description of dorsal horn anatomy and cytoarchitecture, see Willis and Coggeshall (2004); we will briefly mention here only some relevant aspects of lamina II organization.
Lamina II is also referred to as the substantia gelatinosa. This name was coined by the ancient anatomist Luigi Rolando due to the overall gelatinous appearance of this area of the spinal cord, which, as it became apparent with the development of ultrastructural analysis, is directly correlated to the almost complete absence of myelinated fibers. Lamina II is characterized by a large amount of densely packed small round neurons. Rexed (1952) divided cat lamina II into an outer part (II_o), with particularly high cellular density, and an inner part (II_i), less cellular and thicker. This subdivision was subsequently shown to hold for all species. Classical works describe lamina II as a ''closed system'' (Szenta- gothai, 1964). In fact, most neurons have short axons that are confined within laminar boundaries (short-axon cells or Golgi type II cells), while a few others have axons that enter the white matter, but, after a short distance, re-enter the lamina II (funicular cells or Golgi type I cells). A substantial output of lamina II neurons is represented by the dorsally-directed dendrites of projection neurons (''antenna'' neurons) residing in deeper laminae, most of which express the preferred substance P receptor NK_1.
Nonetheless in both rats and monkeys, lamina II neurons projecting to higher centers, such as the brain-stem or the thalamus (Giesler et al., 1978; Willis et al., 1978) or to deeper laminae (Light and Kavookjian, 1988b) have also been described.
In the 70 s, Gobel proposed a classification of the gelatinosa neurons, originally based on cat medullary dorsal horn (Gobel, 1978), which, with some modifications, has been found to be valid also for other species (Todd and Lewis, 1986). The predominant and better characterized Gobel's cell types are stalked and islet cells.
Stalked cells, so-called for their stalk-like spines, are located in lamina II_o, especially at the border with lamina I. In monkeys and humans, dendrites form a ventrally directed cone toward deeper laminae, while major axonal targets are lamina I neurons, and, more rarely, deeper laminae neurons (Light and Kavookjian, 1988a; Schoenen, 1982). In contrast with islet cells, stalked cells do not possess pre-synaptic dendrites (Gobel et al., 1980). Stalked cells are usually considered excitatory interneurons, but some are likely to contain inhibitory endogenous opioids in cats (Cruz and Basbaum, 1985). Finally, electrophysiological recordings indicate that they are either wide dynamic range (WDR) or nociceptive specific neurons (Gobel et al., 1980).
Islet cells have large rostro-caudally directed dendritic trees that extend within the whole lamina II. Cell bodies can be found in both lamina II_o and lamina II_i. Notwithstanding, dendrites predominantly located in lamina II_o are supposed to belong to nociceptive specific neurons, whereas those located in lamina II_i to mechanoreceptive cells (Bennett et al., 1980). Axons end primarily within lamina II (Golgi type II) and are classically reported as inhibitory interneurons using the γ-aminobutyric acid (GABA) as their principal neurotransmitter (Gobel, 1978). These GABAergic neurons possess pre-synaptic vesicle-containing dendrites contacting other nearby dendrites and axon terminals at glomeruli (Spike and Todd, 1992). Colocalization of GABA and glycine was demonstrated in a sub-population of these neurons (Todd, 1996).

The PAF input in lamina II comes mainly from fine unmyelinated sensory fibers (C fibers) and thin-myelinated fibers (Aδ fibers).

>Neuropil organization of lamina II is characterized by the ‘‘complex synaptic arrays’’ between PAF endings and the dendrites or axons of intrinsic spinal neurons originally described by Ralston in cat (1965): the synaptic glomeruli (Fig. 1). In rodents, glomeruli are formed by a core, the central axonal bouton (C bouton) corresponding to a PAF ending, surrounded by several dendrites and a few axon terminals (Coimbra et al., 1974; Ribeiro-Da-Silva and Coimbra, 1982). A similar organization was described in rhesus monkeys, where three types of ‘‘scalloped’’ PAF central terminals were distinguished on the basis of their ultrastructural characteristics, size, and distribution of synaptic vesicle population: (i) dense sinusoid axon (DSA) terminals contain medium-sized and large agranular synaptic vesicles; (ii) large dense-core vesicle (LDCV) terminals contain agranular vesicles of heterogeneous diameter and DCVs; and (iii) regular synaptic vesicle (RSV) terminals contain a homogeneous popula- tion of 45–50 nm clear synaptic vesicles (Knyihar-Csillik et al., 1982). According to the classification proposed by Ribeiro-Da-Silva and Coimbra (1982), glomeruli can be split into two main morphological/neurochemical categories. Glomeruli of type I are further subdivided in non-peptidergic type Ia (containing a C bouton corresponding to DSA terminals of Csillik’s classification) and peptidergic type Ib (containing a C bouton corresponding to LDCV terminals). Glomeruli of type II (corresponding to RSV terminals) can also be further subdivided in type IIa and type IIb (Ribeiro-Da-Silva, 2004).

In rats, type I glomeruli are found in the ventral part of lamina II_o, whereas type II (mainly type IIa) are found in lamina II_i. In monkeys, lamina II contains mainly DSA terminals.

The central boutons in type I glomeruli are thought to rise from unmyelinated PAFs of the C type, whereas the central boutons in type IIa and IIb are believed to originate from Aδ and Aαβ fibers, respectively (Coimbra et al., 1984). In type Ib glomeruli, central boutons are particularly enriched with peptides (Merighi, 2002) and BDNF (Salio et al., 2005, 2007). Glomerular dendrites derive from the ‘‘antenna’’ cells of deeper laminae or from lamina II interneurons (Rethelyi and Szentagothai, 1969). Some of islet and ‘‘antenna’’ cell dendrites in rodents and monkeys are vesicle-containing dendrites (Carlton and Hayes, 1990; Spike and Todd, 1992), containing inhibitory amino acids. These pre-synaptic dendrites (commonly referred to as vesicular 1 [V1] profiles) have recently been demonstrated to contain GABA and to express trkB (Bardoni et al., 2007). Axo-axonic synapses also occur, especially in type II glomeruli (Ribeiro-Da-Silva and Coimbra, 1984; Zhu et al., 1981), and most of these surrounding terminals (V2) also contain inhibitory neurotransmitters (Carlton and Hayes, 1990), but are not trkB immunoreactive (Bardoni et al., 2007). The large number of synaptic contacts indicates that glomeruli are important devices for neurotransmission. In particular, concerning nociceptive transmission, a central role is supposed to be played by type Ib glomeruli. Unlike the other glomerular types, peptidergic C boutons are in fact rarely post-synaptic to other neuronal profiles and, therefore, are thought to behave as ‘‘multiplier systems’’ for pain-related information (Ribeiro-Da-Silva and Coimbra, 1984). In these terminals peptides are typically stored within dense core vesicles (DCVs) that make them easy to be recognized at the electron microscopy level. Notwithstanding, glomeruli represent less than 5% of lamina II synapses. The remaining contacts are traditional asymmetric excitatory synapses (some of them containing DCVs) or symmetric inhibitory synapses (Ralston, III, 1971).

1.2.2. Ascending pathways

Ascending spinal pathways are involved in the transmission of nociceptive information

to higher centers. The spinothalamic pathway is traditionally considered the main nociceptive pathway.

1.2.2.1. Spinothalamic tract. The spinothalamic tract (STT) and its relation to pain have been widely described (see for review Millan, 1999; Tracey, 2003; Willis and Coggeshall, 2004). STT neurons are both nociceptive specific and WDR neurons. Schematically, neurons of the STT localized in lamina I and in deeper laminae (IV–VI) especially at the base of the medial aspect of dorsal horn, ascend in the ventrolateral funiculus and terminate in the lateral thalamus.

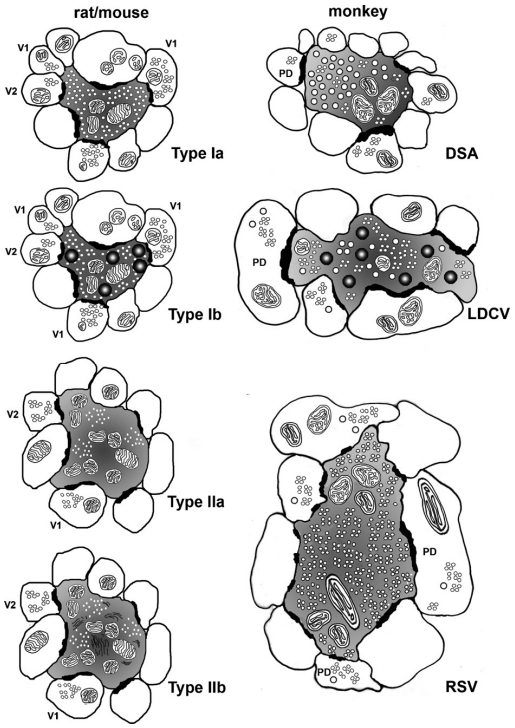

Fig. 1. Ultrastructure of synaptic glomeruli in rodents and monkey. Drawings were obtained from original micrographs and schematized for an easier visualization of similarities/differences between species. Type Ia glomeruli in rodents correspond to DSA glomeruli in monkey. Type Ib and LDCV glomeruli are characterized by the presence of DCVs in central boutons and are, therefore, peptidergic. Type II glomeruli in rodents correspond to RSV glomeruli in monkey. Note that V1 profiles in rodents correspond to PDs in monkey. Plain dendrites are unlabeled. See text for more explanations. DCV, dense core vesicle; DSA, dense sinusoid axon terminal; LDCV, large dense-core vesicle terminal; PD, pre-synaptic dendrite; RSV, regular synaptic vesicle terminal; V1, vesicle-containing dendrite.

Other neurons located at the base of the medial aspect of dorsal horn and in the ventral horn (laminae VII–VIII) ascend in the ventral funiculus and terminate in the medial thalamus (Giesler et al., 1981). Some STT neurons located in lamina I and the lateral spinal nucleus are likely to project to the medial thalamus through the dorsolateral funiculus that is sometimes referred to as dorsal STT (Hylden et al., 1989). Some STT neurons are peptiderigic (particularly those in lamina X) and may contain enkephalin/ dynorphin (Nahin, 1988), enkephalin/galanin/cholecystokinin (Ju et al., 1987), or SP (Battaglia et al., 1992).
Collectively, the terminals of STT neurons exhibit a patchy distribution in three main thalamic regions: (i) the ventrobasal complex; (ii) the medial thalamus including the intralaminar nuclei; and (iii) the posterior complex, an important site for pain integration involved in the cortical-thalamo-cortical loop, whereby the cortex reinforces the excitation of thalamic neurons by pain stimuli and vice versa (Craig, 1996; Eaton and Salt, 1995; Ledoux et al., 1987; Peschanski et al., 1983). It has been suggested that neurons in the ventrobasal complex are mostly responsible for sensory and

discriminative aspects of noxious stimuli, whereas neurons in the intralaminar nuclei/posterior complex are responsible for motivational and emotional aspects of pain, as well as the escape reaction to acute pain (Albe-Fessard et al., 1985; Apkarian, 1995).
The thalamus is the crucial relay for the reception and processing of nociceptive information en route to the cortex (Bushnell, 1995). The thalamo-cortical inputs (Millan, 1999) are mainly conveyed to the first somatosensory area of the postcentral cortical gyrus (S I). However, imaging studies have found other cortical areas with nociresponsive or WDR neurons activated by noxious stimuli, such as: the second somatosensory area (S II; receiving inputs from VPI and intralaminar nuclei); certain regions of the parietal cortex; the insular cortex; the anterior cingulate cortex; and the medial prefrontal cortex (Casey and Minoshima, 1995; Derbyshire et al., 1997; May et al., 1998). These cortical structures are highly interconnected to each other and with limbic structures. Their activation is predominantly contralateral to the noxious stimulus, except for some bilateral activations (i.e., in the prefrontal and anterior cingualte cortex), perhaps associated with emotional-cognitive factors (Jones and Derbyshire, 1996).

1.2.2.2. Other ascending pathways. Other pain-related ascending pathways are less well characterized and, probably, less critical for sensory-discriminative aspects of pain, but are, nonetheless, important for the general dimension of pain and pain control.
The spinoreticular tract originates from nociceptive specific neurons (and some WDR) in laminae I, V/VI, X. It reaches the lateral reticular nucleus or the medial nuclei of the pontomedullary reticular formation (Menetrey et al., 1983; Peschanski and Besson, 1984). The spinoreticular tract has an important projection to the medullar dorsal reticular nucleus, which is critical for descending control (Lima, 1990; Villanueva et al., 1991, 1995). Some projections from the reticular nuclei can reach the medial thalamus. The spinoreticular tract is involved in the motivational and cognitive aspect of pain processing, as well as in the activation of the descending inhibition.
The spinomesencephalic tract originates from neurons found in almost all dorsal horn laminae. Their fibers reach the periaqueductal grey (PAG), the superior colliculus and the parabrachial nucleus. This pathway is involved in motor responses to pain and in affective aspects (Millan, 1999).
The spinohypothalamic tract originates from nociceptive specific and WDR neurons in laminae I, V and X. Axons reach the contralateral hypothalamus (Giesler et al., 1994). This pathway is involved in neuroendocrine/autonomic responses to pain stimuli.
The spinoparabrachial tract originates from nociceptive specific lamina I/II neurons sending their axons to the parabrachial nucleus (Bernard et al., 1996). About 50% of them contain dynorphin and enkephalin (Standaert et al., 1986) and are contacted by CGRP- immunoreactive PAF terminals (Nahin et al., 1991). This is a sizable tract at least in cat, considering that in this species the number of spinoparabrachial neurons in lamina I seems to largely exceed that of SST neurons (Klop et al., 2005). Third-order projection neurons of parabrachial nuclei send their axons to the amygdala, the bed nucleus of the stria terminalis and the hypothalamus. Terminals reaching the central nucleus of the amygdala have been recently found to contain BDNF co-stored with substance P and CGRP (Salio et al., 2007). This pathway is mainly dedicated to the motivational- affective representation of pain information.

1.2.3. Descending pathways
Descending pathways mainly originate in the rostroventral medulla and play an important role in the modulation and integration of nociceptive information within the dorsal horn. Most descending pathways exert an inhibitory control onto the excitability of dorsal horn neurons, but some facilitatory effects have also been described.

1.2.3.1. Descending inhibition. The main inhibitory descending pathway is the raphespinal tract, originating in the medullary raphe magnus nucleus (RMN) (see for review Fields and Basbaum, 1994; Gebhart, 2004; Millan, 1999; Rosenfeld, 1994). Other descending pathways are likely to originate from neurons within the medullary reticular nuclei of the gigantocellular complex (Martin et al., 1985) and from the locus coeruleus (West et al., 1993).
RMN neurons are primarily activated by inputs from the PAG, which, in turn, is activated when the tonic inhibition of local enkephalinergic neurons is removed. This inhibition of inhibitors is mediated by the intervention of hypothalamic afferents that release opioid peptides (b-endorphin) in response to specific sensory inputs from ascending pathways (namely the spinoreticular tract). Also amygdala projections may disinhibit PAG neurons, especially in fearful situations. Most RMN neurons are serotoninergic neurons, eventually leading to excitation/ inhibition depending upon the array of receptor sub-types in target neurons. They reach the ipsilateral dorsal horn via the dorsolateral funiculus, and tend to make synapses onto enkephalinergic inhibitory neurons. When activated, these inhibitory interneurons exert a pre- and post-synaptic inhibition onto the dorsal horn projection neurons. Other possible effects, consequent to the activation of the inhibitory descending system, are consistent with a direct inhibition due to the release of inhibitory neurotransmitters, resulting in (i) inhibition of neurotransmitter release from nociresponsive PAF terminals; (ii) inhibition of local excitatory interneurons; and (iii) direct inhibition of spinal projection neurons. Certain studies have also shown that descending inhibition onto WDR neurons preferen- tially inhibits the Aδ and C fiber-mediated excitation rather than the Aαβ (Fields and Basbaum, 1994; Millan, 1997). This differential inhibition may reinforce the local inhibitory effect of Aαβ fibers on nociresponsive neurons. The inhibitory descending system is not somatotopically arranged, and for this reason the pain relief effect produced as a consequence of its activation is quite diffuse.

1.2.3.2. Descending facilitation. Facilitatory (and inhibitory) aminergic neurons have been indistinctly observed in the medullary nucleus reticularis, in the parabrachial nucleus, in the cortex and in other brain areas (Monhemius et al., 1997; Suzuki et al., 2002; Zhang et al., 1996). It has been suggested that they can exert alternatively excitatory and inhibitory actions on individual neurons via distinct receptor subtypes (Boess and Martin, 1994). Other medullary nuclei (i.e., the rostroventromedial nucleus, RVM) seem to have separate inhibitory and facilitatory systems, which likely reach the dorsal horn via different pathways (Zhuo and Gebhart, 1997).
Suzuki et al. (2002) have recently shown a pronociceptive spino-bulbo-spinal loop that may underlie some forms of central sensitization. According to their model, NK_1-expressing lamina I/III neurons activated by nociceptive PAFs are likely to excite serotoninergic neurons in the RMN that, in turn, play a facilitatory action onto WDR spinal neurons.
In general, descending inhibitory influences are predominant and often tonically active, whereas facilitatory influences are induced directly by noxious stimuli, probably in order to maintain the hyperalgesic state and, consequently, protect the damaged tissues (Gebhart, 2004).

2. Anatomical evidence for BDNF as a pain modulator

The availability of specific antibodies and tracing techniques has made it possible to unravel the cellular localization and distribution of BDNF and its high affinity receptor in central and peripheral neurons. Histological data which are relevant to the role of BDNF as a pain modulator are reported in this section.

2.1. Neuronal synthesis, subcellular storage/co-storage and release of BDNF

Neurotrophic factors can be locally synthesized by neurons and/or endocytosed at neuronal somatodendritic domains (transcytosis) to be eventually targeted to terminals by anterograde axonal transport (von Bartheld et al., 2001; von Bartheld, 2004). In the case of BDNF, synthesis and subsequent anterograde transport have been widely documented in neurons, as well as synthesis and release in microglia (Coull et al., 2005). The capability of BDNF to undergo anterograde transport to terminals makes it unusual amongst the other NTs. It should be mentioned that besides BDNF, pro-BDNF could also be anterogradely transported and may have important physiological functions (Zhou et al., 2004).

Examples of central neurons synthesizing and anterogradely transporting BDNF have been found in cerebral cortex, parabra- chial nucleus, hippocampus and locus coeruleus (Altar et al., 1997; Conner et al., 1997; Kohara et al., 2001). As to peripheral neurons, peptidergic small-to medium-sized dark neurons in DRGs (see Section 1.2.1) synthesize and anterogradely transport BDNF to their central terminals in the dorsal horn of the spinal cord (Kerr et al., 1999; Michael et al., 1997).

Until recently the site of subcellular storage of BDNF was controversial. Although this issue has been the subject of several investigations, most of the work in this field has been done using confocal microscopy on isolated neurons and/or cell lines (Mowla et al., 1999; Wu et al., 2004) or by a biochemical approach (Fawcett et al., 1997). Initial ultrastructural localization (Michael et al., 1997) indicated that the NT was packaged within DCVs, but it remained to be determined if DCVs were the sole site of BDNF subcellular storage, in particular because DCVs appeared to be very rare in certain neurons that anterogradely transport BDNF in vivo (Smith et al., 1997), and since agranular vesicles in nerve terminals have also been indicated to contribute to BDNF accumulation (Luo et al., 2001).

We have recently addressed this issue by quantitative high resolution ultrastructural immunocytochemistry and demonstrated that BDNF is solely packaged in DCVs in both central and peripheral neurons (Salio et al., 2007). Our post-embedding staining demonstrated that DCVs have a BDNF content 31 (spinal cord)–36 (amygdala) times higher than agranular vesicles. These results are consistent with Western blot analysis of rat synaptosomes, where BDNF colocalizes with the synaptic marker synaptotagmin (Fawcett et al., 1997), and with the nowadays widely established notion that high and low molecular weight neurotransmitters are respectively packaged in DCVs and agra- nular vesicles (Merighi, 2002). The ability to distinguish between synthesized and endocytosed BDNF in vivo is of relevance to the understanding of the biology of this NT (Lessmann et al., 2003). In vitro, BDNF can avoid degradation after being internalized in the lysosomal compartment, and thus enter a transcytosis pathway that enables it to move across multiple synapses (von Bartheld et al., 2001; von Bartheld, 2004). Nonetheless, transcytosis of BDNF does not seem to be of relevance in vivo, since the NT was never observed within lysosomal structures, albeit this needs further confirmation (Salio et al., 2007).

Many of the neurons that are capable of anterogradely transporting BDNF also synthesize neuropeptides (Michael et al., 1997) that, as mentioned, are typically stored within DCVs. In the case of substance P and CGRP, we have recently shown that they not only are costored in DCVs together with BDNF, but also that costorage occurs in remarkably constant stoichiometric ratios (Salio et al., 2007).

When considering these observations, the functional significance of BDNF anterograde transport appears easier to understand, especially when taking into consideration that BDNF is now regarded as anterograde neuromodulator, with properties that are somewhat similar to that of a neurotransmitter (Altar and Distefano, 1998; Blum et al., 2002).

Studies on BDNF release have shown that many parameters associated with

neuropeptide secretion also apply to BDNF released from neurons. These include the lack of physical docking at synaptic sites, the virtual lack of fusion-competent DCVs, the need for prolonged intracellular Ca^{2+} elevations in the release compartment, and the slow emptying of peptide content from DCVs, (Balkowiec and Katz, 2000; Brigadski et al., 2005; Lessmann et al., 2003). Altogether these parameters make the release of high molecular weight substances stored in DCVs profoundly different from the release of more classical low molecular weight transmitters contained in agranular vesicles. Costorage of BDNF and neuropeptides within individual DCVs leaves the possibility open for similar mechanisms of release that may occur concurrently for both types of molecules. In endocrine cells, it has been demonstrated that DCVs can release some of their cargo by kiss and run, raising the possibility of differential release of low versus high molecular weight stored molecules (Rutter and Tsuboi, 2004; Tsuboi and Rutter, 2003). However, peptide (and likely BDNF) release requires complete vesicle fusion (Barg et al., 2002), and simultaneous capacitance measurements and confocal imaging have shown that a negligible amount of peptides stored in DCVs can be released by kiss and run.

Given that BDNF can be costored with one or more peptides, fusion of a DCV containing, for example, BDNF and substance P should release both molecules. However, Lever et al. (2001) have shown that one pattern of afferent stimulation releases substance P alone, whilst a different pattern releases substance P and BDNF. Possible mechanisms of differential release are suggested by quantitative analysis. Immunogold labeling in spinal cord and amygdala led to the conclusion that all terminal DCVs contain a cocktail of high molecular weight transmitters (Salio et al., 2007), and thus differential release of BDNF and costored peptides in vivo could rely on differences in the relative rate of their dissolution from the DCV core, since this is the critical determinant of the speed of peptide/neurotrophin secretion in vitro (Brigadski et al., 2005).

In sensory neurons DCV-stored BDNF can be released with activity. Such a possibility was first demonstrated after depolarization of cultured nodose-petrosal ganglion cells (Balkowiec and Katz, 2000). Subsequently, as mentioned previously, it was shown that BDNF can be released from C-fiber terminals in spinal cord after appropriate experimental challenge (Lever et al., 2001). Dialysis microprobes indicated that release is higher in superficial laminae (Walker et al., 2001). After peripheral inflammation, the content of BDNF increases in DRGs, together with an increased and more widespread release in dorsal horn (Lever et al., 2001; Walker et al., 2001). This perhaps reflects a de novo expression in RT97-positive medium-to large-sized light neurons (see above) or induction (and subsequent release) of BDNF in microglial cells, as it occurs in certain neuropathic pain models (Coull et al., 2005).

2.2. Distribution of BDNF in nociceptive pathways (see Table 1)

2.2.1. Sensory ganglia and spinal cord

Distributional studies on the occurrence of BDNF in sensory neurons have been mainly focused on DRGs and the trigeminal ganglion (Ichikawa et al., 2006; Kashiba et al., 2003), although the NT has been detected also in the nodose (Kashiba et al., 2003), petrosal (Ichikawa et al., 2007), jugular (Ichikawa et al., 2007) and geniculate (Farbman et al., 2004) ganglia.

Table 1

Neuronal expression of BDNF and trkB (mRNA and/or protein) in nociceptive pathways of normal adult animals

Location		BDNF	fl-trkB	References
Sensory neurons (I order neurons)	DRGs	Peptidergic small-to medium-sized dark neurons	Peptidergic small-to medium-sized dark neurons	Wetmore and Olson, 1995; Luo et al., 2001; Salio et al., 2005
	Encephalic ganglia	Trigeminal, jugular, nodose, Petrosal and geniculate neurons	Trigeminal, petrosal, and geniculate neurons	Matsumoto et al., 2001; Kashiba et al., 2003; Farbman et al., 2004; Ichikawa et al., 2006, 2007
Sensory column nuclei (II order neurons)	Spinal cord	PAF terminals	• PAF terminals • Cell bodies • STT neurons	Zhou et al., 1993, 2004 Conner et al., 1997 Salio et al., 2005, 2007; Slack et al, 2005
	Encephalic trunk	PAF terminals in trigeminal sensory nuclei	Rostral ventromedial medulla	Guo et al., 2006; Ichikawa et al., 2006; Renn et al., 2006
Relay neurons (III order neurons)	Thalamus	• Few terminals and occasional cell bodies in medial thalamus • Scattered terminals and cell bodies in posterior intralaminar nucleus	Cell bodies in all nuclei related to pain processing	Altar et al., 1994; Conner et al., 1997; Yan et al., 1997a, 1997b
	Reticular formation	No	Cell bodies	Yamuy et al., 2000
	PAG	• Mainly fibres	Cell bodies	Altar et al., 1994;Conner et al., 1997
		• Occasional cells		Guo et al., 2006; Renn et al., 2006
	Hypothalamus	• Mainly fibres	Cell bodies, mainly	Altar et al., 1994; Conner et al., 1997
		• Occasional cells	expressing tr-trkB	Yan et al., 1997a, 1997b; Silhol et al., 2005; Arancibia et al., 2007
	Parabrachial nucleus	Cell bodies	Not applicable	Conner et al., 1997
Integrative centers	Amygdala	Occasional to scattered cell bodies	Very abundant cell bodies	Altar et al., 1994; Conner et al., 1997; Yan et al., 1997a, 1997b; Salio et al., 2007
	Somatosensory cortex	Terminals mainly in central neurons Cell bodies in all layers,less abundant in layers I and IV • Processes in neuropil of layers I-III and V	Cell bodies mainly concentrated in layer V with dendrites that extend into supragranular cortex	Pitts and Miller, 1995, 2000; Fryer et al., 1996; Giehl et al., 1998; Robertson et al., 1998; Miller and Pitts, 2000; Bruns and Miller, 2007
Descending pathways	Raphe magnus nucleus	Cell bodies	Cell bodies	Madhav et al., 2001
	Paramedian reticular formation nuclei	Cell bodies	Cell bodies	Conner et al., 1997; Yan et al., 1997a, 1997b; Yamuy et al., 2000
	Locus coeruleus	Cell bodies	Cell bodies	Conner et al., 1997; Numan et al., 1998; King et al., 1999; Yamuy et al., 2000; Akbarian et al., 2002

Data on the distribution of BDNF and trkB in normal animals reported in this table are derived from in situ hybridization and/or immunocytochemistry. Note that not all studies have been carried out with specific probes for specific trkB isoforms. These indications are given only when

available. Abbreviations are listed at beginning of the main text.

It should be, however, kept in mind from the beginning that the pattern of BDNF (and other peptides) in DRGs is not fixed and changes with inflammation and axotomy (see Section 2.4). The type of sensory neurons expressing BDNF, i.e., the peptidergic small-to medium-sized dark neurons (Luo et al., 2001; Salio et al., 2005; Wetmore and Olson, 1995), and the pattern of expression/co-expression, in particular with the trk receptors (see Section 2.4), appear to be similar in different ganglia.
BDNF localization in the spinal dorsal horn is mostly prominent in terminals of laminae I–II, where the NT is stored together with the sensory neuropeptides substance P and CGRP, whereas there are no II order neurons expressing the NT in this location (Salio et al., 2005, 2007; – Section 1.2.1).

2.2.2. Supraspinal centers

Second order neurons of the trigeminal system do not express BDNF, but immunoreactive terminals, likely PAF originating from the trigeminal ganglion, are found in lamina II of the trigeminal spinal nucleus (Ichikawa et al., 2006). Relay III order neurons only occasionally express BDNF with exception of the pontine parabrachial neurons that anterogradely transport the NT to the central nucleus of amygdala (Conner et al., 1997).
Among integrative centers, BDNF-expressing neurons are relatively abundant in several layers of somatosensory cortex (Bruns and Miller, 2007; Fryer et al., 1996; Giehl et al., 1998; Miller and Pitts, 2000; Pitts and Miller, 1995; Pitts and Miller, 2000; Robertson et al., 1998). Neurons of origin of all the three main descending pathways related to supraspinal modulation of pain neurotransmission (see Section 1.2.3.1) express BDNF (Akbarian et al., 2002; Conner et al., 1997; King et al., 1999; Madhav et al., 2001; Numan et al., 1998; Yamuy et al., 2000; Yan et al., 1997a). Still it remains to be established whether or not the NT can be anterogradely transported to the terminals of these neurons located at spinal level.

2.3. BDNF receptors

As mentioned, many of the biological affects of BDNF are mediated by the high affinity receptor trkB. BDNF (and the other members of the NT family) also binds, albeit with much lower affinity, to a different receptor commonly referred to as the common NT low affinity receptor ($p75^{NTR}$). The biological functions mediated by $p75^{NTR}$ remain, for the most, elusive and appear to be mainly related to cell survival (Barrett, 2000) rather than (putative) neurotransmitter function, and therefore are not taken into consideration here.
Binding of BDNF to trkB induces dimerization and autophosphorylation of the receptor, and aggregation of numerous adaptive proteins that lead to activation of several kinase cascades and intracellular transduction pathways (Kaplan and Miller, 2000). Most of these pathways may be of relevance to the explanation of the effects of BDNF at the cell level, although they only partly account for short-term modulation of synaptic strength (see Section 3.2.1).
In developing and adult CNS, three different trkB isoforms are generated by alternative splicing of the trkB mRNA (Barbacid, 1994; Klein et al., 1990b; Middlemas et al., 1991): the full-length trkB (fl-trkB) receptor and two truncated receptor forms (tr-trkB) (Klein et al., 1990a; Middlemas et al., 1991). All trkB isoforms share a common extracellular domain, whereas the truncated isoforms lack the signal transducing intracellular tyrosine kinase domain, and thus do not appear to be able to trigger the intracellular signal transduction pathways that are commonly utilized by BDNF to exert its biological functions (Klein et al., 1990a; Middlemas et al., 1991). This also applies to pain modulation, since repeated intrathecal injections of a specific antibody to fl-trkB, but not to tr-trkB, reversed the thermal hyperalgesia observed in nerve-

ligated mice (Yajima et al., 2002).

2.3.1. Distribution of trkB in nociceptive pathways (see Table 1)

Several studies have reported on the distribution of trkB mRNA in CNS (see for example Altar et al., 1994; Fryer et al., 1996; King et al., 1999; Klein et al., 1990b), but there is considerably less information on the cellular (Yan et al., 1997a; Zhou et al., 1993) and subcellular (Aoki et al., 2000; Drake et al., 1999) localization of the receptor protein. These studies are of course not limited to pain pathways, given the importance of BDNF as a survival factor for central neurons. In general terms, in situ hybridization experiments showed that tr-trkB is prevalently expressed in choroid plexus, ependymal cells and astrocytes, i.e., non neuronal cells, whereas both fl-trkB and tr-trkB are expressed in neurons (Altar et al., 1994; Armanini et al., 1995; Beck et al., 1993; Ernfors et al., 1992; Frisé n et al., 1993; Klein et al., 1990a, 1990b). Given the possibility that tr-trkB is also present in the latter, distributional studies as such should be taken into consideration with some care, because not all RNA probes/antibodies employed possess a univocal specificity for the functional fl-trkB.

2.3.1.1. Sensory ganglia and spinal cord. Most sensory ganglia that express BDNF also express trkB. These include the DRGs, the trigeminal, petrosal and geniculate ganglia (Farbman et al., 2004; Ichikawa et al., 2006, 2007; Kashiba et al., 2003; Matsumoto et al., 2001).

Initial studies at the light microscope level on the distribution of trkB in spinal cord led to the localization of mRNA (Bradbury et al., 1998; Ernfors et al., 1993; Widenfalk et al., 2001) and protein (Garraway et al., 2003; Zhou et al., 1993) in neurons (and glia) distributed throughout the dorsal horn (Mannion et al., 1999; Widenfalk et al., 2001; Zhou et al., 1993), including neurons of the STT (Slack et al., 2005). Bradbury et al. (1998) suggested that trkB was unlikely to be present on PAF terminals, also taking into consideration that only a small proportion of medium-to large-sized DRG neurons expressed the receptor under normal conditions (Koltzenburg et al., 1999; Lewin and Barde, 1996; McMahon et al., 1994; Wright and Snider, 1995), but see Section 2.4. Although in situ hybridization experiments showed that the dorsal horn neurons expressing the trkB mRNA were very numerous, immunocytochemistry revealed that the trkB protein was considerably less abundant or even absent (Mannion et al., 1999; Michael et al., 1999). High levels of fl-trkB expression were only observed following activity, inflammation and/or axotomy in several species (Frisen et al., 1992; Mannion et al., 1999; Michael et al., 1999; Narita et al., 2000; Thompson et al., 1999), whereas increased expression of trkB following partial sciatic nerve ligation was reported in one study only (Narita et al., 2000).

At the ultrastructural level even less information was available (Frisen et al., 1992) until recently, when Salio et al. (2005) carried out an in-depth study on the localization on fl-trkB in lamina II, the precise definition of localization at synapses being crucial to the understanding of the mechanisms of action of BDNF in the modulation of neurotransmission.

This study provided the first ultrastructural description of fl- trkB localization at synapses between first and second order sensory neurons in spinal lamina II, from which it appeared evident that fl-trkB was not only present at somato-dendritic membranes of lamina II neurons, but also at axon terminals. About 90% of these terminals were identified as belonging to PAFs. Moreover, all fl- trkB-immunopositive C boutons in type Ib glomeruli were immunoreactive for BDNF, and, at individual glomeruli and axo- dendritic synapses, fl-trkB was located in a mutually exclusive fashion at pre- or post-synaptic membranes. Thus only a small fraction of fl-trkB-immunoreactive dendrites were post-synaptic to BDNF-immunopositive PAFs, which, on the other hand, also expressed fl-trkB.

The importance of this data in understanding the circuitry involved in the

modulation of lamina II synapses by BDNF is discussed below (Section 3.2); in addition, it is important to recall here that it was previously assumed that local interneurons were the only source of trkB in lamina II, and receptor expression in PAFs was a matter of debate.

2.3.1.2. Supraspinal centers. It is of interest to note (see Table 1) that virtually all supraspinal areas related to nociception contain trkB-expressing neurons. These include not only all the main relay centers (thalamus, reticular formation, hypothalamus, PAG and parabrachial nucleus), which are the sites of III order neurons (Altar et al., 1994; Arancibia et al., 2007; Conner et al., 1997; Guo et al., 2006; Renn et al., 2006; Silhol et al., 2005; Yamuy et al., 2000; Yan et al., 1997a), but also the integrative centers (cortex and amygdala), which represent the end point of the ascending pathways (Altar et al., 1994; Bruns and Miller, 2007; Conner et al., 1997; Fryer et al., 1996; Giehl et al., 1998; Guo et al., 2006; Miller and Pitts, 2000; Pitts and Miller, 1995, 2000; Renn et al., 2006; Robertson et al., 1998; Salio et al., 2007; Silhol et al., 2005; Yan et al., 1997a), and neurons in the most important sites of origin for descending pathways (Akbarian et al., 2002; Conner et al., 1997; King et al., 1999; Madhav et al., 2001; Numan et al., 1998; Yamuy et al., 2000; Yan et al., 1997a). The reason for such an interest lies in the possibility that BDNF can reach at least some of these areas following anterograde transport, which, as mentioned, is of relevance for its transmitter role (see Section 2.1).

2.4. Plasticity of sensory neurons in regard to concurrent BDNF/trkB expression

Primary sensory neurons display a widely documented neurochemical and functional plasticity that relates to the onset of certain exaggerated and/or altered conditions of pain perception. This issue has been reviewed elsewhere (Binder and Scharfman, 2004; Hucho and Levine, 2007; McMahon and Jones, 2004; Pezet and McMahon, 2006; Price et al., 2006; Woolf and Ma, 2007), but some novel relevant finding should be mentioned here.

Salio et al. (2005) have recently demonstrated that a subpopulation of peptidergic small-to medium-sized dark DRG neurons (about 10% of the total DRG neurons) co-expressed BDNF+trkB but not trkA in normal rat and mouse. Therefore, under normal conditions the DRG neurons containing BDNF fall into two different subpopulations: the first (about 2/3) expresses trkA and is thus NGF-sensitive; the second (the remaining 1/3) expresses trkB, and thus is insensitive to NGF. This finding is of relevance since it has been previously established that following peripheral inflammation, BDNF mRNA and protein are upregulated by an NGF-dependent mechanism in trkA expressing peptidergic small-to medium-sized dark neurons (Pezet et al., 2002b), which, at that time, were believed to represent the total population of BDNF containing neurons in DRGs. The issue of BDNF localization is further complicated by the fact that the NT in sensory neurons may also be dysregulated in several different experimental conditions including several models of neuropathic pain (Fukuoka et al., 2001; Ha et al., 2001; Obata et al., 2002, 2003; Obata and Noguchi, 2004; Onda et al., 2003; Zhao et al., 2003), axotomy (Michael et al., 1999), and inflammation (Onda et al., 2004).

Since the results of experimental manipulations often yield rather contrasting results (see Section 4), data on expression of BDNF in sensory neurons under different experimental conditions must be considered with caution, in particular in those circumstances where activation of trkA receptors is likely to occur.

3. **Functional evidence for BDNF as a pain modulator**

The seminal paper by Kafitz et al. (1999) was one of the first to show that BDNF excited neurons in the hippocampus, cortex and cerebellum as rapidly as the

neurotransmitter glutamate, even at a more than thousand-fold lower concentration. This opened the way to a series of investigations aiming to dissect out the role of the NT as a fast synaptic transmitter. Functional data on the role of BDNF at synapses involved in pain neurotransmission are reported below.

3.1. Primary sensory neurons

A few studies have been reported so far about the effects of BDNF on the activity of DRG neurons in relation to pain. Peripherally, the NT seems to be able to interfere both with tactile mechanotransduction and nociception, and sensitivity of slowly adapting mechanoreceptors is dependent on the availability of the NT. BDNF-deficient mice exhibited, in medium- and large-diameter DRG neurons, downregulation of the ASIC2 sodium channels, which are necessary for normal mechanotransduction (McIlwrath et al., 2005).

Several lines of evidence indicate that nociceptive DRG neurons are sensitized by BDNF. Intraplantar injections of BDNF caused a transient thermal hyperalgesia that persisted up to 5 h after the treatment. NT-4/5, the other neurotrophin acting at trkB, was even more effective (Shu et al., 1999). In the same study, application of BDNF to the receptive field, in a skin-nerve preparation, sensitized the electrical responses of C fibers to noxious thermal stimulation. The mechanisms underlying both the behavioral and electrophysiological effects are not clear: the neurotrophin could directly interact with the nociceptors or, alternatively, modulate their activity indirectly, by binding to non-neuronal cells.

Modulation of DRG neuron function by BDNF has been investigated in normal animals and neuropathic pain models. Delivery of BDNF directly to DRGs induced mechanical allodynia in normal rats; the same behavior, produced by sciatic nerve transection, was attenuated by direct application to injured DRGs of an anti-BDNF antibody (Zhou et al., 2000). Chronic constriction or transection of the sciatic nerve caused a down-regulation of voltage-dependent potassium channels in DRG neurons (Kim et al., 2002; Park et al., 2003); exogenous application of BDNF to cultured DRG neurons had a similar effect on potassium channel expression, suggesting a role of the NT in the changes of electrical activity observed in injured primary sensory neurons. Finally, BDNF delivered in vivo directly to the transected sciatic nerve in axotomized rats reduced the increase of $GABA_A$ receptor-mediated conductance caused by the injury. On the other hand, the shape of action potentials generated by DRG neurons was affected by NGF, but not by BDNF (Oyelese et al., 1997).

3.2. Spinal cord

Undoubtedly the vast majority of functional data supporting the role of BDNF as a pain neurotransmitter derive from studies carried out on rodent spinal cord. An issue of importance was, initially, the demonstration of the existence of a basal release of the NT in the superficial dorsal horn (Walker et al., 2001). In parallel it was shown that release could be evoked by a specific pattern of electrical stimulation (short bursts of high frequency) of C fibers with intervention of the NMDA receptors (Lever et al., 2001), and that capsaicin was also able to induce a dose-dependent release of BDNF. In both cases, release was increased after systemic or intrathecal NGF treatment. Similarly it was altered during pathological pain. Walker et al. (2001) observed, after sciatic nerve transection, that BDNF release was detected throughout the whole dorsal horn, rather than being limited to superficial laminae. In another model of neuropathic pain, the loose ligation of the sciatic nerve, BDNF release in dorsal horn was increased together with the appearance of thermal hyperalgesia (Miletic and Miletic, 2002).

All these observations converged to indicate that PAFs are capable of releasing the NT in dorsal horn, although it should be considered that they are not the only source of BDNF in this area, since Coull et al. (2005) have recently demonstrated that BDNF can also be released from glia.
Once released in dorsal horn, the NT modulates excitatory (glutamatergic) and inhibitory (GABAergic/glycinergic) neurotransmission.

3.2.1. Modulation of glutamatergic transmission

The circuitry at the basis of the effects of BDNF at glutamatergic synapses is quite complex and remains, for the most, to be established, since a parallel histological and functional analysis has been carried out only in a few studies. Nonetheless, both pre- and post-synaptic modulatory effects have been demonstrated, and we will summarize below the main functional data in support.

3.2.1.1. Post-synaptic mechanisms. Several data converge to demonstrate that binding of BDNF with post-synaptic trkB receptors – expressed by different populations of spinal cord neurons – determines synaptic facilitation. Application of exogenous BDNF in an isolated spinal cord preparation produced the increase of the ventral root potential (VRP) evoked by stimulation of the nociceptive primary afferents (Kerr et al., 1999; Fig. 2A, ❶). This observation was in keeping with the histological data on the (post-synaptic) trkB localization in motor neurons (Yan et al., 1997b). The nociceptive reflex was also modulated by endogen- ously released BDNF, since BDNF-deficient neonatal mice exhibited reduced VRPs compared to controls (Heppenstall and Lewin, 2001). Wind-up, a frequency-dependent facilitation of VRP evoked by repetitive stimulation and related to sensitization of spinal cord circuits, was also decreased in these mice. In addition, Kerr et al. (1999) showed that BDNF sequestration by the antibody trkB-IgG caused a significant depression of VRP, but only in NGF-pretreated rats (where BDNF was upregulated).
The BDNF-mediated potentiation of excitatory transmission in spinal cord ventral horn appeared to be related to activation of NMDA receptors expressed on motoneurons. Namely, BDNF induced the facilitation of glutamatergic EPSPs evoked by dorsal root stimulation, and its effect was inhibited by the intracellular block of NMDA receptors with MK-801 (Arvanian and Mendell,

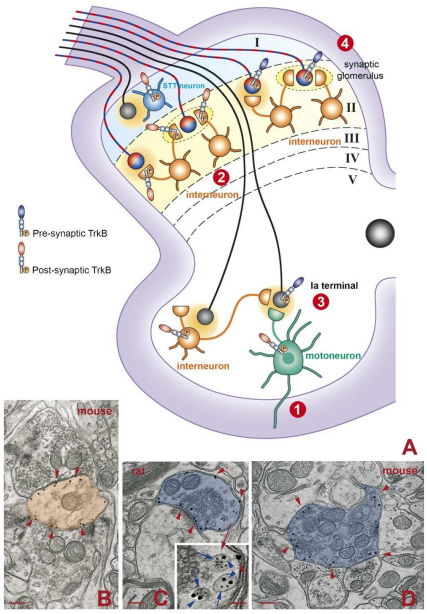

Fig. 2. Modulatory mechanisms of excitatory neurotransmission in spinal cord, mediated by BDNF. (A): Schematic representation of circuits involved in modulation of glutamatergic synaptic transmission by BDNF in dorsal and ventral horn. At the dorsal horn level (laminae I and II), the NT

acts on both pre- and post-synaptic trkB. Activation of post-synaptic trkB triggers several intracellular pathways, involving both post-translational (such as potentiation of NMDA receptors) and transcriptional mechanisms. Pre-synaptic trkB are expressed on primary afferent fibers of both peptidergic and non-peptidergic type. Both types of fibers may be engaged in glomeruli and axo-dendritic synapses in lamina II (see also Fig. 5): BDNF binding to these receptors modulates glutamate and peptide release. Some peptidergic terminals express both BDNF and trkB receptors: at these synapses trkB could act as an autoreceptor, regulating the NT release. At the ventral horn level, both a pre- and a post-synaptic modulation by BDNF have been observed, likely involving NMDA receptors. The post-synaptic effect is due to activation of trkB on motoneurons, while the pre-synaptic action could occur at the terminals of large muscle spindle (Ia) fibers or, indirectly, through the involvement of a ventral horn interneuron. See text for further details and references. Color codes: PAF terminals: non-peptidergic, blue; peptidergic, red (the two colors are used together to indicate the possibility that both types are involved); uncharacterized, black. Pre-synaptic trkB, blue; post-synaptic trkB, orange; Axons are indicated by circles, dendrites by half-circles. (B–D): electron micrographs showing the pre- and post-synaptic ultrastuctural localization of trkB (red arrowheads) in mouse (B, D) and rat (C) neuronal processes within lamina II. Superimposition of same colors used in A has been used to highlight the different types of profiles engaged in axo-dendritic contacs (B–C) or a glomerulus (D), as identified by immunocytochemistry. The axon terminal in C displays fl-trkB (red arrowheads) and contains three DCVs immunolabeled for BDNF (10 nm gold, blue arrows) and CGRP (20 nm gold, blue arrowheads). Bars = 250 nm; insert = 100 nm. See text for further details and references.

2001). Moreover, the depolarizing response of motoneurons directly evoked by NMDA was enhanced after application of the NT.

Similar mechanisms have been described in the dorsal horn. Glutamatergic EPSCs recorded from lamina II neurons and evoked by dorsal root stimulation were rapidly potentiated by BDNF (Garraway et al., 2003; Fig. 2A, ❷). Subsequent ultrastructural observations were confirmative about the presence of post- synaptic trkB receptors in lamina II neurons (Salio et al., 2005). Similar to what was observed in the ventral horn, the BDNF effect was blocked by intracellular MK-801, indicating the involvement of post-synaptic NMDA receptors (Garraway et al., 2003).

The intracellular pathways linking the activation of post- synaptic trkB receptors to the modulation of NMDA receptors have been studied quite extensively, and results obtained from studies on isolated neurons and/or other locations of CNS are remarkably similar to those in spinal cord (Fig. 3). BDNF binding to trkB causes receptor autophosphorylation and the subsequent activation of several intracellular transduction pathways (Fig. 3, ❶). In keeping with observations in vitro, trkB phosphorylation in spinal cord was significantly increased after noxious mechanical, thermal or chemical stimulation in neonatal or adult rats, while innocuous stimulation was ineffective (Lever et al., 2003b; Pezet et al., 2002b). The level of trkB phosphorylation was maximal 2 min after stimulation and decreased within 30 min. Increased trkB phosphorylation was also observed after intraplantar capsaicin injection or NGF pre-treatment.

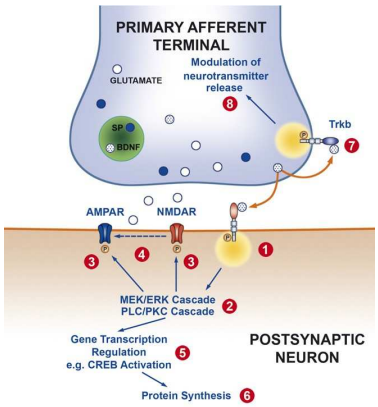

Fig. 3. Summary of the mechanisms by which BDNF potentiates glutamatergic transmission in spinal cord. For clarity, a simple axo-dendritic synapse formed by a primary afferent terminal and a post-synaptic neuron is illustrated. ❶ Following interaction with BDNF at the post-synaptic membrane, trkB is activated by autophosphorylation; ❷ TrkB activation triggers MEK/ERK and PLC/PKC cascades. ❸ Second messengers of these pathways phosphorylate NMDA and AMPA receptors modulating their activity. ❹ NMDA receptors are probably involved in BDNF-induced AMPA receptor potentiation, by regulating receptor phosphorylation and trafficking. Second messengers also regulate ❺ gene transcription and ❻ subsequent protein synthesis. ❼ BDNF-induced trkB activation at pre-synaptic level leads to ❽ modulation of neurotransmitter release.

One major consequence of trkB activation is the phosphorylation of NMDA receptors. Several intracellular pathways are involved, i.e., MEK/ERK, PLC/PKC, and, likely, other still incompletely characterized pathways leading to activation of other down-stream kinases (Fig. 3, ❷).
The MAP kinase ERK is activated by nociceptive stimulation and contributes to pain hypersensitivity (Ji et al., 1999). Incubation of spinal cord slices with BDNF or intrathecal administration of the NT induced the increase of ERK phosphorylation in superficial dorsal horn (Pezet et al., 2002a; Pezet et al., 2002b). Conversely, the use of a MEK inhibitor reduced the phosphorylation of NR1 caused by BDNF application (Slack et al., 2004). Activation of ERK by BDNF was particularly characterized in STT neurons (Slack et al., 2005).
PLC and PKC inhibitors blocked the NMDA-mediated facilitation of EPSCs, induced by BDNF in lamina II neurons (Garraway et al., 2003) (see above), and incubation of rat hemicords in a PKC inhibitor reduced the phosphorylation of NR1 produced by BDNF stimulation (Slack et al., 2004). PKC activation was involved in long-lasting thermal hyperalgesia and tactile allodynia induced in mice by intrathecal administration of BDNF, and mediated the $[Ca]_i$ increases observed in cultured spinal neurons following application of the NT (Yajima et al., 2005).
The BDNF-induced phosphorylation of NMDA receptors (Fig. 3, ❸) leads to modification of channel properties (for example by increasing the open probability) and regulation of other cellular mechanisms, such as receptor trafficking. Namely, superfusion of the isolated hemisected spinal cord in BDNF for 20 min caused a significant increase of NMDA receptor phosphorylation localized to lamina I, II and V of the dorsal horn (Slack and Thompson, 2002). Different subunits of the receptor can be targeted by activated trkB. For example, endogenous BDNF released by burst stimulation of the dorsal roots produced a significant increase of phosphorylation of the NMDA receptor subunit NR1 (Slack et al., 2004), whereas incubation with BDNF of post-synaptic densities purified from rat spinal cord induced the phosphorylation of NR2A and NR2B (Di Luca et al., 2001). Finally, NMDA receptors were reported to contribute to the hyperalgesic responses induced by intrathecal administration of BDNF in normal mice (Groth and Aanonsen, 2002) and phosphorylation of the NR2B subunit was observed in a model of chronic inflammation (Guo et al., 2002).
A cross-talk between NMDA and AMPA receptors (Fig. 3, ❹) maybe also responsible of short-term changes in response properties of the post-synaptic membrane (for review see Derkach et al., 2007).

3.2.1.2. Pre-synaptic mechanisms. Experimental support for a possible pre-synaptic action of BDNF on glutamate release has been provided both in ventral and dorsal horn. In the already mentioned study by Arvanian and Mendell (2001), the initial facilitation of evoked EPSPs, induced in motoneurons by BDNF, was followed by a long-lasting depression. The effect was NMDA-dependent, as it was inhibited by extracellular NMDA receptor blockers. However, the intracellular block of motoneuron NMDA receptors was ineffective, suggesting a pre-synaptic site of action for the NT. The circuitry involved has not been investigated histologically: BDNF could interact with NMDA receptors expressed on primary afferent terminals (Fig. 2A, ❸), located on spinal cord interneurons or both.

Two studies have recently described the effects of BDNF on spontaneous glutamate release in lamina II (Fig. 2A, ❹). Unfortunately, these two studies cannot be directly compared, since they have employed completely unrelated experimental approaches. Nonetheless, both indicate that BDNF acts pre-synaptically to increase the release of transmitters from PAFs. In adult rats treated with an intraplantar injection of complete Freund's adjuvant (a model of chronic inflammation), a brief application of BDNF increased the frequency of miniature EPSCs (mEPSCs) recorded from lamina II neurons (Matayoshi et al., 2005). The effect was resistant to tetrodotoxin, but sensitive to lidocaine, a non-selective blocker of sodium channels. Therefore rapidly activated tetrodotoxin-resistant channels, such as Nav1.8 and NaN, which are expressed on nociceptors and up-regulated in pathological states, appeared to be implicated. In untreated neonatal rats, a sustained application of BDNF elicited an increase of mEPSC frequency in lamina II neurons (Merighi et al., 2008). The amplitude and kinetic properties of mEPSCs were unchanged. Incubation of slices with BDNF induced the release of glutamate and peptides from PAFs, evoking long-lasting calcium oscillations in lamina II neurons, as demonstrated after confocal analysis of calcium fluxes, quantitative immunocytochemistry, and electron microscopy.
Taken together, the two studies support the notion that activation of pre-synaptic trkB receptors in PAF terminals (Fig. 3, ❼) leads to modulation of release of coexisting transmitters (Fig. 3, ❽).

3.2.2. Modulation of GABAergic and glycinergic transmission

Evidence is also accumulating in support for a role of BDNF as a modulator of inhibitory synapses in dorsal horn, since the release of both GABA and glycine appears to be regulated (among others) by the NT. Initial studies showed that superfusion of the isolated dorsal horn from adult rats increased the release of GABA induced by potassium-mediated depolarization (Pezet et al., 2002a; Fig. 4A,). More recently we have reported that the NT increased the spontaneous release of GABA and glycine in lamina II neurons from neonatal rats, although the evoked release of the two neurotransmitters was depressed (Bardoni et al., 2007). The mechanisms responsible were, at least in part, elusive. However, trkB receptors were detected on GABAergic terminals of inhibitory interneurons, giving a strong support to the notion that BDNF acts as pre- synaptic modulator at glomeruli (Fig. 4A(❷), B, and C).
An additional consequence of the capability of BDNF to modulate the release of GABA appears to be a depression of peptidergic transmission. In the isolated dorsal horn, BDNF has been shown to inhibit substance P (but not glutamate) release,

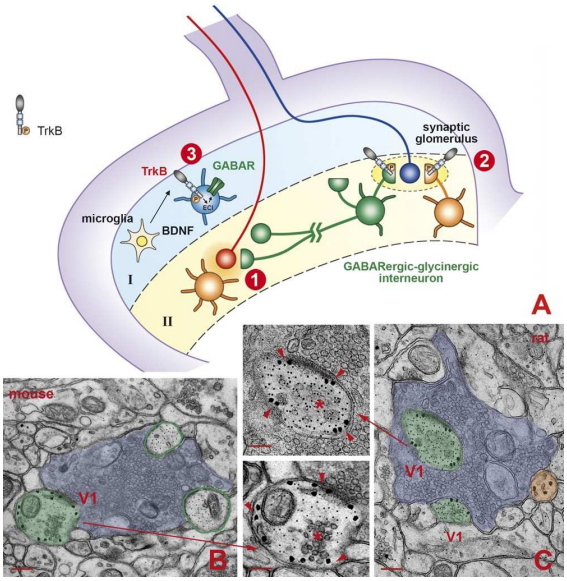

Fig. 4. Modulatory mechanisms of inhibitory neurotransmission in superficial spinal cord dorsal horn, mediated by BDNF. (A): lamina I: activation of microglia during pathological pain induces BDNF release that, in turn, converts the action of GABA on lamina I projection neurons from inhibitory to excitatory; lamina II: inhibitory interneurons (islet cells, green) express trkB receptors on their vesicular dendrites (V1) in glomeruli (see B and C). These profiles are likely to be involved in a direct modulation of GABA and glycine release. TrkB on GABAergic interneurons could also indirectly control the release of other neurotransmitters (glutamate, substance P) from primary afferent terminals, but the circuitry remains to be clarified (as represented by an interrupted process). Some dendritic profiles in lamina II also express trkB (unclassified cells, orange). Color codes: PAF terminals: non peptidergic, blue; peptidergic, red; uncharacterized, black. Post-synaptic trkB, orange. Axons are indicated by circles, dendrites by half-circles. (B-C): electron micrographs showing the ultrastuctural localization of trkB and GABA in mouse (B) and rat (C) glomeruli. Superimposition of same colors used in A has been used to highlight the different types of profiles forming the glomeruli, as identified by immunocytochemistry. Two V1 profiles are shown at higher magnification in the inserts. They are labeled for fl-trkB (arrowheads) and contain clusters of agranular vesicles immunolabeled for GABA (asterisk). Bars = 250 nm; inserts = 100 nm. See text for further details and references.

evoked by electrical dorsal root stimulation or by capsaicin. Since the effect was blocked by a $GABA_B$ receptor antagonist, the activation of $GABA_B$ receptors expressed on primary afferent terminals was proposed to be responsible for inhibition of peptide release (Meyer-Tuve et al., 2001; Pezet et al., 2002a) (Fig. 4A, ❶).

A link between BDNF and inhibitory neurotransmission was demonstrated also in neuropathic pain, where altered levels of endogenous BDNF are observed (see Section 2.4). Rats subjected to the ligation of a spinal nerve (SNL) exhibited a hypersensitivity to thermal and mechanical stimuli that was reduced by intrathecal administration of BDNF (Lever et al., 2003a). After SNL a reduced release of BDNF and GABA was demonstrated, whereas normal levels of GABA release were restored upon superfusion with the NT. These observations led to the conclusion that a reduction of BDNF availability occurs in neuropathic pain and causes the impairment of GABAergic neurotransmission in dorsal horn, contributing to hyperexcitability and central sensitization.

The role of BDNF-mediated regulation of inhibitory transmission in neuropathic pain has also been recently investigated by Coull et al. (2005) (Fig. 4, ❸). In this study, intrathecal administration of microglia activated by ATP reproduced the mechanical allodynia observed after peripheral nerve injury. The same pain behavior was observed after intrathecal injection of BDNF. Both treatments produced an alteration of the concentration of anions (principally chloride) in lamina I neurons, likely through the reduced synthesis of the potassium-chloride co-transporter KCC2. Anion reversal

potential was shifted toward values more positive than the neuron resting potential, and thus the effect of GABA receptor activation was depolarizing under these conditions. Therefore, BDNF was proposed as a link molecule between microglia and neurons: ATP-stimulated microglia releases BDNF, and interaction of the NT with lamina I neurons contributes to their hyperexcitability in neuropathic pain.
In summary, the functional studies reported so far suggest a predominant excitatory effect of BDNF as modulator of synaptic transmission in spinal cord. In particular, in superficial dorsal horn glutamatergic transmission is potentiated by BDNF, both through an increase of glutamate release and by enhancing synaptic efficacy at the post-synaptic level. Similarly, motor responses to nociceptive inputs are facilitated by the NT, although a depressing pre-synaptic effect has been observed in immature spinal cord.
The modulation of inhibitory transmission seems to be more complex: post-synaptic modulation of chloride concentrations in lamina I neurons and pre-synaptic inhibition of evoked GABA and glycine release in lamina II would suggest a general effect of disinhibition exerted by BDNF. On the other hand, facilitation of spontaneous, or potassium-evoked, GABA and glycine release indicates that additional yet unraveled mechanisms of regulation occur in dorsal horn.

3.2.3. Alteration of gene transcription

Besides rapid acting at synapses, BDNF is capable of inducing ‘‘long-term’’ effects that require hours to occur and are related to alterations in gene transcription. Kerr et al. (1999) showed that intrathecal administration of BDNF for 3 h induced the increase of immunoreactivity to the immediate early gene c-fos in dorsal horn neurons. A separate study (Jongen et al., 2005) reported that intrathecal injection of BDNF produced a significant increase of the expression of the early gene products c-fos, c-Jun and Krox-24 in the adult rat, particularly in superficial dorsal horn. Local application of BDNF onto the dorsal horn in vivo elicited activation of cyclic AMP response element binding protein (CREB) by phosphorylation, within 2 h (Fig. 3, ⑤). Administration of k-252a (a general antagonist of kinase activity) reduced CREB phosphor- ylation observed after loose ligation of the sciatic nerve. Therefore, activation of CREB by BDNF could be involved in central sensitization and synaptic plasticity phenomena occurring after periph- eral nerve injury (Miletic et al., 2004).

3.2.4. Circuitry by which BDNF exerts its neuromodulatory action at the synapses formed by PAF terminals on post-synaptic neurons in lamina II of normal animals

One of the major obstacles in understanding the role of BDNF as a modulator of synapses in spinal cord is the relative paucity of information on the circuitry involved in such an action of the NT. As mentioned, lamina II plays a pivotal role in the integration of nociceptive stimuli at the synapse between I and II order neurons (see Section 1.2.1.2). Therefore we have devoted a considerable effort in the analysis of these synapses by combined neurochemical, ultrastructural and functional approaches. Data obtained from these and other studies are summarized in Figs. 2 and 4–5.
As mentioned, PAFs of the C type originating from nociceptive small- to medium-sized peptidergic DRG neurons represent the only source of endogenous BDNF in lamina II (and the entire spinal cord). About 2/3 of these fibers express trkA receptors, and the remaining 1/3 are fl-trkB immunoreactive (Salio et al., 2005). Fl- trkB expression also occurs in non-nociceptive RT97-positive medium- to large-sized non-peptidergic neurons. The large majority (90%) of fl-trkB immunoreactive axon terminals in lamina II display a glomerular configuration (Fig. 5). The origin of the remaining 10%, which are arranged in axo-dendritic contacts, may be heterogeneous since they can be identified as C-fiber endings only when displaying peptide-containing LGVs. Fl-trkB-immunoreactive axons which are devoid of LGVs may correspond to endings of the RT97-positive DRG neurons, but also of spinal cord interneurons and/or descending

fibers. Given their low numbers in both rats and mice, peptidergic Ib glomeruli undoubtedly remain the key structures where BDNF exerts a pre-synaptic modulatory role of I to II order neuron synapses. Immunocytochemistry, electrophysiology, real time calcium imaging at the confocal microscope and release/depletion measurements on acute slices converged to demonstrate that BDNF is capable of evoking a sustained release of peptides and glutamate from PAFs by acting on pre-synaptic fl-trkB (Merighi et al., 2008). The reported observations were carried out following a challenge with exogenous BDNF, but glomeruli represent multi-synaptic sites which are ideally structured to amplify the response of second order neurons to activation of individual PAF endings, and it is thus possible that a pre-synaptic modulation of these synapses also occurs in vivo following release of endogenous BDNF. Experimentally, the NT is released endogenously after burst stimulation of C fibers (Lever et al., 2001), and a similar pattern of C fiber activation has been observed after noxious stimuli of high intensity in vitro (Adelson et al., 1996, 1997). Thus it is reasonable to hypothesize that BDNF is released under normal pain conditions, but only as a consequence of an intense stimulation of nociceptors. In this case the NT could exert a local modulation of synaptic transmission, by acting on trkB expressed at glomeruli and non-glomerular endings.

As mentioned, peripheral inflammation causes the increase of BDNF expression in peptidergic primary afferent terminals expressing trkA receptors, in an NGF-dependent manner (Apfel et al., 1996; Mannion et al., 1999; Michael et al., 1997). The level of fl-trkB is also increased in inflammatory pain (Mannion et al., 1999a). This up-regulation of BDNF and its receptors could determine a stronger and more generalized action of the NT in superficial dorsal horn, as a consequence of augmented local release at synapses, and diffusion by volume transmission. The latter could be particularly important in the modulation of GABAergic transmission (Bardoni et al., 2007), given that expression of post-synaptic fl-trkB on GABAergic interneurons has been

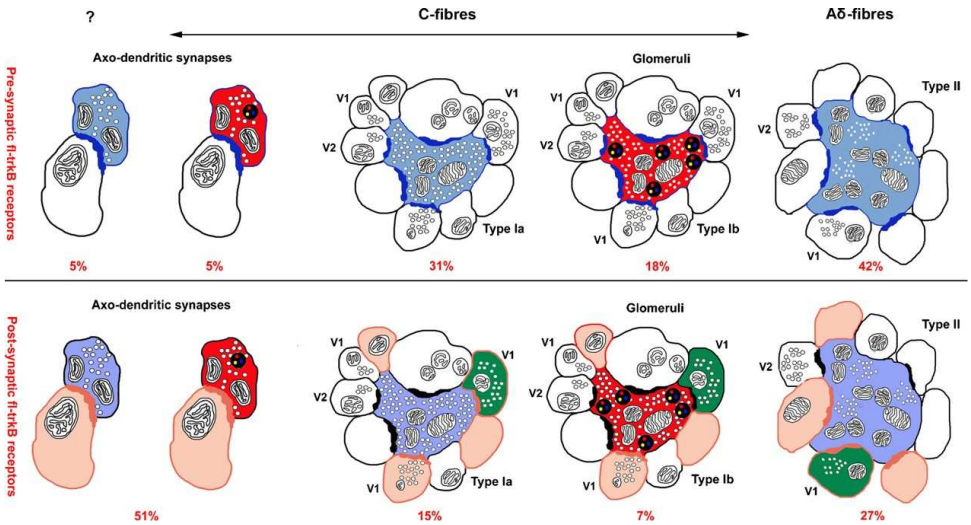

Fig. 5. Circuitries involved in the modulation of lamina II synapses by BDNF. For simplicity type II glomeruli have not been divided into type IIa and IIb (see Ribeiro-Da-Silva, 2004). Top: ultrastructural localization of pre-synaptic fl-trkB; bottom: ultrastructural localization of post-synaptic fl-trkB. Please note that there are no synapses where pre- and post-synaptic receptors are concurrently present (see Salio et al., 2005). Axons and dendrites expressing pre-synaptic fl-trkB have blue membrane. Post-synaptic fl-trkB immunopositive dendrites are orange. BDNF is co-stored with peptides (colored dots) within DCVs (black) contained in peptidergic terminals (red) engaged in axo-dendritic contacts or type Ib glomeruli. Non-peptidergic glutamatergic axons of primary afferent origin are light blue. GABAergic V1 profiles (pre-synaptic vesicle-containing dendrites) in glomeruli are green. Uncolored profiles have uncharacterized neurochemical content as related to BDNF or fl-trkB expression. These include V2 profiles (pre-synaptic vesicle-containg axons) at glomeruli. Red numbers at the bottom of each drawing indicate the percentages (means of rat and mouse) of immunoreactive fl-trkB profiles after quantification (see Salio et al., 2005).? = means that no data are available on the

origin of these profiles.

detected only in glomeruli of the non peptidergic type (Fig. 5), and thus diffusion of BDNF from nearby glomeruli could be important for the activation of these receptors.
To conclude, current anatomical and functional evidence converges to indicate that: (i) pre-synaptic fl-trkB on PAFs participates in the modulation of excitatory (glutamate, peptides) neurotransmitter release, whereas post-synaptic fl-trkB is mainly concerned with inhibitory neurotransmission; (ii) BDNF modulation of excitatory and inhibitory synapses occurs at different specific types of glomeruli. However, further studies are clearly needed to understand the modalities by which BDNF is released and interacts with its own receptors under different pain conditions.

3.3. Supraspinal centers

Although BDNF and trkB have been detected in several areas in the brain involved in nociception (Table 1), the functional role of the NT in pain elaboration at supraspinal level is still poorly understood.
Early studies had reported an analgesic effect when BDNF was infused into the midbrain, close to PAG and dorsal raphe nuclei (DRNi), including the RMN. In these experiments the NT caused a decrease of the response to thermal and chemical (formalin) stimuli. These effects were accompanied by increased levels of several neurotransmitters (such as opioids and peptides) in the brain and spinal cord. In particular, serotoninergic activity was enhanced, and an effect on serotonin synthesis was hypothesized (Siuciak et al., 1994, 1995, 1998). Infusion of BDNF into PAG and DRNi determined also a change in action potential firing of serotoninergic neurons, producing a more irregular pattern, maybe related to an elevated serotonin turnover (Celada et al., 1996).
In keeping with these results, Frank et al. (1997) showed that continuous infusion of high doses of BDNF at the level of PAG and DRNi caused analgesia, producing increased tail-flick latencies within 24 h. The levels of the trkB protein, but not of mRNA, were decreased in the site of infusion after 6 and 12 days of treatment (although the analgesic effect was not attenuated).
Analgesic effects of the NT during noxious thermal stimulation were also obtained following a single intracerebroventricular administration of BDNF (Cirulli et al., 2000). Chronic inflammatory conditions (induced by CFA injection in the hindpaw) reduced both neurogenesis in hippocampal dentate gyrus and levels of NK-1 receptor and BDNF mRNAs in CA1-CA3 pyramidal neurons. (Duric and McCarson, 2006). Similar effects were obtained after treatment with experimental models of stress, supporting the involvement of hippocampal BDNF in the affective- cognitive aspects of pain.
An interesting study about BDNF's role in modulating a descending facilitatory pathway has been recently reported by Guo et al. (2006). PAG in the brainstem presents high levels of BDNF protein and mRNA. PAG neurons form synapses into the rostroventromedial nucleus (RVM), a relay area that projects to the spinal cord. TrkB expression (both protein and mRNA) has been detected on RVM projecting neurons. Electrical burst stimulation of ventrolateral PAG evoked BDNF release, causing phosphorylation of trkB in RVM. Peripheral inflammation (induced by CFA injection) produced an enhanced expression of BDNF in PAG and trkB phosphorylation in RVM. Microinjections of low doses of BDNF into RVM reproduced the hyperalgesic conditions, while higher doses caused analgesia, consistent with the studies described above (Frank et al., 1997; Siuciak et al., 1994). BDNF binding to trkB on RVM neurons induced the phosphorylation of the NR2A subunit, through the activation of the PLC-PKC pathway. The BDNF mediated potentiation of NMDA receptors is likely involved in the increase of facilitatory drive to spinal cord that occurs in persistent pain (Ren

and Dubner, 2002, 2007; Vanegas and Schaible, 2004).

4. BDNF actions on pain behavior

Behavioral data on the effects of BDNF in mediating nociceptive responses are summarized in Table 2. The involvement of the endogenous NT in acute pain is still controversial: although some studies reported a change in nociceptive behavior by neutralizing BDNF in dorsal horn, the high frequency stimulation pattern required for NT release would argue against an important role in basal nociception. Intrathecal administration of BDNF seemed to evoke, in some cases, a pro-nociceptive response, while in other studies an anti-nociceptive effect was observed. This discrepancy could be due to several factors, such as the heterogeneity of BDNF doses and behavioral tests used in the different studies. In particular, high concentrations of BDNF seem to have a pre- dominant antinociceptive effect (Groth and Aanonsen, 2002; Guo et al., 2006), maybe due to the interaction with lower affinity binding sites and/or the activation of different intracellular pathways.

The effects of BDNF in inflammatory models of pain have been better defined: BDNF plays a pro-nociceptive role, and is likely involved in the genesis of the hyperalgesic responses observed in this pain condition.

The correlation between BDNF and neuropathic pain is also still unclear. Due to the multiplicity of experimental approaches, a correct interpretation of the results obtained by the various groups is often difficult. Several patterns of alteration in BDNF expression have also been observed, depending on the different model of

Table 2
BDNF modulation of pain behavior in normal and pathological conditions

Preparation/pain model	Treatment	Behavioral effects	General effect of BDNF	References
Normal animals				
Rat	Intrathecal TrkB-Fc (chimera Protein able to sequester BDNF	No change in basal pain or hyperalgesia (peripheral capsici)	No effect	Mannio et al 1999
Rat	Intrathecal BDNF	Transient increase of threshold to Termal stimulation	Anti-nociceptive	Pezet et al 2002b
Mouse	(1)Intrathecal BDNF (2) BDNF antisense oligonucleotide (depletion endogenous BDNF)	(1) Thermal hyperalgesia (blocked by D-APV) (2) antinociception (thermal stimulus)	Pro-nociceptive	Groth and AAnonsen, 2002
Rat	Intrathecal administration of BDNF, BDNF-transducing adenovirus, ATP-stimulated microglia	Decrease threshold response to mechanical stimulation (allodynia)	pro-nociceptive	Coull et al., 2005
Mouse	Intrathecal BDNF	Thermal hyperalgesia, tactile allodynia	Pro-nociceptive	Yajima et al 2005
Inflammatory pain models				
Rat (1)formalin test (2)carrageenan injection	trkB-IgG	(1) Decrease of phase 2 only in NGF-pretreated animals (2) Decrease of thermal hyperalgesia	Pro-nociceptive	Kerr et al., 1999
Rat.Peripheral inflammation (CFA)	Intrathecal TrkB-Fc	Block of hypersensitivity to tactile stimuli	Pro-nociceptive	Mannion et al., 1999
Mouse. Carrageenan injection	Antisense oligodeoxynucleotide treatment (BDNF and trkB)	Antisense treatment reduces hyperalgesia in inflamed rats	Pro-nociceptive	Groth and Aanonsen, 2002
Rat. CFA injection	Intraperitoneal anti-BDNF antiserum	Reduction of mechanical hyperalgesia	Pro-nociceptive	Matayoshi et al., 2005
Mouse: (1) Formalin test (2) Carrageenan injection	Conditional deletion of BDNF in nociceptors	(1) Attenuation of 2nd phase (2) Inhibition of thermal hyperalgesia	Pro-nociceptive	Zhao et al., 2006
Neuropathic pain models				

Rat. Chronic constriction injury	BDNF-secreting neuronal grafts in spinal cord	Reduction of allodynia (tactile and cold) and hyperalgesia (mechanical and thermal)	Anti-nociceptive	Cejas et al., 2000
Rat. L5-L6 spinal nerve ligation	Systemic infusion of BDNF	1 mg/h: attenuation of mechanical hyperalgesia 20 mg/h: increase of mechanical hyperalgesia	Both pro- and anti-nociceptive	Miki et al., 2000
Rat. Chronic constriction injury	Viral vector mediated overexpression of BDNF in spinal cord	Reversal of thermal hyperalgesia and tactile allodynia	Anti-nociceptive	Eaton et al., 2002
Rat. Spinal nerve ligation	Intrathecal BDNF	Reduction of thermal hyperalgesia, on mechanical hyperalgesia	Anti-nociceptive	Lever et al., 2003a, 2003b
Rat. Peripheral nerve injury	Intrathecal anti-trkB or trkB-Fc	Reversal of mechanical allodynia	Pro-nociceptive	Coull et al., 2005
Mouse. Sciatic nerve ligation (SNL)	(1) Intrathecal TrkB/Fc (2) BDNF (±) knockout	SNL induces thermal hyperalgesia and tactile allodynia, suppressed by TrkB/Fc or in BDNF (±) knockout mice	Pro-nociceptive	Yajima et al., 2005

Effects of BDNF on nociceptive behavior in normal animals and in animal models of pain. Where more than one treatment is present, the effects are listed with the same number. Abbreviations are listed at beginning of the main text.

lesion, suggesting that the action of the NT in mediating neuropathic pain states can be very complex. An interesting study by Zhao et al. (2006) has recently shown that BDNF released by nociceptors is not involved in neuropathic pain, but only in the nociceptive behavior caused by inflammation. As reported by Coull et al. (2005), BDNF of non-neuronal origin (released by microglial cells) could play an important role in the development of neuropathic pain conditions.

5. Future perspectives and concluding remarks

The picture emerging from currently available data on the modulatory role of BDNF in pain pathways is still largely incomplete and fragmentary.
This is true at not only the supraspinal level, but also in spinal cord and particularly lamina II, which represents the most widely investigated area so far.
Although many of the cellular mechanisms by which the NT exerts it function at synapses have been clarified, still it remains to understand the circuitry that is ultimately responsible for the transfer of information along the polyneuronal chain that eventually leads to the perception of pain in normal and altered conditions.
It would be of interest for example to localize trkA receptors at the ultrastructural level, given the widely recognized importance of NGF in the plasticity of the system under pathological conditions. Also it would be interesting to assess whether or not up-regulation of fl-trkB following inflammation, which has been demonstrated in spinal cord by Western and Northern blotting, leads to concurrent expression of receptors at pre- and post- synaptic partners of the same synapse, albeit preliminary data have not been supportive about this possibility.
These are only a couple of examples that emphasize the need of a combined multidisciplinary approach to fully understand the role of BDNF in pain, and, hopefully, to set the grounds for future therapeutic developments.

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