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Review

BDNF: a neuromodulator in nociceptive pathways?

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Abstract

During development, brain-derived neurotrophic factor (BDNF) supports the survival of certain neuronal population in central and peripheral nervous system. In adulthood, BDNF has been suggested to act as an important modulator of synaptic plasticity. This article reviews and discusses its potential role as neuromodulator in the spinal dorsal horn. BDNF is synthesized in the cell body of primary sensory neurons (pre-synaptic neurons) and its expression is regulated in models of inflammatory and neuropathic pain. The high affinity receptor for BDNF, tropomyosine receptor kinase B (TrkB), is expressed by post-synaptic neurons of the dorsal horn. Stimulation of pre-synaptic nociceptive afferent fibres induces BDNF release and consequent activation of TrkB receptors leading to a post-synaptic excitability. Electrophysiological recordings showed that BDNF enhances the ventral root potential induced by C-fibre stimulation in an in vitro preparation. In addition, behavioural data indicate that antagonism of BDNF attenuates the second phase of hyperalgesia induced by formalin (in nerve growth factor-treated animals) and the thermal hyperalgesia induced by carageenan, suggesting that BDNF is involved in some aspects of central sensitisation in conditions of peripheral inflammation. In conclusion, BDNF meets many of the criteria necessary to define it as a neurotransmitter/neuromodulator in small diameter nociceptive neurons.

Theme: Sensory systems

Topic: Spinal cord processing

Keywords: Neuroplasticity; Sensory neuron; Dorsal horn; Neurotrophic factor release; TrkB; Phospho-ERK

Contents

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1. Introduction

Acetylcholine was the first neurotransmitter to be identified and characterised. Since then, numerous other compounds have been proposed to be neurotransmitters. A series of criteria have been established against which these claims are judged. These can be summarised as follows:

(i) A putative transmitter should be synthesised and released from neurones.
(ii) The pre-synaptic neurone should contain both the neurotransmitter and the appropriate enzyme required for its synthesis.
(iii) The substance should be released from the nerve terminal in a chemically or pharmacologically identifiable form.
(iv) The putative neurotransmitter should reproduce post-synaptically cell-specific events observed to occur upon stimulation of the pre-synaptic neurone. These effects should be obtained at concentrations that approximate those seen after release of neurotransmitter from nerve stimulation.
(v) Known competitive antagonists of the transmitter should block the effect of putative neurotransmitter in a dose-dependent manner.
(vi) There should be appropriate active mechanisms to terminate the action of the putative transmitter.

While numerous exceptions have been reported, these criteria still provide a useful framework to assess putative neurotransmitters.

Nowadays the list of identified neurotransmitters includes L-amino acids, D-amino acids, peptides, gases and lipids. Recently, evidence has accumulated that the neurotrophic factor BDNF (brain derived neurotrophic factor) could be considered a neurotransmitter. In the hippocampus, BDNF has been shown to rapidly depolarise neurones and capable of modulating synaptic strength. It has been suggested that BDNF is a mediator of long-term potentiation (LTP) induction in this system [31]. In the current review we will consider to what extent BDNF meets these criteria in the context of nociceptive transmission in the dorsal horn of the spinal cord.

2. Expression of BDNF in primary sensory neurones

A neurotransmitter by definition is synthesized by pre-synaptic neurons and accumulated at synaptic sites in those neurons. In the pain-signalling system, the first relay of nociceptive information takes place in the dorsal horn of the spinal cord where primary afferent neurons form the pre-synaptic neurons, and local and projection neurons located in the spinal dorsal horn make up the post-synaptic element. In the following sections we will describe how BDNF is synthesized in a sub-population of primary sensory neurons and transported to their central terminals in the spinal cord.

2.1. Protein and mRNA studies in DRG

Among neurotrophins, BDNF is the most abundant and widely distributed in the central nervous system. In primary sensory neurones, BDNF mRNA [55] and protein [10,55,57] are present in a subpopulation of dorsal root ganglion (DRG) cells representing 20–30% of all such cells. BDNF is mainly found in small to medium sized neurons, predominantly those containing neuropeptides such as calcitonin gene-related peptide (CGRP) [41,45]. BDNF is anterogradely transported from the cell bodies of these cells to terminals in the spinal cord [60]. Strong immunolabelling of sensory terminals is seen in laminae I–II of the spinal dorsal horn [45,60]. This band is lost after dorsal rhizotomy, when primary afferent terminals degenerate. Weaker immunostaining is observed in deeper laminae of the dorsal horn [57].

2.2. Ultrastructural studies

Pre-embedding immunohistochemistry has shown that BDNF is located in axons and axon terminals of primary sensory neuron in superficial laminae of the dorsal horn, and more precisely in individual large dense-cored vesicles and the membrane of agranular vesicles [45]. More recently, Luo et al., confirmed these observations and revealed in addition that terminals immunoreactive for BDNF belong to both group I and group II glomeruli [41]. BDNF was not found in post-synaptic dendrites, except occasionally in association with post-synaptic membranes [41].

2.3. Regulation of expression

The expression of BDNF mRNA and protein is dramatically up-regulated in models of inflammatory pain. Intraplantar injection of Freund's adjuvant produces a local swelling and greatly increased sensitivity to painful stimuli, which typically persists for many days. BDNF increases in sensory neurones innervating the inflamed paw, so that up to half of all such neurones become BDNF immunoreactive [16]. The average levels of BDNF mRNA also increased dramatically [17]. The increase is largely restricted to small diameter nociceptive neurones, and protein levels in the terminals of these afferents in the dorsal horn also increase markedly [16]. Most of the increase in BDNF seems to due to the effects of another neurotrophin, nerve growth factor (NGF) [7,45], the synthesis of which increases in inflamed tissues [6]. Thus molecules, which block NGF action prevent the inflammation-induced increase in BDNF [16]. Conversely, supplying exogenous NGF is a very effective method of increasing BDNF in sensory neurons [45]. In the latter

case, essentially all neurones that express NGF receptor TrkA begin to synthesise BDNF. This amounts to about 45% of all sensory neurones that are known to be nociceptive in nature [52].

In conditions of nerve damage and associated neuropathic pain, several groups showed that BDNF is down-regulated in small and medium DRG neurons [58] and over-expressed in uninjured small neurones following spinal nerve ligation [23]. Furthermore, medium and large DRG neurons start expressing de novo synthesised BDNF [15,58]. After chronic constriction of the sciatic nerve, all three categories of small, medium and large DRG neurons show a significant increased BDNF immunoreactivity [24]. An increased immunoreactivity of BDNF was observed in central terminals of these neurons, in deep dorsal horn layers and in dorsal column nuclei [17].

3. Release of BDNF from primary sensory neurones

Many of the sensory neurones that express BDNF also express the neuropeptide substance P (SP). Like SP, BDNF is packaged in large dense core synaptic vesicles in the cell bodies of small diameter-sensory neurone in the dorsal root ganglia and anterogradely transported to axon terminals in the dorsal horn [45,60]. It is not surprising then, that BDNF is found in laminae I and II of the spinal cord, the known termination site of SP-containing C-fibres [45]. These neurons utilize glutamate (stored in clear vesicles) as a fast neurotransmitter. Some of them also release SP from dense-core vesicles when they fire repetitively [21,43]. We have recently found that primary sensory neurons release BDNF in the dorsal horn following defined patterns of electrical stimulation of the dorsal roots or after capsaicin activation of nociceptive terminals [36]. We used an isolated dorsal horn-with dorsal roots attached preparation and quantified BDNF in dorsal horn superfusates by a sensitive enzyme-linked immunosorbent assay (ELISA; optimised Promega kit). A number of different electrical stimulation protocols of the dorsal roots were applied in an attempt to provoke release of BDNF from the dorsal horn.

We found that the release of this trophic factor from nociceptive neurones is dependent on the pattern of primary afferent activity rather than the total number of pulses given or the frequency of synaptic stimulation (Fig. 1) [36].

This is still the case even when BDNF terminal content has been enhanced by NGF treatment. Surprisingly perhaps, we found that simple tetanic stimulation of C-fibres at frequencies up to 100 Hz did not promote detectable BDNF release. It is not clear if this failure of release is due to activation of inhibitory mechanisms, block of the firing of some C-fibres following sustained high frequency stimulation or simply an inappropriate activity pattern (see below). BDNF release from sensory neurones could be evoked by burst stimuli at C-fibre intensity of stimulation.

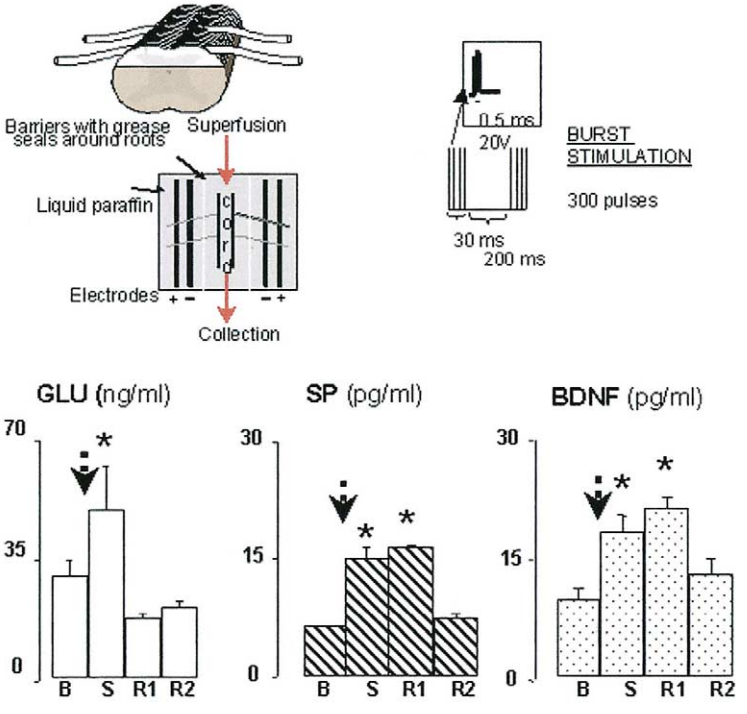

Fig. 1. Burst-stimulation of the dorsal roots induced co-release of glutamate, SP, and BDNF from the isolated dorsal horn. Top left: schematic representation of the isolated dorsal horn-with dorsal root preparation. Top right: electrical stimulation protocol. Bottom: pattern of release for glutamate, SP and BDNF. Transmitter basal contents (B), contents during electrical stimulation (S) and contents in recovery samples (R1 and R2) (adapted from Ref. [36]).

The most effective burst stimuli were a total of 300 pulses delivered in 75 trains at 100 Hz (Fig. 1). The release of BDNF evoked by burst stimulation of the dorsal roots was Ca^{2+}-dependent and could be blocked by tetrodotoxin (TTX) suggesting that it was released from synapses. BDNF and SP could be both released by capsaicin superfusion of dorsal horn slices isolated in vitro. However, the EC_{50} of capsaicin to produce BDNF release was 80 μM, several orders of magnitude higher than capsaicin EC_{50} to evoke SP release (100 nM) (Fig. 2). There are several explanations for this discrepancy: firstly, capsaicin may stimulate BDNF release by activating receptors other than VR1, which may have lower affinity for capsaicin. Secondly, the effect of capsaicin on BDNF might be indirect (we do not know whether VR1-expressing neurones contain BDNF), for example, mediated by glutamate release and nitric oxide production. Finally, BDNF content in the dorsal horn is lower than SP content, and modest release might not be detectable. Importantly, BDNF immunoreactivity in the dorsal horn was substantially reduced after applying capsaicin to dorsal horn slices isolated in vitro indicating that primary afferent BDNF pools can be readily depleted. The physiological relevance of our findings, that BDNF is released by nociceptive fibres activated by either high intensity stimuli delivered in bursts or capsaicin, is underscored by observations that C-fibres can adopt a bursting firing pattern when the intensity of nociceptive stimuli increases over a certain threshold [3,4]. For example, we have shown that small unmyelinated fibres in the tibial nerve develop bursting activity after an injection of capsaicin in the footpad [36]. Other studies have shown C-fibre bursting activity in visceral nerves in response to hydrogen peroxide or bradykinin [3,4] and suggested that the transition to bursting might be involved in the release of a different transmitter. Therefore, it is likely that certain noxious stimuli produce bursting activity in C-fibres that in turn elicits BDNF release in the dorsal horn. It is generally assumed that co-storage of transmitters

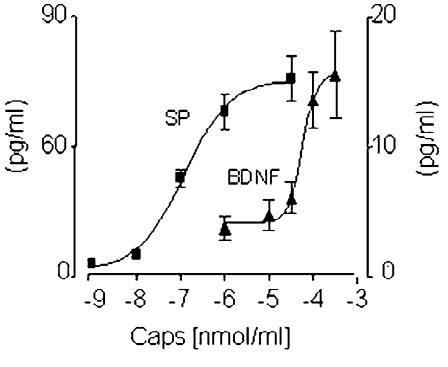

Fig. 2. Capsaicin-induced release of SP and BDNF from the isolated dorsal horn. Capsaicin was superfused through the isolated dorsal horn preparation (see diagram in Fig. 1) after collection of basal outflow fractions. Values represent SP (left *y* axis) and BDNF content (right *y* axis) in fractions challenged with capsaicin after subtraction of contents in basal fractions.

results in co-release [18]. We had the opportunity to test the release conditions of co-existing transmitters, glutamate, SP and BDNF in primary afferent terminals [36]. Whilst all stimulation protocols could evoke the release of SP only burst stimuli could induce co-release of BDNF in the dorsal horn. This was true even when we used tetanic and burst stimuli that released roughly the same amount of SP. Confirming this, the burst stimuli and tetanic stimuli used produced similar amounts of NK_1 internalisation in the superficial dorsal horn, an indicator of NK_1 receptor activation by released tachykinins. Continual C-fibre stimulation at low frequency (1 Hz) also produced a substantial amount of SP release, but no detectable BDNF. Hence, SP appears to be released by a wider range of firing patterns than BDNF. This suggests that different firing patterns of primary afferents may encode the release of different neuromodulators, assuming our ability to detect these compounds is comparable. Notably, neuropeptide release is likely to depend on stimulation frequency [11] whilst neurotrophin release appears to depend on stimulation patterns [36,9]. One consequence may be that different transmitters/modulators which co-exist in nociceptive fibre terminals are released in different pain states, and this could have important implications for targeting analgesic therapies. Given the fact that SP and BDNF are likely to be present in the same population of primary afferents [45], and they may be co-packaged in large, dense-core synaptic vesicles, one could speculate that dissociation of SP and BDNF from the holding matrix inside the secretory vesicles might occur under different conditions. Some peptides may still be retained after opening because they remain in aggregate or solid state [26]. This means that co-storage of peptides in vesicles does not guarantee co-release [34]. For example in neuroendocrine cells different forms of prolactin hormone are secreted under different conditions [5]. Alternatively, BDNF may be stored in a different population of dense-core vesicles from SP that undergoes exocytosis following burst-stimulation. It is possible that the high frequency action potentials of each burst lead to a substantial build-up of calcium concentrations in the pre-synaptic terminals, and that these elevated calcium levels are necessary for BDNF release.

4. Inactivation of synaptically released BDNF

To satisfy the criteria of a neurotransmitter, BDNF should be degraded by specific enzymes or taken up to terminate its action after release from primary sensory neurone terminals. We do not as yet have any definitive data regarding this point. However, there are several testable possibilities and some circumstantial evidence.

One possible mechanism is that BDNF's actions are terminated because of uptake by TrkB receptors. It is known that BDNF binding to TrkB is followed by internalisation of the receptor–ligand complex [13]. There are

both full length and truncated forms of the TrkB receptor, only the former of which appears capable of signal transduction. The full-length receptor appears to be fairly widely distributed in the spinal cord (see below) but truncated receptors are even more abundant (unpublished data). It is possible that both forms of receptor contribute to BDNF removal from synaptic sites. Also, there is evidence that TrkB receptors can translocate from cytoplasmic to membrane sites with activity [44]. Thus it is possible that an extra pool of receptors becomes available to bind and remove BDNF at exactly those sites where it is released.

A second possibility is that BDNF's actions are terminated by simple diffusion away from release sites. It certainly appears that at least some BDNF does diffuse intact from release sites, since this is the fraction detected in our release studies (described in Section 3 above). It is also true that there is relatively little BDNF that has to be removed since it has such high affinity for its receptor. However, it is not clear whether the amounts recovered in superfusates represent a small or large proportion of that released.

A third possibility is that BDNF is cleaved locally at synapses. It is known that some neurotrophins can be cleaved in vivo—for instance even large amounts of NT3 given systemically to animals cannot be detected in blood, presumably because it is so effectively degraded. However, this might not be the case for BDNF as it has been shown to be stable in blood after intravenous injection [47]. In the synaptic cleft the extent to which BDNF can be degraded and what enzymes might be responsible, are unknown. Our experience is that in order to detect BDNF in spinal cord superfusates, it was necessary to include a cocktail of protease inhibitor in the superfusing medium [36].

5. Post-synaptic effects of synaptically released BDNF

To satisfy the criteria as a neurotransmitter, receptors for BDNF should be present on post-synaptic neurons and the activation of appropriate synapses should activate this receptor.

5.1. BDNF receptors are present on post-synaptic structures

The high affinity receptor for BDNF is the tropomyosine receptor kinase B (TrkB). It has the classical conformation of a transmembrane receptor, an extracellular domain with many sites of glycosylation, a unique transmembrane segment and an intracellular domain characterised by a tyrosine kinase activity.

TrkB can be expressed in cells under two forms: (i) full length, which is the one described above, and frequently referred to as $gp145^{trk}$ (Fig. 3), or (ii) a truncated form called $gp95^{trk}$ because its molecular weight is 95 kDa. $gp95^{trk}$ differs from $gp145^{trk}$ by the lack of the tyrosine kinase (TK) domain (see Fig. 3). The absence of a tyrosine kinase domain (which appears to be the key enzyme in the intracellular cascade activated by BDNF) leaves the potential function of $gp95^{trk}$ still an open question. Several lines of evidence suggest that when both types of receptor are expressed in the same cell, binding of BDNF on $gp95^{trk}$ acts in a negative dominant fashion to the activation and down-stream action of $gp145^{trk}$ activation [13,22,25].

Both forms of the receptor are present in cells of the spinal cord. Immunolabelling performed using an antiserum directed against the extracellular domain of the receptor (therefore not distinguishing the different forms of TrkB) revealed that receptors are expressed in neurons throughout the grey matter of the spinal cord, motorneurons being the strongest labelled neurons. In the grey and the white matter, glial cells also express the receptor, but with a lower abundance [56,59]. Later, in situ hybridization studies confirmed a widespread expression of transcripts for both full length and truncated receptors in spinal cord [14,30,33,39]. Interestingly, the expression of TrkB appears to be markedly up-regulated in dorsal horn cells in some models of persistent pain [35,42,46].

5.2. Activation of TrkB

It is known from previous studies performed in other systems that binding of BDNF to TrkB leads to dimerization of the receptor and its auto-phosphorylation on tyrosine residues. As considered below, activation of different tyrosine residues leads to the activation of different intracellular transduction pathways, and therefore has different physiological implications. But as a general rule, increased phosphorylation of TrkB is a marker of an increased activation of the receptor. Fig. 3 summarizes the different transduction pathways activated in response to phosphorylation of different tyrosine residues of TrkB.

Using a biochemical approach, we showed that peripheral chemical, thermal or mechanical noxious stimulation (but not innocuous stimuli) increased the phosphorylation of TrkB in the spinal dorsal horn [48]. Since TrkB is mainly contained in dorsal horn neurons (with only modest expression on the terminals of primary sensory neurones) the increased phosphorylation is most likely to reflect effects on post-synaptic neurons. The activation of TrkB is a quick event, seen within 2 min of stimulation, and decaying rapidly too (lost within 30 min after stimulation). It occurs in somatotopically appropriate regions of the spinal cord. An example is shown in Fig. 4, a Western blot showing increased phosphorylation of TrkB at various times after application of a chemical noxious stimulus, in an anaesthetized animal. This evidence suggests that BDNF, via an interaction with its receptor, is specifically implicated in cellular activation in nociceptive relays in the spinal cord. As increased activation of the receptor is due

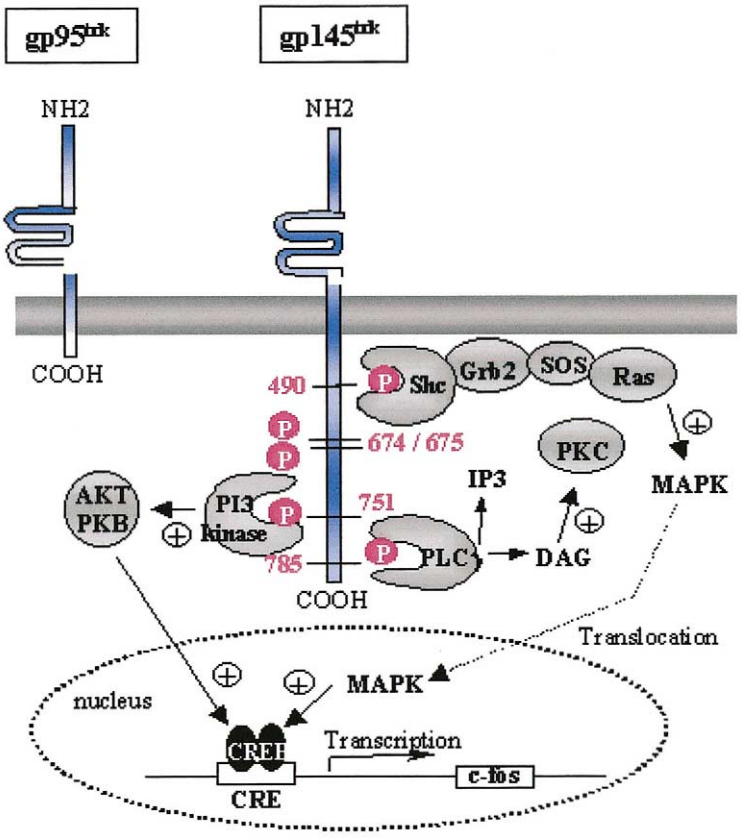

Fig. 3. Cartoon showing the structure of truncated ($gp95^{trk}$) and full length ($gp145^{trk}$) TrkB receptors. The sites of phosphorylation of $gp145^{trk}$ are indicated as well as the transduction pathways activated by phosphorylation on these different sites.

to an action of the ligand on its receptor, increased phosphorylation of TrkB can be considered as further and independent evidence for release of BDNF from primary afferent fibres following C-fibre stimulation. These data therefore support the release data discussed above and suggest the BDNF can be released with nociceptor activation in vivo as well as in vitro.

5.3. Activation of second messengers

Experiments on cultured cells (including both PC12 cells and primary cultures of DRG) have shown that activation of Trk receptors by neurotrophic factors leads to auto-phosphorylation of receptors in specific tyrosine residues. The exact location of the different tyrosine residues differs slightly between the different Trk receptors, but the nature of the transduction cascade activated by phosphorylation of each residue is conserved. In each receptor, phosphorylation of different groups of tyrosine leads to the activation of distinct adaptive proteins and subsequently distinct transduction pathways. In the spinal cord, direct evidences of second messenger activation induced by BDNF are the activation of the MAP kinase ERK pathway [48] and subsequent transcription of the proto-oncogene *c-fos* [32] and nitric oxide [12].

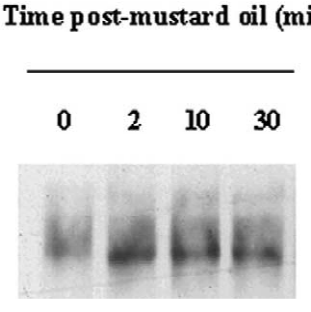

Fig. 4. Example of Western blot showing the time-course if increased spinal phosphorylated state of TrkB induced by chemical noxious stimulation (painting of mustard oil on both hind paws). Adapted from Ref. [48].

We have observed that BDNF induces phosphorylation of the MAP kinase ERK in neonatal rat spinal cord slices maintained in vitro [32]. We have also found that microinjection of BDNF in the intact spinal cord of anaesthetized adult rat is an effective stimulus. ERK activation is rapid (within minutes) and persists for only tens of minutes. It is mainly restricted to neurones. Phospho-ERK is also seen in dorsal horn neurons after noxious stimulation, and is necessary for the full development of inflammatory heat and mechanical hyperalgesia [28,29]. Using the sequestering molecule for BDNF, TrkB-IgG, we showed that about 40% of the phospho-ERK elicited by noxious stimulation can be attributed to endogenous release of BDNF [48]. Thus, it seems likely that BDNF

released with nociceptor activation can contribute to the development of central changes in pain processing, a topic considered below.

5.4. Modification of spinal nociceptive processing

At the post-synaptic level, binding of neurotransmitters to specific receptors induces a cascade of events including ion channel opening, membrane hyperpolarisation or depolarization, and activation of second messenger systems. These can lead to a rapid neuronal activation and slower regulation of gene transcription. In the spinal dorsal horn, many transmitters are released from the primary sensory neuron terminals. Among them, the excitatory amino acid glutamate, as well as neuromodulators such as CGRP, SP, and somatostatin. Different types of metabotropic and ionotropic receptors of glutamate are present on dorsal horn neurones and they are all now implicated in the development of pain responses. However the ionotropic NMDA receptor plays a special role in the development of 'wind-up' and central sensitization [20,54]. This ligand-gated ion channel is an heterooligomeric complex formed with subunits NR1, NR2A–D, NR3. They consist of one or more NR1 subunits (likely the glycine-binding site) and a glutamate binding subunit (NR2 or NR3). Activity of NMDA receptor can be regulated by either intra- or extracellular mechanisms and phosphorylation is a key intracellular mechanism of its regulation. In the hippocampus, BDNF is known to potentiate synaptic efficacy by increasing the activity of the NMDA receptor [37]. This action is mediated by NR2A and 2B subunits [38,40,51]. In the spinal cord, where BDNF is released after C-fibre stimulation, BDNF potentiates the ventral root potential induced by C-fibre stimulation [32,53]. In the immediate post-natal period (but not beyond the first week of life), monosynaptic connections between muscle afferents and spinal motoneurones show post-synaptic facilitation and pre-synaptic depression with BDNF, and in an NMDA-dependent fashion [8]. The potentiating effects of BDNF are likely to be due to a phosphorylation of NR2A and 2B subunits [19], but recent data suggest that the NR1 subunit can also be phosphorylated by BDNF [50].

Some of the numerous spinal changes observed after noxious stimulation can be mimicked by application of exogenous BDNF. For instance, both BDNF and acute nociceptive stimulation induce in dorsal horn neurons activation of ERK within minutes [43,28] and transcription of *c-fos* within hours [1,2,32].

6. Antagonism of BDNF action

A key question for the hypothesis examined in this chapter is whether endogenous BDNF contributes to pain-related responses. This question has not been easy to address because of the lack of relevant tools. There are currently no available specific BDNF receptor antagonists (although some compounds such as K252a are non-specific antagonists). However, BDNF can be sequestered with a synthetic fusion protein consisting of the extracellular domain of and the TrkB receptor fused to a portion of an IgG molecule. This creates a stable and soluble protein with the correct stereochemistry to bind BNDF (as well as the other TrkB ligand, neurotrophin-4/5) with very high affinity [49]. We have examined the effects of TrkB-IgG on nociceptive afferent-evoked reflex responses in the spinal cord. We have used an in vitro hemisected spinal-cord preparation and measured the long-latency ventral root potentials elicited by stimulation of C-fibres in the dorsal root. We increased levels of BDNF within small sensory neurones by pre-treating animals with NGF. Under such circumstances, this nociceptor-evoked response is reduced significantly after superfusion of the preparation with TrkB-IgG [32], strongly suggesting that BDNF is released from afferent fibres under these conditions. To date, most of the work that has examined the effects of BDNF on synaptic transmission has been undertaken on isolated preparations in vitro. There is evidence from work on the hippocampus that BDNF will induce long-lasting enhancement of synaptic transmission in vivo. We have tested this idea in the context of pain processing by assessing the effect of TrkB-IgG on nociceptive behavioural responses in adult rats. Behavioural responses evoked in standard model of persistent pain (subcutaneous injection of dilute formalin into one hind paw) were significantly reduced by prior intrathecal administration of TrkB-IgG [32,53]. Both phases of the formalin response were affected, although the second phase, known to depend in part on the induction of central sensitization, was reduced to a greater degree. Thus, these findings show that under some conditions, particularly those that model persistent pain states (e.g., inflammation), BDNF is released with activity from nociceptive afferent terminals and contributes to the post-synaptic responses in spinal-cord neurons.

Recently, Heppenstall and Lewin [27] have used a different approach to study the role of endogenous BDNF. They have studied ventral root responses in mice with null mutations in the BDNF gene. They studied animals in the first few postnatal weeks (these animals do not survive into adulthood). They also used an electrophysiological approach to characterise nociceptor-evoked reflexes recorded in the ventral roots of isolated preparations. They found that BDNF knock-out mice had smaller C-fibre reflexes and smaller potentiation of these reflexes with repeated nociceptor stimulation (wind-up) [27]. These observations provide an independent measure of BDNF's actions. However, in the knock-out mice the actions of BDNF did not appear to depend on an NMDA-sensitive mechanism.

7. Conclusions

As we review here, BDNF meets many of the criteria necessary to establish it as a neurotransmitter/neuro-

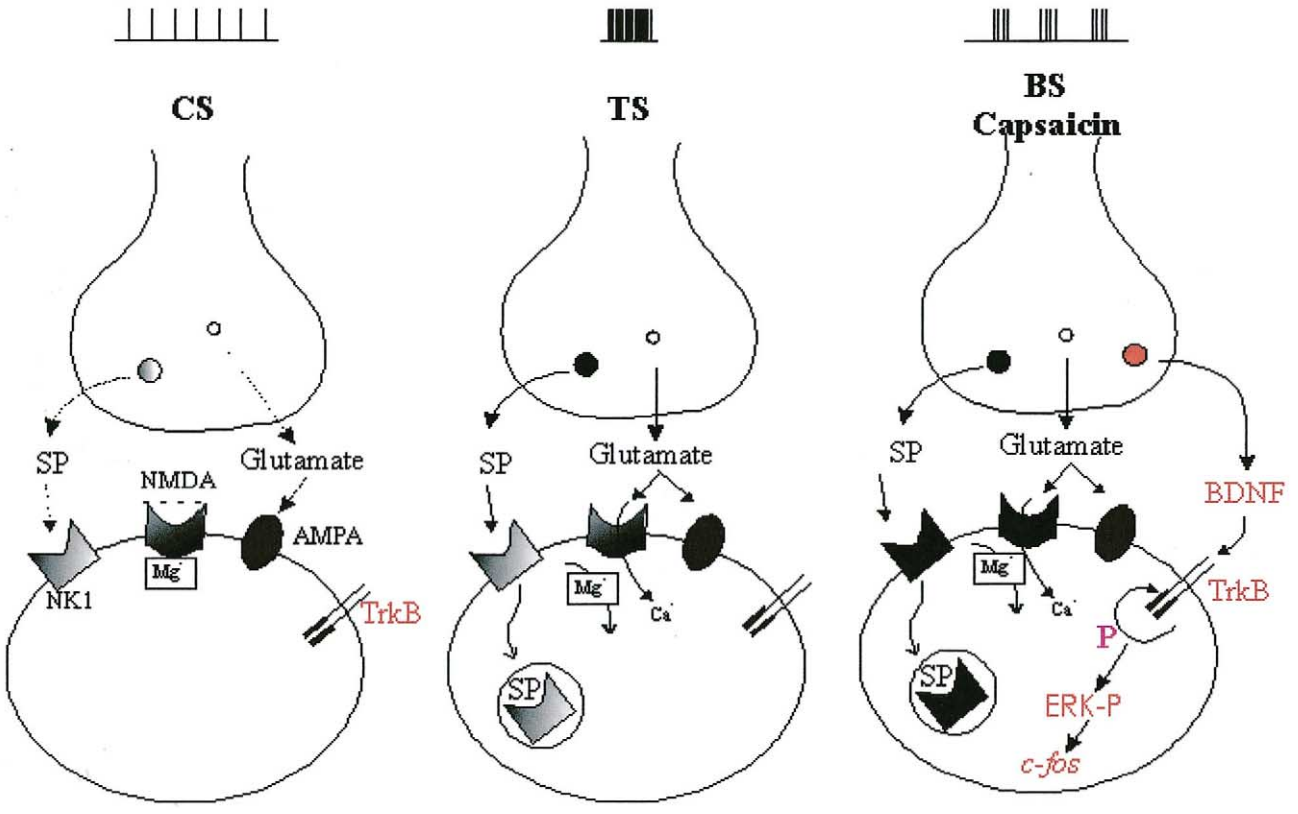

Fig. 5. Glutamate, SP and BDNF are released from nociceptors to activate second order dorsal horn. Distinctive firing patterns of primary afferents encode the release of different transmitters and subsequent neuronal activation in the spinal dorsal horn. Continuous stimulation (CS) leads to the activation of NK_1 and AMPA receptors on post-synaptic neurons. Tetanic stimulation (TS) recruits AMPA, NMDA, NK_1 receptors. After burst stimulation (BS) or capsaicin treatment, in addition to NK_1, AMPA and NMDA receptors, TrkB receptors are recruited due to BDNF release. This leads to down-stream phosphorylation of ERK and transcription of *c-fos*.

modulator in small diameter nociceptive neurons. It is synthesized by these neurons and packaged in dense core vesicles. The BDNF expressing nociceptive afferents terminate mostly in the superficial dorsal horn, and the post-synaptic cells in this region express full-length TrkB receptors. Spinal neurons are responsive to exogenous BDNF, as evidenced both histochemically (by activation of TrkB and ERK, and the induction of *c-fos*) and electrophysiologically (by an increased excitability to nociceptive inputs). BDNF is thus sufficient to elicit changes in post-synaptic excitability. The release of BDNF from nociceptive fibres with activity has now been shown. There are both electrophysiological and behavioural data indicating that antagonism of BDNF at least partially prevents some aspects of central sensitisation. Thus, BDNF released from nociceptors along with SP and glutamate appears necessary for the full activation of second order dorsal horn neurons (Fig. 5).

Acknowledgements

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References

[1] C. Abbadie, J.M. Besson, *c-fos* expression in rat lumbar spinal cord during the development of adjuvant-induced arthritis, Neuroscience 48 (1992) 985–993.
[2] C. Abbadie, J.M. Besson, *c-fos* expression in rat lumbar spinal cord following peripheral stimulation in adjuvant-induced arthritic and normal rats, Brain Res. 607 (1993) 195–204.
[3] D.W. Adelson, J.Y. Wei, L. Kruger, H2O2 sensitivity of afferent splanchnic C fiber units in vitro, J. Neurophysiol. 76 (1996) 371–380.
[4] D.W. Adelson, J.Y. Wei, L. Kruger, Warm-sensitive afferent splanchnic C-fiber units in vitro, J. Neurophysiol. 77 (1997) 2989–3002.
[5] J.K. Angleson, A.J. Cochilla, G. Kilic, I. Nussinovitch, W.J. Betz, Regulation of dense core release from neuroendocrine cells revealed by imaging single exocytic events, Nat. Neurosci. 2 (1999) 440–446.
[6] S.C. Apfel, Neurotrophic factors and pain, Clin. J. Pain 16 (2000) S7–11.
[7] S.C. Apfel, D.E. Wright, A.M. Wiideman, C. Dormia, W.D. Snider, J.A. Kessler, Nerve growth factor regulates the expression of brain-

derived neurotrophic factor mRNA in the peripheral nervous system, Mol. Cell. Neurosci. 7 (1996) 134–142.
[8] V.L. Arvanian, L.M. Mendell, Acute modulation of synaptic transmission to motoneurons by BDNF in the neonatal rat spinal cord, Eur. J. Neurosci. 14 (2001) 1800–1808.
[9] A. Balkowiec, D.A. Katz, Cellular mechanisms underlying activity-dependent release of endogenous BDNF from hippocampal neurons, in: 31st Annual meeting of the Society for Neuroscience, San Diego, CA, 10–15 November 2001.
[10] I. Barakat-Walter, Brain-derived neurotrophic factor-like immunoreactivity is localized mainly in small sensory neurons of rat dorsal root ganglia, J. Neurosci. Methods 68 (1996) 281–288.
[11] T. Bartfai, K. Iverfeldt, E. Brodin, S.O. Ogren, Functional consequences of coexistence of classical and peptide neurotransmitters, Prog. Brain Res. 68 (1986) 321–330.
[12] D.L. Bennett, J.S. French, J.V. Priestley, S.B. McMahon, The effect of BDNF on *c-fos* and NOS expression in dorsal horn neurons of the adult rat spinal cord, in: 26th Annual Meeting of the Society for Neuroscience, November 1996.
[13] S. Biffo, N. Offenhauser, B.D. Carter, Y.A. Barde, Selective binding and internalisation by truncated receptors restrict the availability of BDNF during development, Development 121 (1995) 2461–2470.
[14] E.J. Bradbury, V.R. King, L.J. Simmons, J.V. Priestley, S.B. McMahon, NT-3, but not BDNF, prevents atrophy and death of axotomized spinal cord projection neurons, Eur. J. Neurosci. 10 (1998) 3058–3068.
[15] H.J. Cho, J.K. Kim, H.C. Park, J.K. Kim, D.S. Kim, S.O. Ha, H.S. Hong, Changes in brain-derived neurotrophic factor immunoreactivity in rat dorsal root ganglia, spinal cord, and gracile nuclei following cut or crush injuries, Exp. Neurol. 154 (1998) 224–230.
[16] H.J. Cho, J.K. Kim, X.F. Zhou, R.A. Rush, Increased brain-derived neurotrophic factor immunoreactivity in rat dorsal root ganglia and spinal cord following peripheral inflammation, Brain Res. 764 (1997) 269–272.
[17] H.J. Cho, S.Y. Kim, M.J. Park, D.S. Kim, J.K. Kim, M.Y. Chu, Expression of mRNA for brain-derived neurotrophic factor in the dorsal root ganglion following peripheral inflammation, Brain Res. 749 (1997) 358–362.
[18] S. De Biasi, A. Rustioni, Glutamate and substance P coexist in primary afferent terminals in the superficial laminae of spinal cord, Proc. Natl. Acad. Sci. USA 85 (1988) 7820–7824.
[19] M. Di Luca, F. Gardoni, A. Finardi, S. Pagliardini, F. Cattabeni, G. Battaglia, C. Missale, NMDA receptor subunits are phosphorylated by activation of neurotrophin receptors in PSD of rat spinal cord, Neuroreport 12 (2001) 1301–1305.
[20] A.H. Dickenson, Spinal cord pharmacology of pain, Br. J. Anaesth. 75 (1995) 193–200.
[21] A.W. Duggan, R.C. Riley, M.A. Mark, S.J. MacMillan, H.G. Schaible, Afferent volley patterns and the spinal release of immunoreactive substance P in the dorsal horn of the anaesthetized spinal cat, Neuroscience 65 (1995) 849–858.
[22] F.F. Eide, E.R. Vining, B.L. Eide, K. Zang, X.Y. Wang, L.F. Reichardt, Naturally occurring truncated trkB receptors have dominant inhibitory effects on brain-derived neurotrophic factor signaling, J. Neurosci. 16 (1996) 3123–3129.
[23] T. Fukuoka, E. Kondo, Y. Dai, N. Hashimoto, K. Noguchi, Brain-derived neurotrophic factor increases in the uninjured dorsal root ganglion neurons in selective spinal nerve ligation model, J. Neurosci. 21 (2001) 4891–4900.
[24] S.O. Ha, J.K. Kim, H.S. Hong, D.S. Kim, H.J. Cho, Expression of brain-derived neurotrophic factor in rat dorsal root ganglia, spinal cord and gracile nuclei in experimental models of neuropathic pain, Neuroscience 107 (2001) 301–309.
[25] A. Haapasalo, E. Koponen, E. Hoppe, G. Wong, E. Castren, Truncated trkB.T1 is dominant negative inhibitor of trkB.TK+-mediated cell survival, Biochem. Biophys. Res. Commun. 280 (2001) 1352–1358.
[26] W. Han, D. Li, A.K. Stout, K. Takimoto, E.S. Levitan, Ca2+-induced deprotonation of peptide hormones inside secretory vesicles in preparation for release, J. Neurosci. 19 (1999) 900–905.
[27] P.A. Heppenstall, G.R. Lewin, BDNF but not NT-4 is required for normal flexion reflex plasticity and function, Proc. Natl. Acad. Sci. USA 98 (2001) 8107–8112.
[28] R.R. Ji, H. Baba, G.J. Brenner, C.J. Woolf, Nociceptive-specific activation of ERK in spinal neurons contributes to pain hypersensitivity, Nat. Neurosci. 2 (1999) 1114–1119.
[29] R.R. Ji, K. Befort, G.J. Brenner, C.J. Woolf, ERK MAP kinase activation in superficial spinal cord neurons induces prodynorphin and NK-1 upregulation and contributes to persistent inflammatory pain hypersensitivity, J. Neurosci. 22 (2002) 478–485.
[30] A. Josephson, J. Widenfalk, A. Trifunovski, H.R. Widmer, L. Olson, C. Spenger, GDNF and NGF family members and receptors in human fetal and adult spinal cord and dorsal root ganglia, J. Comp Neurol. 440 (2001) 204–217.
[31] H. Kang, A.A. Welcher, D. Shelton, E.M. Schuman, Neurotrophins and time: different roles for TrkB signaling in hippocampal long-term potentiation, Neuron 19 (1997) 653–664.
[32] B.J. Kerr, E.J. Bradbury, D.L. Bennett, P.M. Trivedi, P. Dassan, J. French, D.B. Shelton, S.B. McMahon, S.W. Thompson, Brain-derived neurotrophic factor modulates nociceptive sensory inputs and NMDA-evoked responses in the rat spinal cord, J. Neurosci. 19 (1999) 5138–5148.
[33] V.R. King, E.J. Bradbury, S.B. McMahon, J.V. Priestley, Changes in truncated trkB and p75 receptor expression in the rat spinal cord following spinal cord hemisection and spinal cord hemisection plus neurotrophin treatment, Exp. Neurol. 165 (2000) 327–341.
[34] K. Langley, N.J. Grant, Are exocytosis mechanisms neurotransmitter specific?, Neurochem. Int. 31 (1997) 739–757.
[35] S.L. Lee, J.K. Kim, D.S. Kim, H.J. Cho, Expression of mRNAs encoding full-length and truncated TrkB receptors in rat dorsal root ganglia and spinal cord following peripheral inflammation, Neuroreport 10 (1999) 2847–2851.
[36] I.J. Lever, E.J. Bradbury, J.R. Cunningham, D.W. Adelson, M.G. Jones, S.B. McMahon, J.C. Marvizon, M. Malcangio, Brain-derived neurotrophic factor is released in the dorsal horn by distinctive patterns of afferent fiber stimulation, J. Neurosci. 21 (2001) 4469–4477.
[37] E.S. Levine, R.A. Crozier, I.B. Black, M.R. Plummer, Brain-derived neurotrophic factor modulates hippocampal synaptic transmission by increasing *N*-methyl-D-aspartic acid receptor activity, Proc. Natl. Acad. Sci. USA 95 (1998) 10235–10239.
[38] E.S. Levine, J.E. Kolb, Brain-derived neurotrophic factor increases activity of NR2B-containing *N*-methyl-D-aspartate receptors in excised patches from hippocampal neurons, J. Neurosci. Res. 62 (2000) 357–362.
[39] D.J. Liebl, W. Huang, W. Young, L.F. Parada, Regulation of Trk receptors following contusion of the rat spinal cord, Exp. Neurol. 167 (2001) 15–26.
[40] S.Y. Lin, K. Wu, E.S. Levine, H.T. Mount, P.C. Suen, I.B. Black, BDNF acutely increases tyrosine phosphorylation of the NMDA receptor subunit 2B in cortical and hippocampal postsynaptic densities, Brain Res. Mol. Brain Res. 55 (1998) 20–27.
[41] X. Luo, R.A. Rush, X. Zhou, Ultrastructural localization of brain-derived neurotrophic factor in rat primary sensory neurons, Neurosci. Res. 39 (2001) 377–384.
[42] R.J. Mannion, M. Costigan, I. Decosterd, F. Amaya, Q.P. Ma, J.C. Holstege, R.R. Ji, A. Acheson, R.M. Lindsay, G.A. Wilkinson, C.J. Woolf, Neurotrophins: peripherally and centrally acting modulators of tactile stimulus-induced inflammatory pain hypersensitivity, Proc. Natl. Acad. Sci. U.SA 96 (1999) 9385–9390.
[43] J.C. Marvizon, V. Martinez, E.F. Grady, N.W. Bunnett, E.A. Mayer, Neurokinin 1 receptor internalization in spinal cord slices induced by dorsal root stimulation is mediated by NMDA receptors, J. Neurosci. 17 (1997) 8129–8136.

[44] A. Meyer-Franke, G.A. Wilkinson, A. Kruttgen, M. Hu, E. Munro, M.G. Hanson Jr., L.F. Reichardt, B.A. Barres, Depolarization and cAMP elevation rapidly recruit TrkB to the plasma membrane of CNS neurons, Neuron 21 (1998) 681–693.

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

[46] M. Narita, Y. Yajima, T. Aoki, S. Ozaki, H. Mizoguchi, L.F. Tseng, T. Suzuki, Up-regulation of the TrkB receptor in mice injured by the partial ligation of the sciatic nerve, Eur. J. Pharmacol. 401 (2000) 187–190.

[47] W. Pan, W.A. Banks, M.B. Fasold, J. Bluth, A.J. Kastin, Transport of brain-derived neurotrophic factor across the blood–brain barrier, Neuropharmacology 37 (1998) 1553–1561.

[48] S. Pezet, I. Lever, M. Malcangio, R.J. Williams, S.B. McMahon, Nociceptive stimulation induces TrkB receptor phosphorylation in the rat dorsal horn, in: 31st Annual Meeting of the Society for Neuroscience, San Diego, CA, 10–15 November 2001.

[49] D.L. Shelton, J. Sutherland, J. Gripp, T. Camerato, M.P. Armanini, H.S. Phillips, K. Carroll, S.D. Spencer, A.D. Levinson, Human trks: molecular cloning, tissue distribution, and expression of extracellular domain immunoadhesins, J. Neurosci. 15 (1995) 477–491.

[50] S. Slack, S. Pezet, M. Malcangio, S.W.N. Thompson, BDNF induces NMDA receptor subunit 1 phosphorylation in rat dorsal horn neurons, in: 31st Annual Meeting of the Society for Neuroscience, San Diego, CA, 10–15 November 2001.

[51] D.L. Small, C.L. Murray, G.A. Mealing, M.O. Poulter, A.M. Buchan, P. Morley, Brain derived neurotrophic factor induction of *N*-methyl-D-aspartate receptor subunit NR2A expression in cultured rat cortical neurons, Neurosci. Lett. 252 (1998) 211–214.

[52] W.D. Snider, S.B. McMahon, Tackling pain at the source: new ideas about nociceptors, Neuron 20 (1998) 629–632.

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

[54] L. Urban, S.W. Thompson, A. Dray, Modulation of spinal excitability: co-operation between neurokinin and excitatory amino acid neurotransmitters, Trends Neurosci. 17 (1994) 432–438.

[55] C. Wetmore, L. Olson, Neuronal and nonneuronal expression of neurotrophins and their receptors in sensory and sympathetic ganglia suggest new intercellular trophic interactions, J. Comp. Neurol. 353 (1995) 143–159.

[56] Q. Yan, M.J. Radeke, C.R. Matheson, J. Talvenheimo, A.A. Welcher, S.C. Feinstein, Immunocytochemical localization of TrkB in the central nervous system of the adult rat, J. Comp. Neurol. 378 (1997) 135–157.

[57] Q. Yan, R.D. Rosenfeld, C.R. Matheson, N. Hawkins, O.T. Lopez, L. Bennett, A.A. Welcher, Expression of brain-derived neurotrophic factor protein in the adult rat central nervous system, Neuroscience 78 (1997) 431–448.

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

[59] X.F. Zhou, L.F. Parada, D. Soppet, R.A. Rush, Distribution of trkB tyrosine kinase immunoreactivity in the rat central nervous system, Brain Res. 622 (1993) 63–70.

[60] X.F. Zhou, R.A. Rush, Endogenous brain-derived neurotrophic factor is anterogradely transported in primary sensory neurons, Neuroscience 74 (1996) 945–953.