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Pain Facilitation and Activity-Dependent Plasticity in Pain Modulatory Circuitry: Role of BDNF-TrkB Signaling and NMDA Receptors

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Abstract Pain modulatory circuitry in the brainstem exhibits considerable synaptic plasticity. The increased peripheral neuronal barrage after injury activates spinal projection neurons that then activate multiple chemical mediators including glutamatergic neurons at the brainstem level, leading to an increased synaptic strength and facilitatory output. It is not surprising that a well-established regulator of synaptic plasticity, brain-derived neurotrophic factor (BDNF), contributes to the mechanisms of descending pain facilitation. After tissue injury, BDNF and TrkB signaling in the brainstem circuitry is rapidly activated. Through the intracellular signaling cascade that involves phospholipase C, inositol trisphosphate, protein kinase C, and nonreceptor protein tyrosine kinases; N-methyl-D-aspartate (NMDA) receptors are phosphorylated, descending facilitatory drive is initiated, and behavioral hyperalgesia follows. The synaptic plasticity observed in the pain pathways shares much similarity with more extensively studied forms of synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD), which typically express NMDA receptor dependency and regulation by trophic factors. However, LTP and LTD are experimental phenomena whose relationship to functional states of learning and memory has been difficult to prove. Although mechanisms of synaptic plasticity in pain pathways have typically not been related to LTP and LTD, pain pathways have an advantage as a model system for synaptic modifications as there are many well-established models of persistent pain with clear measures of the behavioral phenotype. Further studies will elucidate cellular and molecular mechanisms of pain sensitization and further our understanding of principles of central nervous system plasticity and responsiveness to environmental challenge.

Keywords Hyperalgesia · Tissue injury · Neurotrophins · Brainstem · Phosphorylation · Inflammation · Periaqueductal gray · Rostral ventromedial medulla · Signal transduction

After injury, the pain we perceive reflects only partly the level and intensity of a noxious stimulus as it is also modulated by circuitries in the central nervous system (CNS). In addition to well-documented inhibitory control, pain processing can be vigorously facilitated by CNS circuitry, a process that contributes to the development of chronic or persistent pain conditions [1–4]. Consistent with this view, recent studies point out that abnormal pains after injury are linked to an enhanced neuronal activity within CNS structures involved in pain modulation. The increased excitability in the pain modulatory circuitry is dynamic and involves activation of chemical mediators including excitatory amino acids and their receptors, recruitment of intracellular signaling transduction cascades, and long-lasting changes in synaptic efficacy [2]. This activity-dependent plasticity in the pain modulatory circuitry is complementary to mechanisms of hyperexcitability in spinal dorsal horn neurons as well as exhibiting similarity to plasticity observed in hippocampal synapses involved in experimental phenomena such as long-term potentiation (LTP) and long-term depression (LTD) that are thought to mimic functional states of learning and memory [5]. This review focuses on cellular and molecular mechanisms and behavioral outcome of inflammation-induced neuronal hyperexcitability in brainstem pain modulatory circuitry.

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Evidence will be provided for a critical contribution of brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, and its interaction with N-methyl-D-aspartate (NMDA) glutamate receptors, to synaptic plasticity and descending pain facilitation.

Pain Modulatory Circuitry and Behavioral Hyperalgesia

Multiple brain sites and pathways are involved in descending pain modulation, ranging from the cerebral cortex to the caudal medulla [6, 7]. The most well characterized endogenous pain modulatory pathway involves a circuitry linking the midbrain periaqueductal gray (PAG), rostral ventromedial medulla (RVM) and the spinal cord (Fig. 1a). Mainly utilizing animal models of transient nociception, earlier studies found pain inhibitory actions of the brainstem descending circuitry [6, 7]. The most sensitive sites for the analgesic action of morphine are located in the PAG and the adjacent hypothalamic periventricular area [8]. Focal brain stimulation of the PAG produces sufficient analgesia to allow surgery in rats without the use of chemical anesthetics [9]. Importantly, stimulation of the PAG activates a normal function of the brain: *pain inhibition* [10, 11]. Convergent lines of evidence indicate that PAG stimulation-produced

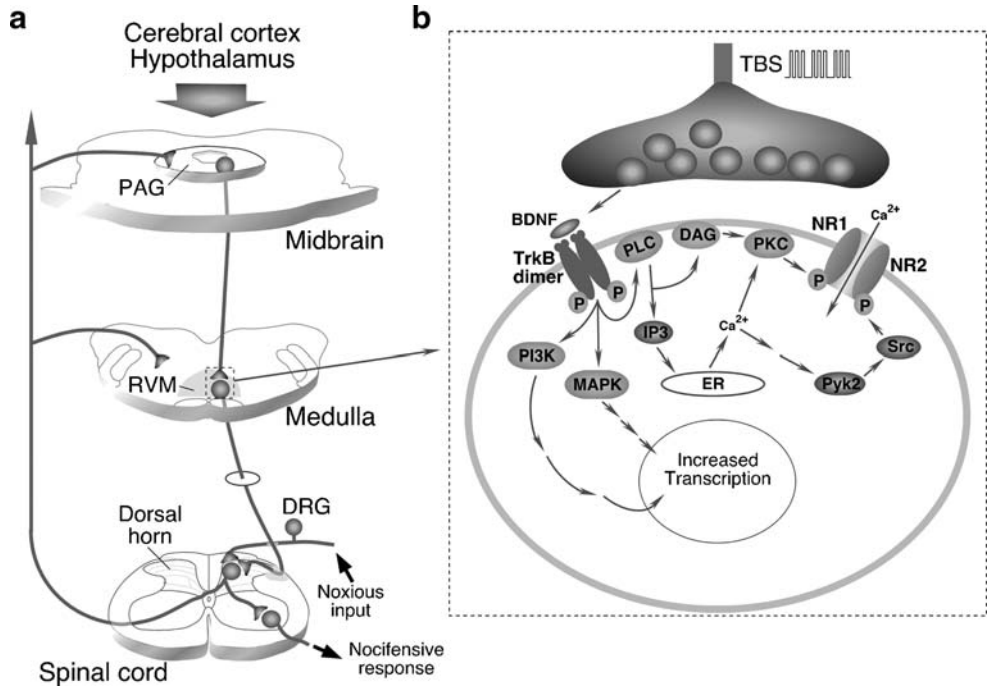

Fig. 1 **a** The descending pathways involved in pain modulation. The *PAG* in the midbrain has efferent projections to the *RVM* and RVM neurons directly project to the spinal dorsal horn where nociceptive input is initially processed. Primary afferent dorsal root ganglion (*DRG*) neurons transmit peripheral noxious input to the spinal dorsal horn. Supraspinal projecting dorsal horn neurons also issue collaterals terminating in pain modulatory structures and the PAG also receives input from forebrain structures including cerebral cortex and hypothalamus. Inflammation induces peripheral sensitization associated with an increased barrage of primary afferent activity and increased activation of dorsal horn neurons, followed by central sensitization and behavioral hyperalgesia measured with nocifensive withdrawal responses. Spinal ascending input activates neurons at the brainstem level leading to expression of activity-dependent plasticity. In addition to BDNF released from primary afferent terminals, inflammation upregulates BDNF in PAG neurons. BDNF is released from terminals in the RVM (enlarged in **b**), resulting in an enhancement of descending facilitation that contributes to spinal dorsal horn neuronal hyperexcitability and a time-dependent maintenance of behavioral hyperalgesia. The brainstem descending pathway also produces descending pain inhibition. (Adapted from *80*, Copyright 2006 by the Society for Neuroscience, with permission). **b** Simplified schematic diagram illustrating coupling of BDNF–TrkB signaling pathways with NMDA receptor activation in the RVM. A PAG–RVM BDNF-containing synapse is enlarged from *dashed rectangle* in **a**. The release of BDNF in the RVM from terminals of PAG neurons after injury can be mimicked by electrical stimulation with TBS. BDNF binds to TrkB and leads to autophosphorylation (*P*) of several tyrosine residues in the cytoplasmic domain of the TrkB receptor. Phosphorylation of Y785 on TrkB recruits phospholipase C-gamma (*PLC*), followed by generation of inositol trisphosphate (IP_3) and *DAG*. Through activation of IP_3 receptors on endoplamic reticulum (*ER*), Ca^{2+} is released from internal stores. DAG and Ca^{2+} activate *PKC* isoforms that phosphorylate serine residues in the C-terminus of the NMDA receptor NR1 subunit. The increased intracellular Ca^{2+} may stimulate the proline-rich tyrosine kinase 2 (*Pyk2*)-Src pathway that leads to tyrosine phosphorylation of the NR2 subunit of the NMDA receptor. Phosphorylation of NMDA receptors will potentiate the NMDA receptor channel gating and increase synaptic strength. Phosphorylation of tyrosine residues in TrkB also creates docking sites for a number of adaptor proteins and activation of phosphatidylinositol-3-OH kinase (*PI3K*) and *MAPK* cascades, leading to increased transcriptional activity. These events contribute to amplification of synaptic input, central sensitization, and the development of hyperalgesia

analgesia is relayed through other brainstem nuclei [12]. The RVM has been identified as the premier relay station between the PAG and spinal dorsal horn. The RVM consists mainly of the midline nucleus raphe magnus and the adjacent gigantocellular reticular nucleus, alpha part [6, 13]. Brainstem descending pathways linking the PAG, the RVM, and the spinal cord constitute a major mechanism in descending inhibition of pain transmission.

Recent studies have focused on animal models of persistent pain and revealed that the output of pain modulatory circuitry also exerts a facilitatory effect on nociceptive transmission, particularly after injury. Animals exhibit exaggerated nocifensive behavior after peripheral tissue or nerve injury, described as hyperalgesia (an increased response to noxious stimulus) and allodynia (a nocifensive response to a normally nonnoxious stimulus). Hyperalgesia/allodynia also develops in tissues distant from the site of injury, so-called "secondary hyperalgesia" [14]. Emerging evidence indicates that descending *facilitation* not only parallels inhibition but also can be a driving force for the development of behavioral hyperalgesia/allodynia [1, 15]. Local anesthesia of RVM attenuates mustard oil-induced hyperexcitability of spinal dorsal horn neurons [16] and behavioral hyperalgesia [17]. Lesions of the RVM suppress formalin-induced nocifensive behavior [18], inhibit secondary hyperalgesia produced by topical application of mustard oil [15], and attenuate (secondary) mechanical hyperalgesia after masseter muscle inflammation induced by complete Freund's adjuvant (CFA) [19]. The same phenomenon occurs in models of neuropathic pain. Suzuki et al. [20] show that descending facilitatory control of mechanically evoked responses is enhanced in dorsal horn neurones after nerve injury. The tactile allodynia and cold hypersensitivity after nerve injury depends upon activation of bulbospinal descending facilitatory pathways [21, 22]. These observations point to an ascending–descending loop that is activated in response to prolonged stimulation to facilitate spinal nociceptive processing, leading to behavioral hyperalgesia. The presence of descending inhibition and facilitation originating from the RVM suggests that the RVM output is a net effect of interactions between these systems. Alterations in the activation of these bimodal systems caused by enhanced nociceptive drive from sites of tissue injury, drug manipulations, or RVM lesions can reset this balance from facilitation to inhibition, and vice versa [23].

Plasticity of Pain Modulatory Circuitry after Inflammation

Studies in the past decade or so have come to the conclusion that the CNS structures involved in pain modulation are themselves, modifiable. After peripheral tissue injury, dynamic changes in descending pain modulation occur [24–28]. By monitoring inhibition of paw withdrawal responses to a noxious thermal stimulus, or antinocifensive responses, in lightly anesthetized rats during RVM stimulation, Terayama et al. [29] examined the potency of descending inhibition during the development of inflammation. After a unilateral CFA-induced hind paw inflammation, the stimulus–response function curve for the inflamed paw was initially shifted to the right of the noninflamed paw at 3 h, suggesting a reduced net inhibition, and then gradually shifted to the left and reached maximal potency at 24 h after inflammation. These findings indicate that inflammation induces dramatic changes in the excitability of RVM pain-modulatory circuitry. Early in the development of inflammation there is an increased descending facilitation, which reduces the net effect of the inhibition. Over time, the level of descending inhibition increases, or descending facilitation decreases, leading to a net enhancement of antinociception. Direct stimulation of the spinal dorsolateral funiculus that bypasses brainstem synaptic mechanisms does not produce a change in excitability indicating that the changes are caused by supraspinal mechanisms at the level of the RVM or higher. Thus, the activity of descending pathways exhibits considerable plasticity.

The plasticity of the pain modulatory circuitry also involves enhanced RVM neuronal activity. Using nocifensive withdrawal responses as a behavioral correlate, RVM neurons have been characterized as "on cell," "off cell," and "neutral cell" [30, 31]. An on cell typically shows a burst of activity immediately before the onset of a nocifensive response, and an off cell exhibits a pause in activity just before a nocifensive response. These two types of cells are pain-modulatory neurons. The activity of neutral cells has no clear relationship to nocifensive responses and the role of neutral cells in pain modulation is less clear. Apparently, the time-dependent plasticity in descending pain modulatory circuitry involves changes in the response profiles of RVM neurons. Montagne-Clavel and Oliveras [32] have shown changes in RVM neuronal properties after inflammation in the awake, freely moving rat, which suggests an increase in the population of neurons involved in pain modulatory activity. Through continuous recordings during the development of inflammation, Miki et al. [33] showed that some neutral-like cells changed their response profile and could be reclassified as on or off cells. The switch in the response profile of RVM neurons correlates with the temporal changes in excitability in the RVM after inflammation. This phenotypic change of RVM neurons was verified in a population study that showed a significant increase in the percentage of on and off cells, and a decrease in the neutral-like cell population 24 h after inflammation as compared to control animals [33]. The

studies of RVM neurons support the view that enhanced descending modulation after inflammation involves both facilitation and inhibition as there are changes in the responses of both on and off cells [33, 34]. The enhanced RVM neuronal activity likely results from an increased synaptic input and receptor activation after injury.

Involvement of NMDA Receptors

Activation of excitatory amino acid receptors in pain modulatory neurons in the RVM mediates morphine analgesia [35]. Glutamatergic synapses in descending circuitry also play a critical role in response to injury [15, 36]. Injection of NMDA, the prototype NMDA receptor agonist, into the RVM produces pain facilitation or inhibition that is dependent upon the postinflammatory time period. At 3 h postinflammation, low doses of NMDA produce facilitation of the response to noxious heat of the inflamed and noninflamed hind paws and tail, indicating that descending facilitatory effects are NMDA receptor dependent and occur early after inflammation [36]. Higher doses of NMDA at 3 h postinflammation only produce inhibition. At 24 h postinflammation, NMDA produces only inhibition. All of these effects are blocked by administration of NMDA receptor antagonists [36]. These findings suggest a contribution of NMDA receptors to changes in synaptic strength and potency in the RVM after inflammation. Thus, as with experimental phenomena of LTP and LTD [5], at least one form of experience or activity-dependent plasticity in the RVM is NMDA receptor dependent.

The increased sensitivity of NMDA receptors in the descending circuitry during the development of inflammatory hyperalgesia is related to transcriptional and translational modulation of the receptors. The native NMDA receptor is likely a tetramer that consists of two NR1 and two NR2 subunits. Examination of the mRNA expression of the NR1, NR2A and NR2B subunits of the NMDA receptor in the RVM reveals an upregulation that parallels the time course of the RVM excitability changes. This is accompanied by an increase in NMDA receptor protein levels [33].

Protein phosphorylation is a major mechanism for regulation of receptor sensitivity and changes in synaptic strength. Phosphorylation of multiple sites in the cytoplasmic C-termini of the NR1 and NR2 subunits, including tyrosine, serine, and threonine residues, is known to modulate NMDA receptor activity, affect synaptic transmission, and contribute to behavioral hyperalgesia [37–41]. There was a time-dependent increase in NR2A tyrosine phosphorylation in the RVM after inflammation as compared to naïve noninflamed rats (Fig. 2). Interestingly,

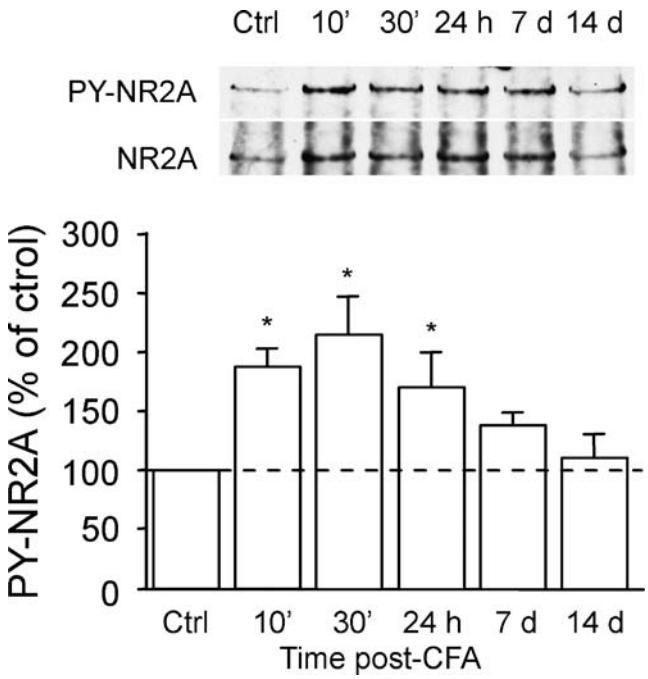

Fig. 2 Western blot illustrating an increase in NR2A tyrosine phosphorylation in the RVM after CFA-induced inflammation. RVM tissues were punched out and proteins were isolated from noninflamed (*Ctrl*) rats and rats at 10 min (*10'*) to 14 d after inflammation. To examine tyrosine phosphorylation of the NR2 subunits, protein samples from RVM were first immunoprecipitated with anti-NR2A or anti-NR2B antibodies. The eluted NR2A or NR2B proteins were then incubated with an anti-phosphotyrosine antibody (clone 4G-10). The tyrosine phosphorylation, as indicated by the immunoblot against 4G-10, was associated with a band of 180 kDa that correlates with the NR2 subunit of the NMDA receptor in RVM tissues. The *top blot* shows the immunoreactive bands against anti-phosphotyrosine 4G-10 (*PY-NR2A*) after immunoprecipitation of extracted proteins with anti-NR2A antibodies. The *bottom blot* shows immunobands against NR2A antibodies after stripping and reprobing the same membrane previously probed with 4G-10 antibodies. The levels of tyrosine phosphorylation are compared by normalizing to the NR2A-immunoreactive bands. The relative phosphotyrosine protein levels (mean±SEM) after inflammation are expressed as a percentage of the control. Asterisks indicate significant differences from the control ($p<0.05$, $n=5$). *Dashed line* indicates the control levels. (Adapted from *80*, Copyright 2006 by the Society for Neuroscience, with permission)

inflammation induces an increase in NR2B, but not NR2A subunit tyrosine phosphorylation in the spinal cord [40]. These changes in phosphorylation involve intracellular signaling pathways that include mGluR activation by glutamate, diacylglycerol (DAG)-inositol trisphosphate (IP_3) second messenger pathways, the release of calcium from intracellular stores, and activation of Src family kinases, with resulting coupling of mGluR activation to NMDA receptor NR2 subunit phosphorylation [40, 42]. The similarity of these cell-signaling pathways to those found associated with LTP is apparent and suggests common cell signaling mechanisms underlying synaptic plasticity associated with LTP and inflammatory hyperalgesia.

A time-dependent increase in NR1 serine 896/897 phosphorylation has also been identified in the RVM after hind paw inflammation [43]. A common feature of the changes in NMDA receptor phosphorylation is that it occurs rapidly, as early as 10–30 min after inflammation, and persists for up to a week. The time course of changes in NMDA receptor phosphorylation in the RVM correlates well with changes in excitability in descending circuitry after inflammation.

In summary, it is clear that the synaptic plasticity observed in pain pathways shares much similarity with more extensively studied forms of synaptic plasticity such as LTP and LTD, which typically express NMDA receptor dependency and regulation by trophic factors. LTP and LTD are experimental phenomena, which can be used to demonstrate synaptic plasticity and long term changes in synaptic circuitry. However, it has been difficult to prove that LTP /LTD occurs in vivo in response to behavioral experience and subserve functional roles [5]. Although mechanisms of synaptic plasticity in pain pathways have typically not been related to LTP or LTD, pain pathways have an advantage as a model system for eliciting LTP or LTD-like synaptic modifications as there are many well-established models of persistent pain with clear measures of the behavioral phenotype.

BDNF and Persistent Pain

In the adult mammalian brain, BDNF facilitates excitatory synaptic transmission and long-term synaptic plasticity [44]. The effects of BDNF are mediated through its binding to a subtype of the tropomyosin-related kinase, TrkB, and subsequent activation of downstream signaling pathways [45, 46]. The BDNF–TrkB-signaling pathway plays an essential role in activity-dependent synaptic plasticity underlying LTP and LTD [44–47]. BDNF is also considered a neuromodulator in spinal nociceptive processing [48–51]. Thus, BDNF–TrkB signaling is likely involved in synaptic mechanisms underlying both memory and pain [52].

Evidence has accumulated that BDNF is involved in chronic or persistent pain, although the focus has been mainly on the periphery and BDNF released from primary sensory neurons into the spinal cord (see [50, 53]). In fibromyalgia patients, mean serum levels of BDNF are significantly increased as compared to healthy controls [54]. After peripheral inflammation, BDNF mRNA and protein levels are upregulated in the dorsal root ganglion and spinal cord in rats [55, 56]. There may be a phenotypic switch of BDNF expression to large primary sensory neurons associated with inflammation [57]. Inflammatory hyperalgesia is attenuated by sequestration of endogenous BDNF systemically [58] or in the spinal cord [56, 59], antisense treatment against BDNF and TrkB [60], and conditional knock-out of BDNF in primary sensory neurons [61]. The involvement of BDNF-TrkB signaling in pain transmission is selective. Although both BDNF and neurotrophin-4 (NT-4) are ligands of the TrkB receptor, NT-4 is not involved in nociceptive plasticity. In NT-4 null mutants, the activity-dependent plasticity of the ventral root potential evoked by stimulation of nociceptive primary afferents remains normal [62]. In fact, NT-4 may modulate morphine analgesia via TrkB [63].

Sciatic nerve injury upregulates BDNF mRNA and protein expression in dorsal root ganglion cells [64–67]. It has been reported that neuropathic pain in animals after nerve injury also involves activation of the BDNF-TrkB pathway [68–71]. However, Zhao et al. [61] show that selective deletion of BDNF in primary sensory neurons does not affect the development of neuropathic pain in mice. This finding suggests that, if BDNF were involved in neuropathic pain, it would come from other sources such as spinal activated microglia. In rats with neuropathic pain, there is a shift in the neuronal anion gradient [72]. This shift of the anion gradient converts normally inhibitory anionic synaptic currents to excitatory currents and leads to increased excitability of dorsal horn neurons and behavioral hyperalgesia. Interestingly, BDNF from activated microglia produces a similar shift in neuronal anion gradient, as indicated by a shift in the anion reversal potential in spinal lamina I neurons [73]. As a result, at the resting membrane potential, gamma-amino butyric acid (GABA) induces depolarization, instead of hyperpolarization, in nerve-injured rats. These results suggest that microglia-derived BDNF may be an important contributor to dorsal horn neuronal hyperexcitability after nerve injury.

BDNF Signaling in Pain Modulatory Circuitry Facilitates Inflammatory Hyperalgesia

BDNF is widely expressed throughout the adult mammalian nervous system [74–76]. Notably, studies have shown high levels of BDNF mRNAs and proteins within the PAG [75, 77]. Abundant TrkB mRNAs and proteins are observed in RVM neurons projecting to the spinal dorsal horn [78, 79]. The distribution of the BDNF–TrkB system in the PAG–RVM circuitry suggests their role in synaptic activity related to pain modulation [53]. Our recent work demonstrates that enhanced supraspinal BDNF–TrkB-receptor signaling contributes to the development of persistent pain after tissue injury [80].

PAG neurons provide major synaptic input to the RVM [81, 82]. In the ventrolateral PAG, more than 60% of RVM-projecting neurons express BDNF [80]. These BDNF-containing neurons in the PAG are involved in the response

to injury. After hind paw inflammation, both immunostaining and Western blot show significant increases in BDNF-like immunoreactivity in the PAG [80]. The upregulation of BDNF in PAG neurons is associated with the development of behavioral hyperalgesia. Accompanying the increase in BDNF in PAG, the levels of full-length TrkB proteins and their phosphorylation also exhibit time-dependent increases after inflammation in the RVM (Fig. 3).

Electrical stimulation of the peripheral nerve can induce BDNF release into the spinal cord [83, 84]. This release of BDNF is stimulus parameter dependent [50, 85]. It appears that primary afferents carry distinctive firing patterns, which encodes the release of different transmitters and subsequent neuronal activation in the spinal dorsal horn. Continuous electrical stimulation leads to release of substance P and glutamate and the activation of neurokinin1 (NK1) and alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors on postsynaptic spinal neurons. When tetanic stimulation is employed, additional recruitment of NMDA receptors occurs. Only after trains of burst stimulation (TBS) or stimulation at C-fiber strength, in addition to NK1, AMPA and NMDA receptors, TrkB receptors are recruited because of BDNF release in the spinal dorsal horn [50, 85]. This parameter-dependent property of stimulation-evoked BDNF release is very similar to that described in LTP [86, 87]. It associates BDNF release with long-term modification of synaptic strength and activity-dependent neuronal plasticity.

The TBS protocol that induces BDNF release in the spinal cord also is sufficient to evoke BDNF release in PAG-RVM synapses. Because TrkB undergoes immediate autophosphorylation after binding to released BDNF, the increase in TrkB tyrosine phosphorylation (pTrkB) can be used as a measure of increased activation of the receptor and provides evidence for the release of BDNF from presynaptic terminals [88, 89]. We have applied TBS to the ventrolateral PAG to activate PAG neurons in isoflurane anesthetized rats and assessed the levels of pTrkB in the RVM [80]. Compared with the rats not receiving stimulation, the pTrkB level in the RVM was significantly increased at 30 min after electrical stimulation of PAG (Fig. 4a). Double immunostaining of Tyr490 (anti-phospho-tyr490-TrkA/B antibodies) and TrkB with RVM tissues showed that electrical stimulation of the PAG with TBS induced an increase in the number of pTrk (Tyr490)-immunoreactive puncta in the cytoplasm and proximal dendrites of TrkB-labeled RVM neurons. These observations are consistent with the upregulation of BDNF in PAG and pTrkB in RVM neurons after peripheral inflammation and suggest functional release of BDNF in pain modulatory circuitry during maintained ascending input after injury.

What is the functional significance of activation of the PAG-RVM BDNF-containing neurons in pain processing? Results from behavioral pharmacological studies indicate that RVM–BDNF signaling produces pain facilitation [80]. First, injection of presumably physiological doses of BDNF (10–

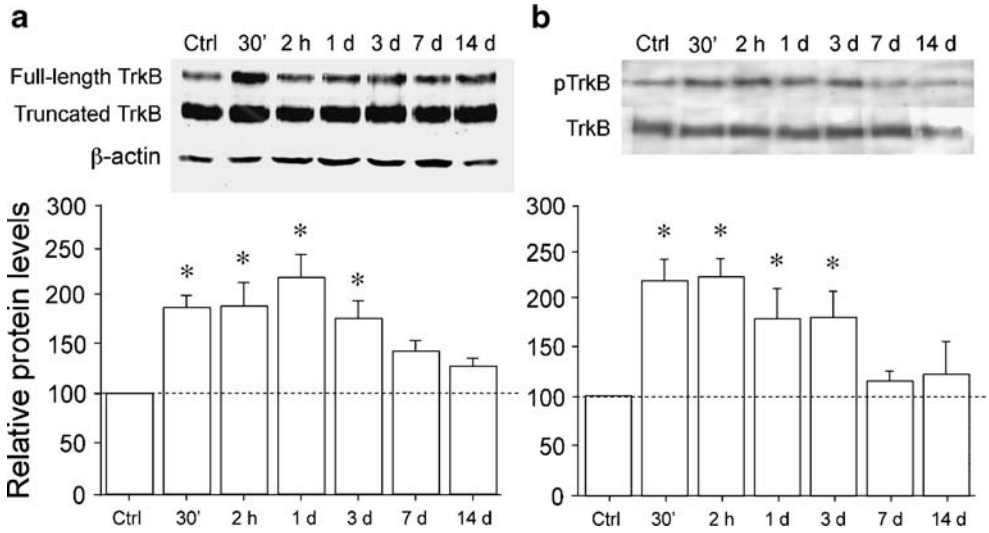

Fig. 3 Peripheral inflammation upregulates TrkB and TrkB phosphorylation in the RVM. **a–b**: Western blot shows the time-dependent increase of the TrkB expression (**a**) and tyrosine phosphorylation of TrkB (*p-TrkB*, **b**) in the RVM after inflammation. The *upper blots* show examples of the immunoreactive bands against anti-TrkB that identifies both full-length and truncated TrkB (**a**) and 4G-10, the antiphosphotyrosine antibody (**b**). The *lower blots* shows immunobands against beta-actin and TrkB antibodies, respectively, after stripping and reprobing the same membrane. The *bottom bar graphs* show the mean levels of the full-length TrkB and p-TrkB normalized to beta-actin (**a**) and TrkB (**b**). The relative TrkB and p-TrkB levels (mean±SEM) after inflammation are expressed as a percentage of the controls. *Asterisks* indicate significant differences ($p<0.05$) from the control ($n=4$ per time point). (Adapted from *80*, Copyright 2006 by the Society for Neuroscience, with permission)

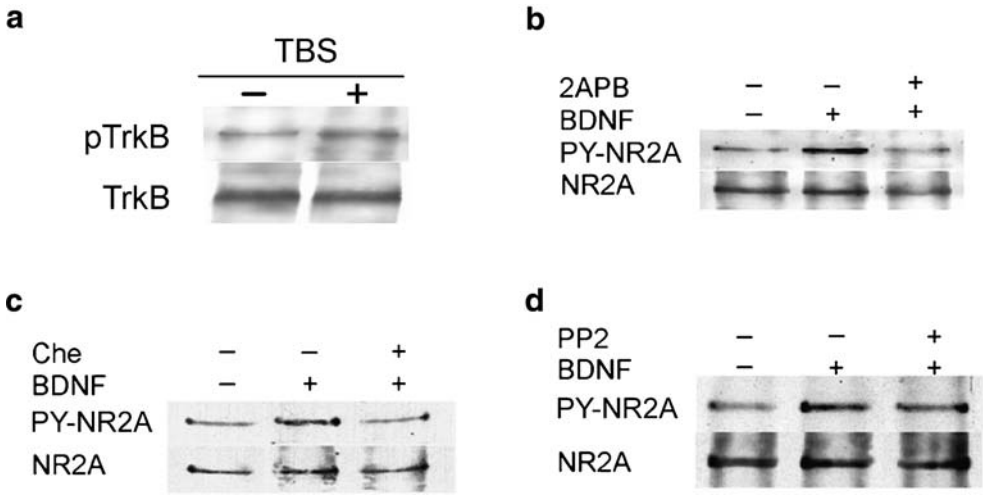

Fig. 4 **a** Western blot illustrating an example of the increased phosphorylation of TrkB receptor (*pTrkB*) in RVM after electrical stimulation of PAG. Proteins from RVM tissues were first immunoprecipitated with TrkB and subsequently probed against 4G-10. Compared to the sham control (−), there was an increase ($p<0.01$, $n=4$ per group) in pTrkB in the RVM at 30 min after electrical stimulation (+). Trains of burst stimulation (*TBS*) protocol: 60 trains, square pulses of 0.5 ms, 67 Hz, 0.25 mA; for a total of 10 min, 1 sec on and 9 sec off. **b–d**. BDNF-induced NR2A tyrosine phosphorylation in RVM. The transverse brainstem slice including RVM was obtained from adult 8–10-week-old rats. The slices were incubated with BDNF (18.5 nM) for 10 min before protein extraction. Representative immunoblots against anti-4G-10 (*PY-NR2A*) and anti-NR2A antibodies are shown. Pretreatment with an IP_3 receptor antagonists 2-aminoethoxydiphenyl borate (*2APB*, 0.036 mM, **b**), a PKC inhibitor chelerythrine (*Che*, 0.01 mM, **c**), a Src family tyrosine kinase inhibitor PP2 (0.04 mM, **d**), blocked or attenuated the BDNF-induced increase in PY-NR2A in the RVM. (Adapted from *80*, Copyright 2006 by the Society for Neuroscience, with permission)

100 fmol) into the RVM produces behavioral hyperalgesia as indicated by a reduction of paw withdrawal latencies to a noxious thermal stimulus. Second, neutralizing RVM BDNF with antiBDNF antiserum or TrkB-IgG fusion protein attenuates inflammatory hyperalgesia. Third, knockdown of RVM TrkB receptors by RNA interference leads to attenuation of behavioral hyperalgesia. Thus, BDNF–TrkB signaling in supraspinal circuitry is complementary to the well-documented contribution of BDNF anterogradely transported from dorsal root ganglion neurons to the spinal cord to pain hypersensitivity [56, 59]. BDNF is likely associated with RVM neurons or circuitry that contribute to the net descending pain facilitation. The chemical signature(s) of descending inhibitory vs facilitatory circuitries needs to be further studied. It is of interest to determine whether serotoninergic neurons in the RVM participate in BDNF-produced descending pain facilitation [90].

The effect of BDNF on pain behavior is dose dependent. Whereas BDNF facilitate nociception in the fmol dose range [60, 80], studies have shown that exogenously applied high doses of BDNF (in the nmol range) into the cerebral ventricle [91], PAG [92–94], RVM [80], or intrathecal space [88, 95] produce analgesia or hypoalgesia. Applying high doses of BDNF to the spinal cord produces inhibition of evoked activity of 75% of dorsal horn nociceptive neurons [96]. Overexpression of BDNF in the rat spinal cord suppresses neuropathic pain [97]. It is estimated that the fmol dose of BDNF mimics physiological concentration and likely produces an effect through TrkB signaling [80]. On the other hand, the analgesia produced by high doses of BDNF (nmol) may be related to (1) downregulation of TrkB receptors [80, 93, 98] or (2) activation of p75 neurotrophin receptor that produces synaptic inhibition [99]. BDNF may also upregulate endogenous opioids to produce analgesia [94]. It should be noted again that the net effect of descending modulation is a result of the interaction between facilitatory and inhibitory drives. For example, the TBS of the PAG that produces release of BDNF in the RVM should produce antinociception when a behavioral end point is used. Although BDNF is released, its facilitatory effect on pain transmission can be overridden by simultaneous activation of descending inhibition produced by intense PAG electrical stimulation [9, 10].

Interaction with NMDA Receptors

BDNF exerts its effects via interactions with other receptors and ion channels [100, 101]. The facilitation of BDNF on glutamatergic synaptic transmission constitutes an important mechanism for activity-dependent long-term synaptic plasticity in the CNS [44, 102–105]. The interrelationship between the TrkB receptor and NMDA receptors in pain transmission has also received attention. BDNF enhances phosphorylation of NMDA receptor subunits in the spinal dorsal horn [85, 106]. Consistently, there is a concurrent

abolition of NR1 serine 896/897 phosphorylation, but not AMPA receptor subunit GluR1 ser831 phosphorylation, in the spinal dorsal horn after conditioned knock out of BDNF in primary sensory neurons [61]. BDNF potentiates NMDA-evoked ventral root responses [59] and facilitates synaptic current of superficial dorsal horn neurons, an effect dependent upon NMDA receptor activity [107]. Intrathecal coadministration of an NMDA receptor antagonist with BDNF dose dependently inhibits BDNF-induced hyperalgesia [60].

The interaction of BDNF–TrkB signaling with NMDA receptors is likely a mechanism underlying injury-induced plasticity in the brainstem pain modulatory circuitry. This view is supported by convergent lines of evidence [33, 80]: (1) Inflammation induces upregulation of TrkB and NMDA receptor subunits and enhanced phosphorylation of both TrkB and NMDA receptor subunits in the RVM; (2) BDNF applied to RVM slices produces an increase in NR2A tyrosine phosphorylation (Fig. 4b–d); (3) Preadministration of NMDA receptor antagonists AP-5 and MK-801 abolishes intra-RVM BDNF-induced facilitation of the paw withdrawal response to noxious heat; and (4) TrkB colocalizes with NR2A in RVM neurons.

The activation of the NMDA receptor after BDNF-TrkB signaling involves several intermediate cellular pathways [45, 50]. Our recent results indicate that IP_3 and protein kinase C (PKC) are involved in BDNF-induced and Src-mediated NR2A tyrosine phosphorylation in RVM neurons (Fig. 4b–d). Thus, this signaling pathway is likely initiated through phosphorylation of the tyrosine residue (Y785) on TrkB that is related to activation of phospholipase C (for comprehensive description of TrkB activation and related cytoplasmic adaptor proteins, see [45, 46, 50]), which is followed by IP_3 and DAG formation, intracellular calcium release, and PKC activation. These events may lead to Pyk2 (proline-rich tyrosine kinase 2) and Src activation, and NR2A tyrosine phosphorylation [42, 108] (Fig. 1b). These cell-signaling pathways involving BDNF and TrkB are quite similar to those associated with neuronal plasticity in the hippocampus and other sites [44, 45]. The findings indicate the usefulness of models of persistent hyperalgesia in the study of cell signaling and its relationship to changes in synaptic strength so important in learning.

Distinct features are noticed for inflammation-induced NR2 tyrosine phosphorylation in the RVM when compared to the spinal dorsal horn. First, the NR2A subunit, but not the NR2B subunit, is tyrosine phosphorylated by BDNF. This is likely related to the differential distribution of the two subunits in the brain [109]. Second, group I mGluRs do not appear to play a role in BDNF-induced NR2A phosphorylation, suggesting different signaling pathways [42, 80]. The mitogen-activated protein kinase (MAPK) signaling pathway is likely involved in mediating the modulatory effect of BDNF on NMDA receptors. It has been shown that BDNF activates extracellular signal-regulated kinases (ERK) in the spinal dorsal horn [88]. The MEK inhibitor U0126 reduces BDNF-induced NR1 serine 897 phosphorylation in an in vitro spinal cord preparation [85]. Further, cyclic AMP response element binding protein shows increased phosphorylation after local application of BDNF in the spinal dorsal horn [110]. Thus, the activation of the MEK/ERK pathway contributes to BDNF-induced NR1 phosphorylation in the spinal cord and may lead to increased transcriptional regulation of nociception.

BDNF may bind to postsynaptic TrkB receptors and enhance NMDA receptor activity through intermediate cellular-signaling pathways. In the pain modulatory circuitry, the postsynaptic mechanism of BDNF is supported by findings that activation of PAG neurons induces internalized and phosphorylated TrkB, and the NMDA receptor subunit colocalizes with TrkB in RVM neurons [80]. In the spinal dorsal horn, antagonism of postsynaptic NMDA receptors with intracellular MK-801 prevents BDNF-produced facilitation of synaptic current [107]. It is also understandable that BDNF is capable of modulating synaptic plasticity through both pre and postsynaptic mechanisms [46, 53, 111]. In the rat hippocampus, the full-length TrkB receptor is located at plasma membrane of dendritic spines, axon initial segments and axon terminals, implicating its pre and postsynaptic localization [112]. The TrkB receptor is located in primary sensory neurons and spinothalamic projection neurons [113, 114]. Pre and postsynaptic localization of full-length TrkB in the superficial dorsal horn has been described [115]. It is unclear whether BDNF can engage presynaptic TrkB to enhance glutamate release from presynaptic terminals in the RVM, resulting in activation of NMDA receptors. A recent report in spinal slices suggests that BDNF modulate dorsal horn neurons through a presynaptic mechanism [58]. However, in rats with nerve injury, BDNF may be released from microglia and induce an increased excitability of dorsal horn neurons through postsynaptic mechanisms [73].

The coupling of BDNF-TrkB signaling with NMDA receptors raises the possibility that NMDA-receptor activation is a site of convergence of intracellular signal transduction via other receptor systems. The AMPA receptor is involved in descending pain inhibition after inflammation [36]. There is an increase in the AMPA receptor GluR1 subunit levels in the RVM postinflammation [116]. GluR1 serine 831 phosphoprotein levels are also increased as early as 30 min after inflammation. Consistent with the above hypothesis, the increase in GluR1 phosphorylation and AMPA-produced descending inhibition of behavioral hyperalgesia is blocked by NMDA receptor antagonists, suggesting that NMDA receptor activation is downstream to AMPA receptor in the RVM circuitry [36,

117]. This scheme may also be applicable to other RVM chemical mediators involved in descending modulation. The importance of the NMDA receptor as an output pathway leading to enhanced hyperalgesia after injury should not be minimized. Intracellular coupling to this receptor leads to changes after persistent injury and not in response to transient protective pain. NMDA receptor activation requires persistent receptor depolarization to remove the magnesium block. Chemical changes after transient injury will not activate this pathway.

Concluding Remarks

Pain modulatory circuitry in the brainstem exhibits considerable plasticity in response to injury. The increased neuronal barrage after injury activates spinal projection neurons that then activate multiple chemical mediators including glutamatergic, opioidergic, and presumably GABAergic neurons at the brainstem level, leading to an increased synaptic strength and facilitatory output. It is not surprising that a well-established regulator of synaptic plasticity BDNF also plays a critical role in pain modulatory circuitry and contributes to the mechanisms of descending pain facilitation. After peripheral tissue injury, BDNF–TrkB signaling in the brainstem descending circuitry is rapidly activated. Through the intracellular signaling cascade that involves phospholipase C, IP_3, PKC, and nonreceptor protein tyrosine kinases, NMDA receptors are phosphorylated and descending facilitatory drive is initiated; and behavioral hyperalgesia follows. The activity-induced plasticity in descending circuitry complements the activity-dependent neuronal plasticity in ascending pain transmission pathways, which also requires BDNF–TrkB signaling and NMDA receptor contribution. It is no coincidence that the synaptic plasticity observed in the pain pathways share much similarity with other forms of synaptic plasticity such as LTP and depression in the CA1 region of the hippocampus, which typically express NMDA receptor dependency and regulation by trophic factors [5]. In fact, the physiological relevance of cell signaling mechanisms involved in pain pathways and leading to activity- and experience-dependent plasticity can serve as model systems for studying the cellular basis of changes in synaptic strength. There are many well-established in vivo models of persistent pain with clear behavioral outcome measures or phenotypes. Further studies will elucidate cellular and molecular mechanisms of pain sensitization and further our understanding of principles of CNS plasticity and responsiveness to environmental challenge.

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