Published in final edited form as:
Brain Res Rev. 2009 April ; 60(1): 135–148. doi:10.1016/j.brainresrev.2008.12.011.

MAP kinase and pain

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Abstract

Mitogen-activated protein kinases (MAPKs) are important for intracellular signal transduction and play critical roles in regulating neural plasticity and inflammatory responses. The MAPK family consists of three major members: extracellular signal-regulated kinases (ERK), p38, and c-Jun N-terminal kinase (JNK), which represent three separate signaling pathways. Accumulating evidence shows that all three MAPK pathways contribute to pain sensitization after tissue and nerve injury via distinct molecular and cellular mechanisms. Activation (phosphorylation) of MAPKs under different persistent pain conditions results in the induction and maintenance of pain hypersensitivity via non-transcriptional and transcriptional regulation. In particular, ERK activation in spinal cord dorsal horn neurons by nociceptive activity, via multiple neurotransmitter receptors, and using different second messenger pathways plays a critical role in central sensitization by regulating the activity of glutamate receptors and potassium channels and inducing gene transcription. ERK activation in amygdala neurons is also required for inflammatory pain sensitization. After nerve injury, ERK, p38, and JNK are differentially activated in spinal glial cells (microglia vs astrocytes), leading to the synthesis of proinflammatory/pronociceptive mediators, thereby enhancing and prolonging pain. Inhibition of all three MAPK pathways has been shown to attenuate inflammatory and neuropathic pain in different animal models. Development of specific inhibitors for MAPK pathways to target neurons and glial cells may lead to new therapies for pain management. Although it is well documented that MAPK pathways can increase pain sensitivity via peripheral mechanisms, this review will focus on central mechanisms of MAPKs, especially ERK.

Keywords

MAPK; neural plasticity; central sensitization; spinal cord; amygdala; microglia; astrocytes; inflammatory pain; neuropathic pain

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1. Introduction

1.1. The MAPK family

The mitogen-activated protein kinases (MAPKs) are a family of intracellular signaling molecules that are evolutionally conserved. This family consists of three major members: extracellular signal-regulated kinase (ERK, including ERK1/2), p38 (including p38α, p38β, p38γ, and p38δ), and c-Jun N-terminal kinase (JNK, including JNK1, JNK2, and JNK3). ERK5 is a new but less-known member of the family (Johnson and Lapadat, 2002). ERK, p38, and JNK represent 3 different signaling cascades that transduce a broad range of extracellular stimuli into diverse intracellular responses by both transcriptional and non-transcriptional regulation (Widmann et al., 1999; Johnson and Lapadat, 2002). All the MAPKs are activated by phosphorylation via different upstream MAPK kinases (MKKs or MEKs), and MKKs are activated by MAPK kinase kinases (MEKKs) (Fig. 1). Development of phospho-specific antibodies for each MAPK pathway has greatly improved our understanding about how MAPKs are activated. ERK was the first family member identified. Early studies indicated a critical role of ERK in regulating mitosis, proliferation, differentiation, and survival of mammalian cells during development (Widmann et al., 1999). Subsequent studies demonstrated that ERK also plays an important role in neuronal plasticity in the adult (Impey et al., 1999). p38 and JNK are activated by proinflammatory cytokines and cellular stress and play essential roles in regulating inflammatory responses, neurodegeneration, and cell death (Widmann et al., 1999; Ji and Woolf, 2001; Kumar et al., 2003; Gao and Ji, 2008). ERK5 has overlapping roles with ERK1/2 (Nishimoto and Nishida, 2006).

1.2. Neuronal and glial regulation of pain by MAPKs

Given the important roles of MAPKs in regulating neural plasticity and inflammatory responses, studies on MAPK regulation of pain have dramatically increased in the last decade, especially in the last several years. A Medline search with the keywords “MAP kinase and pain” has shown 172 related articles in the last 3 years (from July 2005 to June, 2008). These studies have greatly benefited from specific inhibitors available to explore the function of each pathway. Although MAPK inhibitors have been shown to alleviate hyperalgesia and allodynia in inflammatory and neuropathic pain models, these inhibitors have little or no effect on basal physiological pain perception (reviewed in Ji et al., 2007), suggesting a specific role of MAPKs in the development of pain hypersensitivity (abnormal pain, or pathological pain) following tissue and nerve injury.

Early studies on MAPK regulation of pain focused on neuronal mechanisms following intense noxious stimulation and peripheral tissue inflammation (Ji et al., 1999; 2002a,b; Karim et al., 2001; Dai et al., 2002; Pezet et al., 2002). Neuronal activation of MAPKs in nociceptive primary sensory neurons and spinal cord dorsal horn neurons (SCDH) plays an important role in the induction and maintenance of neural plasticity, such as peripheral sensitization (increased sensitivity of primary sensory neurons) and central sensitization (increased sensitivity of SCDH and brain neurons), which underlie heightened pain sensitivity after injuries (reviewed in Ji and Woolf, 2001; Bhave and Gereau, 2004; Obata and Noguchi et al., 2004).

Surprisingly, nerve injury or spinal cord injury induces a profound activation of MAPKs in glial cells in the spinal cord. For example, peripheral nerve injury and spinal cord injury activate p38 and ERK in spinal microglia (Jin et al., 2003; Tsuda et al., 2004; Zhuang et al., 2005; Hains and Waxman, 2006). Nerve injury also activates JNK in astrocytes (Ma and Quirion, 2002; Zhuang et al., 2006). Interestingly, nerve injury activates ERK in microglia and astrocytes in the early-phase (days) and late-phase (weeks), respectively. Importantly, activation of MAPKs in glial cells is necessary for the development and maintenance of neuropathic pain. Although previous studies focused on the responses of neurons and neuronal-

specific mechanisms of hypersensitivity and chronicity of pain, accumulating evidence indicates a critical role of spinal glial cells in the pathogenesis of pain (Watkins et al., 2001; Deleo et al., 2004; Ji and Strichartz, 2004). We will review the evidence showing that MAPKs are critical signaling molecules in glia that can link the activation of multiple glial receptors and production of pronociceptive mediators (Ji and Strichartz, 2004).

ERK5 is a new member of the MAPK family and is activated by MEK5 (Zhou et al., 1995), and MEK5 is activated by MEKK2/3 (Nishimoto and Nishida, 2006). ERK5 (p115) is quite different from ERK1 (p44) and ERK2 (p42) in terms of molecular weight and is also known as big mitogen-activated kinase 1 (BMK1). ERK5 has a unique long carboxy-terminal domain with transcriptional activity that is required for maximum activation of MEF2 (myocyte enhancer factor 2) (Nishimoto and Nishida, 2006). However, ERK5 also shares high homology in the amino-terminal kinase domain with ERK1/2 and contains the Thr-Glu-Tyr (TEY) motif in the activation loop similar to ERK1/2. Nerve injury was shown to activate ERK5 in spinal micorglia, and further, intrathecal injection of ERK5 antisense oligodeoxynucleotides attenuates neuropathic pain (Obata et al., 2007).

Although MAPKs regulate pain sensitization via both peripheral and central mechanisms, we will sharpen our focus on central mechanisms of MAPKs in this review. In particular, we will focus on how ERK regulates central sensitization.

2. ERK and pain

ERK1 (p44 MAPK) and ERK2 (p42 MAPK) have high homology and both are activated by upstream kinase MEK1 and MEK2. Because (1) ERK1 and ERK2 are often activated together, and (2) the MEK inhibitors (e.g., PD98059 and U0126) and phosphoERK (pERK) antibodies do not distinguish between ERK1 and ERK2, we refer ERK1/2 as “ERK” in this review. ERK was originally identified as a primary effector of growth factor receptor signaling, a cascade that involves sequential activation of Ras, Raf, MEK, and ERK (Fig. 1). However, the activation of the ERK cascade is not restricted to growth factor signaling. ERK is activated by persistent neural activity and pathological stimuli (Ji and Woolf, 2001). A growing body of evidence demonstrates an involvement of ERK in neuronal plasticity, such as learning and memory (Impey et al., 1999) and pain hypersensitivity (see below).

2.1. Nociceptive activity-dependent activation of ERK in SCDH neurons

An early study showed that ERK activation (phosphorylation) in spinal cord dorsal horn (SCDH) neurons depends on nociceptive activity (Ji et al., 1999). In non-stimulated naïve animals, very few pERK-positive cells are present in the SCDH. Intraplantar injection of the C-nociceptor activator capsaicin induces a remarkable ERK phosphorylation in SCDH neurons in rats. pERK induction is very rapid (<1 min) after C-fiber activation and demonstrates accurate spatial distribution in that most pERK-immunolabeled neurons are found in the medial part of the superficial SCDH where primary nociceptive afferents from the hindpaw terminate (Fig. 2a). pERK is only induced by noxious stimuli, such as thermal (noxious heat and cold) and noxious mechanical (pinprick) stimuli, but not by innocuous stimuli (e.g., light touch) (Ji et al., 1999). pERK induction is also intensity-dependent: the number of labeled neurons increases following increases in noxious temperature from 45 to 55°C (Ji et al., 1999). It appears that the duration of noxious stimulation is also important; a very brief noxious stimulation (< 10 seconds) may not be sufficient to activate ERK (Wei et al., 2006), although this stimulation is sufficient to induce c-Fos expression in SCDH neurons (Hunt et al., 1987). Thus, ERK is selectively activated by conditions that may cause persistent changes in pain sensitivity.

pERK is also induced in SCDH neurons by intense and persistent noxious input, produced by hindpaw inflammation with formalin (Karim et al., 2001), complete Freund’s adjuvant (CFA,

Ji et al., 2002a; Adwanikar et al., 2004), scorpion BmK venom (Pang et al., 2008), by chronic bladder inflammation (Cruz et al., 2005a), and by monoarthritis in the ankle (Cruz et al., 2005b), as well as by fracture of the femur (Jimenez-Andrade et al., 2007). ERK is also activated in medullary and upper cervical cord neurons following noxious tooth pulp stimulation (Shimizu K et al., 2006).

Although ERK is only activated by high-threshold mechanical stimulation (i.e. activation of C-fibers and Aδ-fibers) in normal conditions, this rule changes after injuries. Thus, low-threshold electrical stimulation (Wang et al., 2004) and tactile stimulation (Hao et al., 2005) can lead to ERK activation in SCDH neurons after nerve injury. Movement of the inflamed joint but not non-inflamed joint also increases pERK in SCDH neurons (Cruz et al., 2005b). Nevertheless, even under injury conditions, ERK activation still depends on nociceptive activity, because low-threshold stimulation elicits pain in injury conditions. These studies indicate that pERK may play an important role in the development of neural plasticity in the spinal cord that underlies the development of tactile allodynia or movement-induced pain, an important feature of chronic pain.

ERK can also be activated in SCDH neurons in an *ex vivo* preparation. In this preparation, an isolated spinal cord slice is prepared with an attached dorsal root so that different types of afferent fibers can be electrically stimulated (Ji et al., 1999; Lever et al., 2003). Consistent with *in vivo* findings, pERK is increased by high-threshold C-fiber and Aδ-fiber stimulation, but not by low-threshold Aβ-fiber stimulation (Ji et al., 1999). C-fiber stimulation can also be mimicked by bath application of capsaicin to spinal slices, which will activate TRPV1 receptors in presynaptic C-fiber terminals to stimulate the release of neurotransmitters acting on postsynaptic receptors, leading to a strong ERK activation in superficial SCDH neurons in spinal slices (Kawasaki et al., 2004, Yanagidate and Strichartz, 2006). This *ex vivo* slice preparation is also convenient to test spinal analgesics using capsaicin-induced pERK as an indicator of central pain activation (Kawasaki and Ji, 2006). Finally, it is worthwhile to mention that in the presence of bicuculline, a GABA-A receptor antagonist, low-threshold electrical stimulation of Aβ fibers is able to induce robust ERK activation in superficial SCDH neurons *ex vivo*, although neither Aβ-fiber stimulation nor bicuculline alone produces significant ERK activation (Baba et al., 2003). Thus, after removal of inhibition (disinhibition), low-threshold stimulation activates ERK in superficial dorsal horn neurons. Since disinhibition also occurs in conditions such as nerve injury (Moore et al., 2002; Coull et al., 2003), this mechanism may account for ERK activation by low-threshold stimulation after nerve injury.

2.2. ERK activation following sensory fiber transmission and activation of various types of postsynaptic receptors in SCDH neurons

The strength of the first synapse formed by the central terminals of primary sensory neurons and SCDH neurons is plastic and modifiable. Following tissue injury, inflammation or nerve injury, multiple changes occur not only peripherally leading to increased primary afferent input (peripheral sensitization), but also centrally leading to hyperactivity of SCDH neurons (central sensitization). The large majority of primary afferents making synaptic contacts in the SCDH, regardless of whether they are myelinated or unmyelinated, contain glutamate, which is released with activity. Glutamate exerts excitatory effects via activation of three distinct receptor classes, the AMPA/Kainate, NMDA, and G-protein linked metabotropic postsynaptic receptors leading to depolarization of spinal neurons.

Specific to nociceptive transmission is the co-release of peptides such as substance P (SP) and calcitonin gene-related peptide (CGRP). Furthermore, the neurotrophin brain derived neurotrophic factor (BDNF), which is constitutively expressed by a subpopulation of sensory neurons, can be co-released with glutamate and SP following a specific pattern of activation of primary afferent fibers (Lever et al., 2001). SP, which plays an important role in nociceptive

transmission, acts preferentially at the G-protein coupled neurokinin-1 (NK1) receptors. Notably, in the dorsal horn NK1 expressing laminae I-III neurons are the first neurons in the central nociceptive pathways from the periphery to the brain. These NK1-expressing neurons are not thought to make extensive local connections within the spinal cord but rather to be predominantly nociceptive-specific projection neurons which terminate extensively within the parabrachial area of the brainstem with limited termination in the periaqueductal gray area, thalamus and reticular formation (Suzuki et al., 2002).

In the *dorsal horn-with dorsal root attached preparation* electrical stimulation of the dorsal roots at high intensity, in order to recruit nociceptive-unmyelinated fibers, evokes release of glutamate, SP and BDNF in dorsal horn superfusates (Lever et al., 2001) (Fig. 3) as well as concomitant activation of postsynaptic neurons and intracellular events. Specifically, phosphorylation of the NR1 subunit of the NMDA receptor, internalization of NK1 receptors and phosphorylation of ERK were all observed (Lever et al., 2001; 2003) (Fig. 3).

Studies from different laboratories have shown that glutamate transmission via NMDA receptors is essential for ERK activation in SCDH neurons (Ji et al., 1999; Lever et al., 2003; Wei et al., 2006). AMPA/Kainate receptors were also implicated in ERK activation in SCDH neurons after C-fiber stimulation (Kawaskai et al., 2004) and glutamate stimulation (Wei et al., 2006) in spinal cord slices, but see Lever et al. (2003). Karim et al. first reported that group I metabotropic glutamate receptors (mGluR1 and mGluR5) are required for inflammation-induced pERK in SCDH neurons (Karim et al., 2001). The essential role of mGluR1/5 in ERK activation has been confirmed in isolated spinal cord slices following high-threshold electrical stimulation (Lever et al., 2003) and capsaicin application (Kawasaki et al., 2004). Furthermore, pharmacologic activation of mGluR5 in the spinal cord leads to ERK activation and ERK-dependent modulation of excitability of superficial dorsal horn neurons, which are known to be involved in nociceptive transmission (Hu et al., 2007).

Bath application of SP to spinal cord slices or intreathecal injection of SP is sufficient to increase pERK staining in SCDH neurons, especially in lamina I neurons (Kawasaki et al., 2004; Wei et al., 2006). pERK was also found in NK-1 containing neurons in the lamina I (Ji et al., 2002a). Polgar et al. (2007) also observed scattered pERK-immunoreactive (IR) neurons in the deep dorsal horn (laminae III and IV) following different types of noxious stimuli. Interestingly, virtually all of the lamina III/IV NK1-IR neurons contain active ERK (pERK) (Polar et al., 2007). Exposure to NK-1 antagonist produces a mild but significant inhibition (around 30%) in the number of pERK-IR neurons in laminae I-II following capsaicin stimulation (Kawasaki et al., 2004; Wei et al., 2006). However, Lever et al. (2003) failed to detect any reduction of the neuronal pERK-IR induced by high-intensity electrical stimulation of dorsal root either following incubation with NK1 antagonists or in the dorsal horn of NK1 knockout mice as compared to wild type mice. This discrepancy may result from different stimulation conditions and different degrees of NK-1 inhibition. Nevertheless, ERK activation in NK-1-IR projection neurons could be important for pain plasticity.

The TrkB receptor for BDNF is widely expressed in the SCDH and can be rapidly phosphorylated by endogenous BDNF released from the central terminals of primary afferent fibers activated by the application of noxious stimuli in the periphery (e.g. intraplantar capsaicin) (Pezet et al., 2002). BDNF activation of TrkB contributes by approximately 40% to the increase in pERK in dorsal horn neurons following peripherally applied noxious stimuli (Pezet et al., 2002) or bath application of capsaicin in spinal slices *ex vivo* (Kawasaki et al., 2004). Sequestering BDNF *in vivo* with a TrkB-IgG fusion molecule significantly reduces the activation of ERK evoked by noxious stimulation (Pezet et al., 2002). Further, BDNF deletion in DRG nociceptive neurons in conditional null animals results in significant reduction of pERK in the dorsal horn, associated with a reduction of the second phase of pain like behavior

in the formalin test (Zhao et al., 2006). Interestingly, pERK staining is increased in spinothalamic tract (STT) projection neurons following intraplantar injection of capsaicin. Microinjections of BDNF into the spinal cord or release of endogenous BDNF activate ERK in TrkB-IR STT neurons (Slack et al., 2005).

Peripheral nerve injury occurs as a result of tissue incision and, perhaps, prolonged retraction during surgery. Local anesthetics are often applied to the neuraxis, by epidural or intrathecal routes, to blunt the pain of surgery and to minimize the consequences of central pain sensitization by surgical trauma. Studies on the effects of a frequently used local anesthetic, bupivacaine, on the activation of ERK in isolated spinal cord showed that it's action was virtually exclusively on ionotropic receptors (Yanagidate and Strichartz, 2006). Activation of ERK via stimulation of presynaptic capsaicin receptors (TRPV1) could be suppressed by bupivacaine, as could activation by postsynaptic NMDA and AMPA receptors, whereas ERK activation by SP (NK-1 receptors) or by mGluR1/5 were unaffected by bupivacaine. Direct activation of ERK, by a phorbol ester, for example, was insensitive to bupivacaine. Although some GPCRs are known to be inhibited by local anesthetics, those involved in ERK activation in SCDH neurons do not seem to be among them (Yanagidate and Strichartz, 2007).

Skin incision has been shown to activate ERK within 10 min (unpublished observation), and sub-cutaneous bupivacaine, injected under the surgical site before the incision, suppresses primary allodynia and virtually abolishes secondary allodynia and hyperalgesia (Duarte et al., 2005). It was shown that the secondary effects resulted from systemic bupivacaine, and has also been demonstrated that secondary tactile allodynia requires transmission through spinal Ca^{2+}-permeable AMPA/kainate receptors (Pogatzki et al., 2003). Interestingly, transmission through NMDA receptors was strongly linked to primary hyperalgesia and only weakly contributed to secondary mechanical sensitivity (Pogatzki et al., 2003). Similarly, systemic bupivacaine was much more effective on secondary than on primary hyperalgesia (Duarte et al., 2005). Combining the in vitro studies of spinal ERK activation with the behavioral studies after incision suggests that both neuraxial and circulating local anesthetics are able to reduce post-incisional spinal sensitization by preventing ERK activation by AMPA/kainate receptors. These results do not mean that other MAPKs are not also inhibited by bupivacaine's actions.

It is also noteworthy that primary afferent-evoked activation of spinal ERK may also require input from an excitatory 5-HT descending pathway. Thus, depletion of spinal 5-HT by intrathecal 5,7-DHT, a serotonergic neurotoxin, reduces formalin-evoked increase in pERK in the dorsal horn. Further, Ondansetron (a 5-HT3 receptor antagonist) at intrathecal doses that inhibit formalin-induced flinching also attenuates spinal ERK activation (Svensson et al., 2006). Nevertheless, ERK certainly can be activated by primary afferent stimulation in isolated spinal cord slices, in the absence of descending influences. Thus, the actual contribution of descending pathways needs further investigation.

2.3. ERK activation integrates multiple signaling pathways in SCDH neurons

Activation of different protein kinases appears to converge on ERK activation in SCDH neurons. ERK was initially known to be activated by growth factors with their receptors being tyrosine kinases such as TrkB. So it is not surprising that BDNF can activate ERK. But protein kinase A (PKA) and protein kinase C (PKC) appear to play a more important role in ERK activation in SCDH neurons. Hu et al. (2003) showed that ERK integrates PKA and PKC signaling to modulate A-type potassium channels (see more discussion below) and neuronal excitability (Hu and Gereau, 2003). Activation of PKA by forskolin or activation of PKC by PMA produces robust ERK activation. So far, the strongest ERK activation observed in SCDH neurons is induced by a combined application of forskolin and PMA (Kawasaki et al., 2004). Conversely, inhibition of either PKA or PKC can almost completely block capsaicin-induced pERK in spinal slices (Kawasaki et al., 2004). Wei et al. (2006) demonstrate that two of the

Ca^{2+}-depenednet adenylyl cyclases, AC1 and AC8 are required for pERK induction by glutamate and capsaicin, in support of the cAMP/PKA pathway for ERK activation. This pathway can be coupled to the Raf/Ras/ERK pathway via a small G-protein Rap (Impey et al., 1999, Fig. 4). PKC may be coupled to the ERK pathway via another kinase, Src, which is also required for C-fiber-induced ERK activation (Kawasaki et al., 2004). Increased intracellular Ca^{2+}following activation of ionotropic and metabotropic glutamate receptors is very critical for ERK activation via several Ca^{2+}-dependent enzymes, such as AC1/8 and PKC. BDNF and Ca^{2+} may activate another kinase, phosphatidylinositol 3-kinase (PI-3K) that will also lead to the activation of ERK in dorsal horn neurons (Pezet et al., 2008). However, the Ca^{2+}-/calmodulin-dependent kianse II (CaMK-II) has little influence on ERK activation (Kawasaki et al., 2004). Taken together, convergence of multiple signal pathways to ERK activation indicates a critical role of ERK in signal transduction in SCDH neurons.

2.4. ERK activation in SCDH neurons contributes to central sensitization and inflammatory pain

Activation of ERK in SCDH neurons is associated with the activation of nociceptive specific sensory fibers and promotes intracellular events that contribute to central sensitization, which can manifest at both behavioral and cellular levels (Woolf and Salter, 2000). ERK activation in SCDH neurons contributes to both behavioral and cellular characteristics of central sensitization (see below). The functional studies of ERK are largely based on two specific MEK inhibitors PD98059 (Alessi et al., 1995) and U0126 (Favata et al., 1998). Inhibition of ERK activation by MEK inhibitors can be readily confirmed by reduction in pERK levels.

Injection of diluted formalin into a hindpaw elicits two-phase nociceptive responses, and the second phase nocicpetive responses are thought to result from central sensitization (Dickenson and Sullivan, 1987; Coderre and Malzack, 1992). Intrathecal injection of the MEK inhibitor PD98059 blocks the central sensitization-mediated second phase of the painful response to formalin injection in rats (Ji et al., 1999) and mice (Karim et al., 2001). These pharmacological studies were further confirmed by a genetic approach: MEK dominant negative mutant mice in which MEK function is suppressed exclusively in neurons show decreased second phase responses in the formalin model (Karim et al., 2006). Intraplantar injection of capsaicin produces both primary and secondary mechanical hypersensitivity, and the secondary mechanical hypersensitivity also results from central sensitization (Torebjork et al., 1992). The MEK inhibitor U0126 prevents capsaicin-induced secondary mechanical allodynia (Kawasaki et al., 2004). Furthermore, intrathecal injection of MEK inhibitors has been shown to inhibit inflammatory heat hyperalgesia and mechanical allodynia following hindpaw injection of CFA (Ji et al., 2002a; Adwanikar et al., 2004); bee venom (Yu and Chen, 2005), and scorpion venom (Pang et al., 2008), after joint monoarthritis (Cruz et al., 2005), and in a model of inflammatory visceral pain (Galan et al., 2003).

It has been proposed that “memory of pain” may underlie chronic pain development (Sandkuhler, 2000; Ji et al., 2003; Malcangio and Lessmann, 2003). It is believed that long-term potentiation (LTP) in hippocampal neurons is a cellular mechanism of memory. LTP is also induced in dorsal horn neurons following intense noxious stimulation (Sandkuhler, 2000) and is regarded as an important feature of central sensitization (Ji et al., 2003). Xin et al. (2006) have shown that ERK is activated in SCDH neurons following the induction of spinal LTP, and further, MEK inhibitor can block spinal LTP when applied before LTP induction and 15 min after LTP inducing stimuli. Activation of ERK is also required for the induction of LTP in superficial dorsal horn neurons in a spinal cord slice preparation *ex vivo* (Wei et al., 2006). Windup is a rapid and transient form of central sensitization evoked during repetitive C-fiber stimulation, which may also be mediated by ERK (Fukui et al, 2007).

NMDA receptors play an essential role in the induction of central sensitization (reviewed in Woolf and Salter, 2000). Interestingly, ERK is required for noxious stimulation-induced phosphorylation of the NR1 subunit (Slack et al., 2004). Recently, Kohno et al. (2008) have shown that ERK is critical for bradykinin-induced enhancement of NMDA currents in lamina II spinal neurons. While NMDA receptors are critical for synaptic plasticity, AMPA receptors are essential for synaptic transmission. Painful stimulation induces trafficking and insertion of AMPA receptor GluR1 subunits to the plasma membrane of SCDH neurons (Galan et al., 2004), and activity-dependent insertion of AMPA subunits requires activation of the MEK/ERK pathway (Qin et al., 2005). ERK is also required for bradykinin-induced enhancement of NMDA currents in lamina II spinal neurons (Kohno et al., 2008).

While posttranslational regulation is sufficient to induce central sensitization, transcriptional regulation is important to maintain central sensitization. In particular, the transcription factor cAMP-response element binding protein (CREB) is pivotal for long-term neuronal plasticity in both hippocampal and dorsal horn neurons (Ji et al., 2003). CREB maintains long-term neural plasticity by inducing gene transcription and by forming new synapses (Lonze and Ginty, 2002). ERK activation is essential for CREB phosphorylation in SCDH neurons after noxious stimulation (Ji and Rupp, 1997; Kawasaki et al., 2004) and during LTP induction (Xin et al., 2006). ERK is required for inflammation-induced up-regulation of prodynorphin and NK-1 (Ji et al., 2002a). CREB is likely to induce the transcription of NK-1 and prodynorphin, as well as other pronociceptive genes such as Cox-2, TrkB, and BDNF (Lonze and Ginty, 2002; Ji et al., 2003; Fig. 4).

2.5. ERK regulation of Kv4.2 potassium channel in the spinal cord

The evidence described above clearly implicates ERK activation in the induction and maintenance of inflammatory pain. The regulation of gene expression as described above can explain central sensitization at late timepoints. However, the rapid induction of ERK activation following noxious stimulation or receptor activation is associated with rapid induction of acute central sensitization that cannot be explained by alterations in gene expression. Studies have demonstrated that ERK activation leads to increases in excitability of spinal superficial dorsal horn neurons (Hu and Gereau, 2003). This increase in excitability is mediated by a reduction in transient outward (A-type) potassium currents in these neurons, and genetic evidence indicates that these currents are largely carried by channels composed of Kv4.2 (Hu et al, 2003; Hu et al, 2006). Thus, ERK-dependent inhibition of A-type currents and increases in excitability of dorsal horn neurons are absent in spinal cords from Kv4.2-/- mice (Hu et al 2006). Consistent with a role for this ERK-dependent modulation of Kv4.2 in the acute phase of central sensitization, mechanical sensitization following formalin- or carrageenan-induced inflammation are absent in Kv4.2-/- mice compared to wild-type littermate mice (Hu et al, 2006). This modulation of Kv4.2-mediated A-type K+ currents, which involves direct phosphorylation of Kv4.2 at S616, can also explain sensitization and pain-related behaviors induced by direct activation of spinal mGluR5 (Hu et al, 2007). The studies described above clearly implicate ERK activation and downstream modulation of Kv4.2-containing K+ channels by direct phosphorylation in mediating the rapid acute phase central sensitization.

2.6. ERK activation in amygdala neurons and inflammatory pain

The studies above have largely focused on ERK activation in the spinal cord and its role in central sensitization. However, ERK activation in the context of pain is not limited to the spinal cord. For example, ERK activation has been shown to occur in the central nucleus of the amygdala several hours after formalin-induced inflammation of the hind paw (Carrasquillo and Gereau, 2007). This ERK activation is temporally correlated with the induction of mechanical hypersensitivity in the paw contralateral to the inflamed paw. Furthermore, inhibition of this ERK activation completely eliminates this contralateral sensitization and

significantly reduced sensitization in the inflamed paw, suggesting that amygdala ERK activation contributes to generalized sensitization following inflammation (Carrasquillo and Gereau, 2007). Interestingly, the ERK activation observed in the CeA was observed only in the right CeA (and not the left), whether inflammation was induced in the right or left paw (Carrasquillo and Gereau, 2007). This hemispheric lateralization was subsequently demonstrated to be functionally significant, as MEK inhibitor administered in the right CeA, but not the left CeA significantly reduced tactile hypersensitivity following formalin injection in both the right or left hindpaw, whereas inhibition of ERK activation in the left CeA had no effect on this sensitization (Carrasquillo and Gereau, 2008). The central nucleus of the amygdala (CeA) is known to receive nociceptive-specific inputs, and sensitization of neurons in the CeA has been demonstrated following the induction of arthritis (Neugebauer et al., 2004; Han et al., 2005) and nerve injury (Ikeda et al., 2007). The extent to which CeA ERK activation contributes to this sensitization is not yet known, however clearly ERK activation in the CeA mediates a component of central sensitization in an intermediate phase of sensitization in the hours shortly after inflammation. A recent study has confirmed an important role of ERK in the CeA for pain-related synaptic plasticity and behavior (Fu et al., 2008).

2.7. ERK activation in spinal glial cells and neuropathic pain

Many studies reviewed above suggest that pERK is predominantly expressed in SCDH neurons after noxious stimulation and tissue inflammation and contributes importantly to the induction and maintenance of inflammatory hyperalgesia. However, after nerve injury, the pattern of ERK activation in the spinal cord is quite different. Ji et al (1998) initially found that 2 days after sciatic nerve transection, pERK was increased all over the spinal cord (Fig. 2b). Because these pERK-IR cells did not look like neurons, they were ignored. At that time, it was difficult to accept that glial cells might play an important role in pain regulation, although the evidence began to accumulate (Meller et al., 1994;Watkins et al., 1997). In 2002, Ma and Quirion reported that partial sciatic nerve ligation induces ERK activation in spinal astrocytes, 3 weeks post-lesion (Ma and Quirion, 2002). Then, Cheng et al. (2003) showed that transection of the dorsal root induces ERK activation in spinal microglia at 24 and 48 hours after nerve injury. However, neither time-dependent ERK activation nor neuropathic pain behavior was examined in these studies.

In 2005, Zhuang et al. examined the pattern of ERK activation in the spinal cord at different time points (2, 10, and 21 days) after spinal nerve ligation (SNL) and further examined neuropathic pain behavior following intrathecal administration of the MEK inhibitor PD98059 at these time points. The following results have been obtained. (1) On day 2, pERK is increased in OX-42-IR microglia. (2) On day 21, pERK is increased in GFAP-IR astrocytes. (3) On day 10, pERK is increased in both microglia and astrocytes. (4) Intrathecal PD98059 at three separate time points can attenuate SNL-induced mechanical allodynia (Zhuang et al., 2005). These results suggest that sequential activation of ERK in microglia and astrocytes is important for the induction and maintenance of neuropathic pain, respectively. Zhuang et al. (2005) also investigated ERK activation immediately after nerve injury, from 10 min to 6 hours, and found that ERK is transiently activated in dorsal horn neurons. This initial neuronal activation of ERK is likely to result from nerve injury-induced surge of spontaneous discharge.

Katsura et al. showed that Src-family kinases are also activated in spinal microglia after SNL and contribute to the activation of ERK but not p38 in spinal microglia (Katsura et al., 2006). Recently, Tsuda et al. (2008) demonstrated that ERK activation is also induced in spinal microglia after streptozotocin (STZ)-induced diabetes, and intrathecal U0126 can suppress STZ-induced neuropathic pain (Tsuda et al., 2008). In addition to nerve injury, spinal cord injury also activates ERK in spinal microglia, and PD98059 reduces neuropathic pain after spinal cord injury (Zhao et al., 2007). Further, PD98059 inhibits the induction of

cyclooxygenase-2 in spinal microglia and spinal release of prostaglandin E2 (Zhao et al., 2007).

Collectively, studies described above indicate that ERK activation in the spinal cord plays an important role in the development and maintenance of both inflammatory pain and neuropathic pain, but via different cellular mechanisms. After nerve injury, ERK is essential for intracellular signaling in glial cells that lead to the production of proinflammatory and pronociceptive mediators. Compared to neuronal mechanisms, glial mechanisms of ERK for pain control are much less known.

3. p38 and pain

There are four different p38 isoforms: p38α, p38β, p38γ, and p38δ. p38α and p38β are two of the major isoforms in the mature nervous system, and their activated forms are recognized by commercial p-p38 antibodies. In particular, p38α is the most abundant isoform in the DRG and spinal cord (Ji et al., 2002b). p38 is typically activated by cellular stress and proinflammatory cytokines and plays a critical role in inflammatory responses. Systematic or intrathecal administration of p38 inhibitors has been shown to effectively alleviate inflammation and arthritis (Kumar et al., 2003; Boyle et al., 2006). The upstream kinases of p38 are MKK3/6 and the activated p38 is translocated to the nucleus, where it can phosphorylate transcriptional factors such as ATF-2 (Fig. 1). The biosynthesis of TNF-α and IL-1β, as well as many other inflammatory mediators is up-regulated by p38 (Kumar et al., 2003).

Unlike the very low level of basal pERK-IR, there is moderate basal p-p38-IR in the spinal cord. Nonetheless, SNL induces a very robust increase in p-p38 in the spinal cord: p-p38 begins to increase at 12 hours, reaches a peak at 3 days, but is maintained at elevated levels even after 3 weeks (Jin et al., 2003; Zhuang et al., 2006). In particular, different laboratories have shown that p-p38 is increased in spinal cord microglia after SNL (Jin et al., 2003; Tsuda et al., 2004; Katsura et al., 2006), spared nerve injury (Wen et al., 2007); partial sciatic nerve ligation (Clark et al., 2007), spinal cord injury (Hains and Waxman, 2006), and surgical incision (unpublished observation). Moreover, intrathecal infusion of p38 inhibitors attenuates neuropathic pain in different animal models (Jin et al., 2003; Milligan et al., 2003; Schafers et al., 2003; Tsuda et al., 2004; Hains and Waxman, 2006). Svensson et al. (2005a) demonstrated that p38β is expressed in spinal microglia and knockdown of p38β with antisense oligonucleotides prevents acute pain sensitization.

How is p38 activated in spinal cord microglia by nerve injury? Table I shows that activation of various types of receptors by multiple pronociceptive mediators can induce p38 activation in spinal cord microglia. After activation, p38 increases the synthesis of several proinflammatory mediators such as COX-2 (Svensson et al., 2003b), IL-1β (Ji and Suter, 2007), and iNOS (Sung et al., 2005) in the spinal cord possibly via transcriptional regulation. p38 activation also causes a rapid release of IL-1β from spinal microglia via posttranslational regulation. For example, lipopolysaccharide application to a spinal slice induces activation of p38 in spinal microglia and secretion of IL-1β. Further, IL-1β secretion is prevented by a p38 inhibitor (Clark et al., 2006). IL-1β contributes importantly to central sensitization by enhancing excitatory synaptic transmission, suppressing inhibitory synaptic transmission, and inducing CREB phosphorylation in SCDH neurons (Kawasaki et al., 2008b). p38 also activates phospholipase A2 (PLA2) via activating its downstream kinase MAPKAP-2 (MAPK activated protein kinase-2). The activation of PLA2 leads to the generation of arachidonic acid for prostaglandin production. Intrathecal application of a p38 inhibitor reduces PGE_2 release in the spinal cord after inflammation (Svensson et al., 2003a).

4. JNK and pain

Compared to ERK and p38, much less is known about how JNK regulates pain. JNK is also called stress-activated protein kinase (SAPK) and plays an important role in neurodegeneration and apoptosis (Gao and Ji, 2008). JNK has three isoforms: JNK1, JNK2, and JNK3. JNK3 is primary found in the brain and has different roles compared to JNK1 and JNK2. A critical role of neural-specific JNK3 for ischemic apoptosis has been demonstrated (Kuan et al., 2003). JNK1 and JNK2, but not JNK3 are heavily expressed in the spinal cord (Ji et al., 2006). However, for the active forms, pJNK1 (p46) is the predominant form in the spinal cord. Further, spinal pJNK1 levels increase after nerve injury (Daulhac et al., 2006; Zhuang et al., 2006).

Unlike the activation patterns of pERK and p-p38, increased pJNK-IR is primarily observed in spinal cord astroctyes. First, pJNK is persistently increased in spinal cord astrocytes at different times (1, 3, 10 and 21 days) after SNL (Zhuang et al., 2006), in support of a previous study showing increased pJNK in astroctyes 3 weeks after partial sciatic nerve injury (Ma and Quirion, 2002). Second, JNK1 is primarily expressed in spinal cord astrocytes (Zhuang et al., 2006; Katasura et al., 2008). Third, c-Jun, the major transcription factor downstream of JNK, is also activated in spinal astrocytes (Zhuang et al., 2006). Fourth, Transforming growth factor-activated kinase 1 (TAK1), a member of the MAPK kinase kinase family, is induced in spinal astrocytes and required for JNK1 activation following SNL (Katasura et al., 2008). Thus, JNK pathway is preferentially activated in spinal astrocytes. This astrocyte activation of the JNK pathway is also critical for the maintenance of neuropathic pain. Intrathecal infusion of the JNK inhibitor SP600125 attenuates neuropathic pain in the SNL model (Obata et al., 2004; Zhuang et al., 2006) and diabetic model (Daulhac et al., 2006). In particular, a peptide inhibitor of JNK, D-JNKI-1 (Borsello et al., 2003) is more potent than SP600125 in suppressing neuropathic pain (Zhuang et al., 2006).

The upstream mechanisms causing JNK activation in the spinal cord have begun to be revealed (Ji et al., 2006). The growth factor FGF-2 (fibroblast growth factor-2), which is up-regulated both in DRG neurons (Ji et al., 1995) and spinal cord astroctyes (Madiai, 2003) and important for neuropathic pain (Madiai et al., 2005), can induce persistent JNK activation in the spinal cord *in vivo* and in astrocytes *in vitro* (Ji et al., 2006). In contrast, TNF-α only induces a transient activation of JNK in astrocytes (Zhang et al, 1996). JNK activation by TNF-α in astrocytes leads to an up-regulation of several chemokines, such as monocyte chemoattractant protein-1 (MCP-1, Gao and Ji, unpublished observation). Accumulating evidence suggests that MCP-1 plays an important role in pain sensitization (White et al., 2007, Zhang et al., 2007), presumably via CCR2 receptor (Abbadie et al., 2001). MCP-1 is also up-regulated in spinal cord astrocytes after nerve injury, and induces central sensitization by enhancing excitatory synaptic transmission in superficial dorsal horn neurons (Gao and Ji, unpublished observation). Thus JNK may promote neuropathic pain via MCP-1 up-regulation.

5. Conclusions

Chronic pain is a major health problem worldwide. It is estimated that chronic pain could affect 20% of the population in the developed countries. Chronic pain results from neural plasticity manifesting as peripheral and central sensitization. Activation of glial cells will enhance and prolong neural plasticity. In the last several years, we have seen a dramatic increase in studies examining how the MAPK pathways regulate pain hypersensitivity in different injury conditions. These studies have greatly improved our understanding of neural and glial mechanisms of pain regulation. Because MAPK pathways are differentially activated in neurons and glia (microglia and astrocytes) under inflammatory pain and neuropathic pain conditions, pMAPKs (e.g., pERK, p-p38, and pJNK) can be used as molecular and cellular

markers for different pain conditions. These markers may also be applied to test the efficacy and mechanisms of new analgesics.

Finally, inhibitors of ERK, p38, and JNK have been shown to effectively alleviate inflammatory and neuropathic pain in different animal models. Those specific MAPK pathway inhibitors, such as PD98059 and SB203580 are very useful reagents for basic research but cannot be used as drugs for reasons of toxicity, pharmacology, or solubility. A number of diseases, including cancer, diabetes, and inflammation are associated with perturbation of MAPK signaling pathways. Multiple MAPK pathway inhibitors have been tested in clinical trails. For example, MEK inhibitors (e.g., CI-1040, PD0325901, and ARRY-142886) have been tested in patients with cancer, and p38 inhibitors (e.g., SB281838, BIRB0796, Ro320-1195, and SCIO-469) were in clinical trails for rheumatoid arthritis (reviewed in Ji et al., 2007). It is surprising to find that MEK inhibitors are well tolerated, given that the ERK cascade has been implicated in many cell functions including cell growth. It is therefore possible that the essential roles of the ERK cascade in cell proliferation and differentiation during development may be far less crucial in adults. D-JNKI-1 is a very potent inhibitor of JNK and shows great efficacy in an animal model of naturopathic pain (Zhuang et al., 2006) and in patients with acute acoustic trauma (Suckfuell et al., 2007). It remains to be tested whether these MAPK inhibitors are beneficial for patients with different pain conditions.

Acknowledgements

The work was supported in part by NIH DE17794 and NS54932 to RRJ, NS61294 and NS48602 to RWG, and Wellcome Trust to MM.

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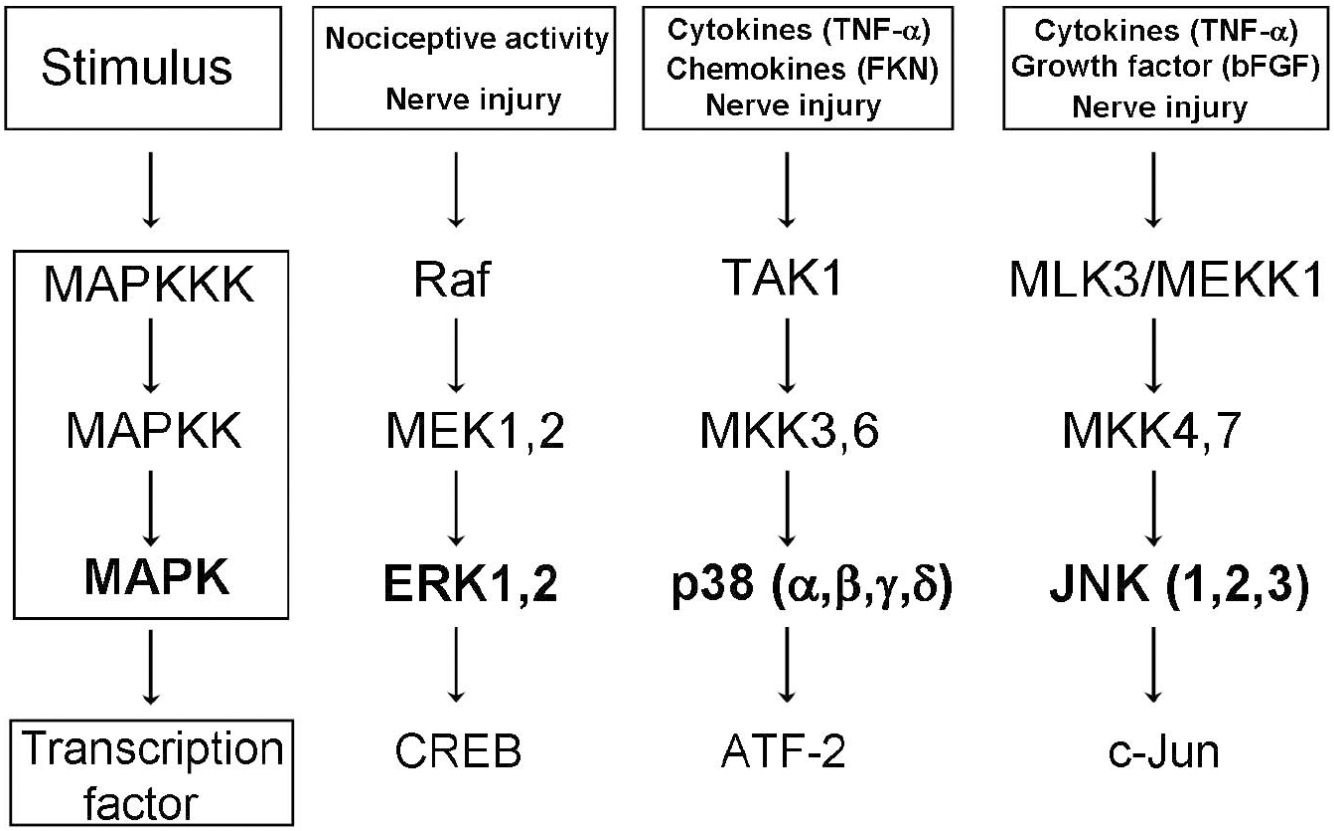

Figure 1.
Schematic of the ERK, p38, and JNK pathways and their upstream activators and downstream effectors. FKN, fractalkine; bFGF, basic fibroblast growth factor.

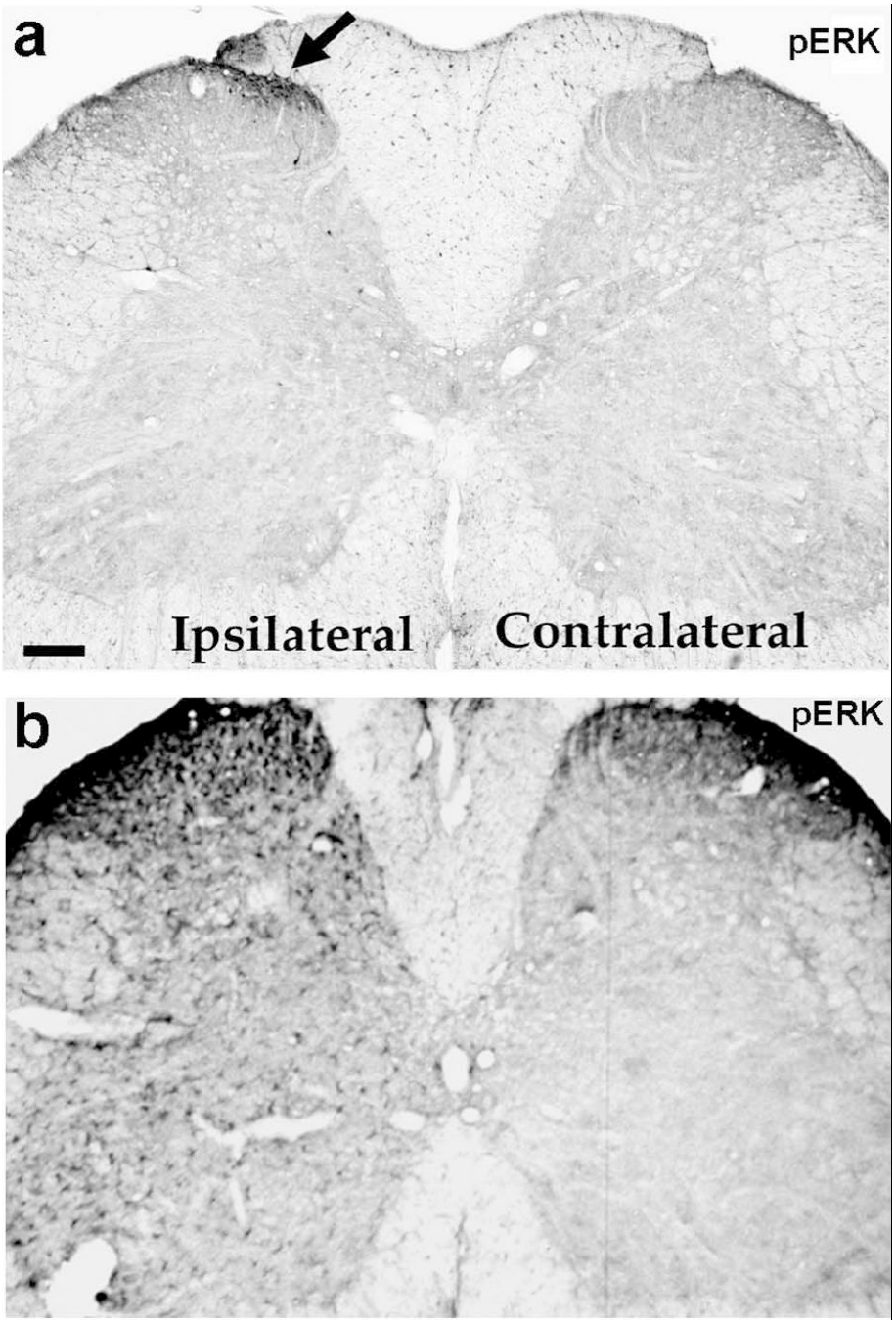

Figure 2.
Activation of ERK in the spinal cord after intraplantar capsaicin injection (a) and sciatic nerve injury (b). Rats were sacrificed 2 minutes after capsaicin injection (75 μg) (a) or 2 days after nerve transaction (b). The L5 spinal cords were collected for immunohistochemistry using pERK antibody. Arrow indicates pERK staining in the medial superficial SCDH on the ipsilateral side. Scale, 100 μm. Fig 2a is modified from Ji et al., 1999.

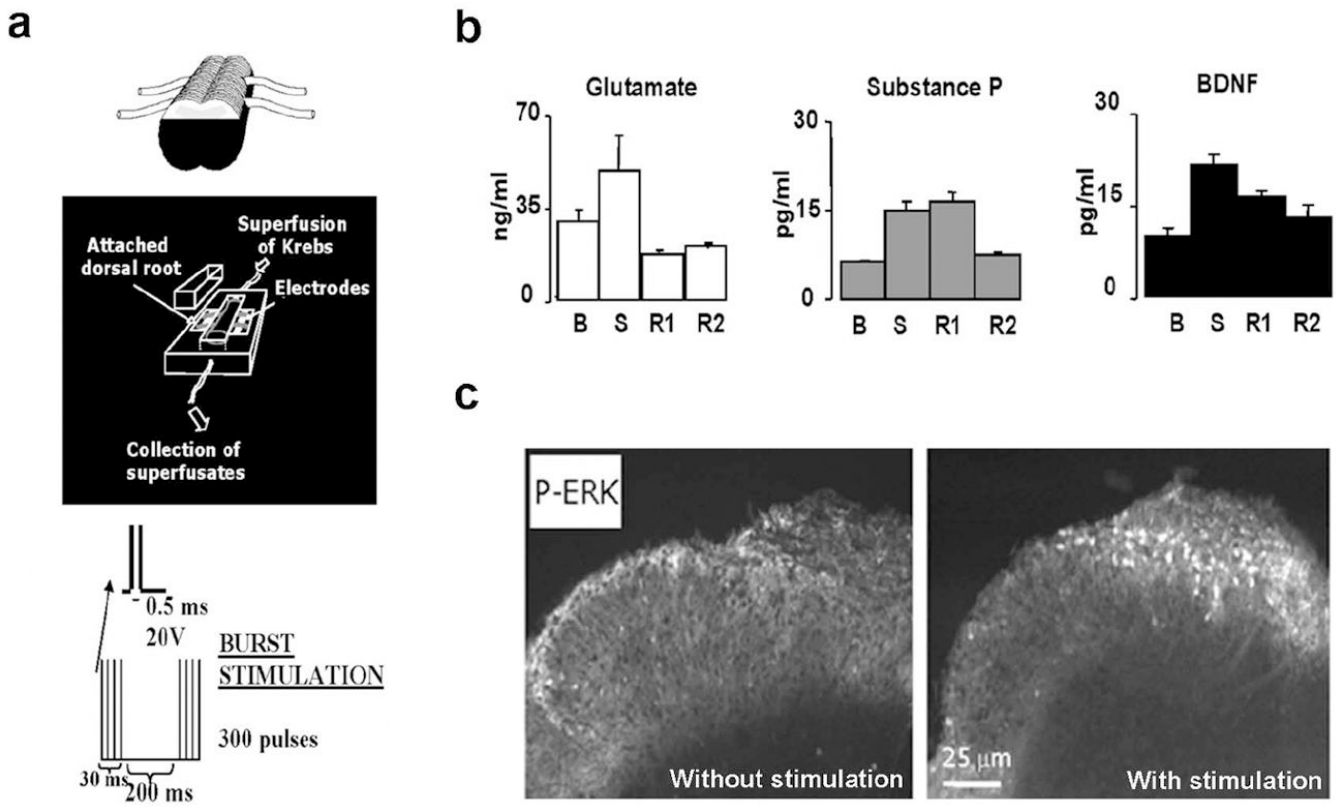

Figure 3.

High-intensity of electrical stimulation of the dorsal roots, in an isolated spinal cord segment (a) evokes release of glutamate, substance P and BDNF in dorsal horn superfusates (b), and phosphorylation of ERK in the superficial dorsal horn (c). B= basal levels in superfusates, S= levels following electrical stimulation; R1 and R2= levels in recovery periods after stimulation. Modified from Lever et al., 2001, 2003.

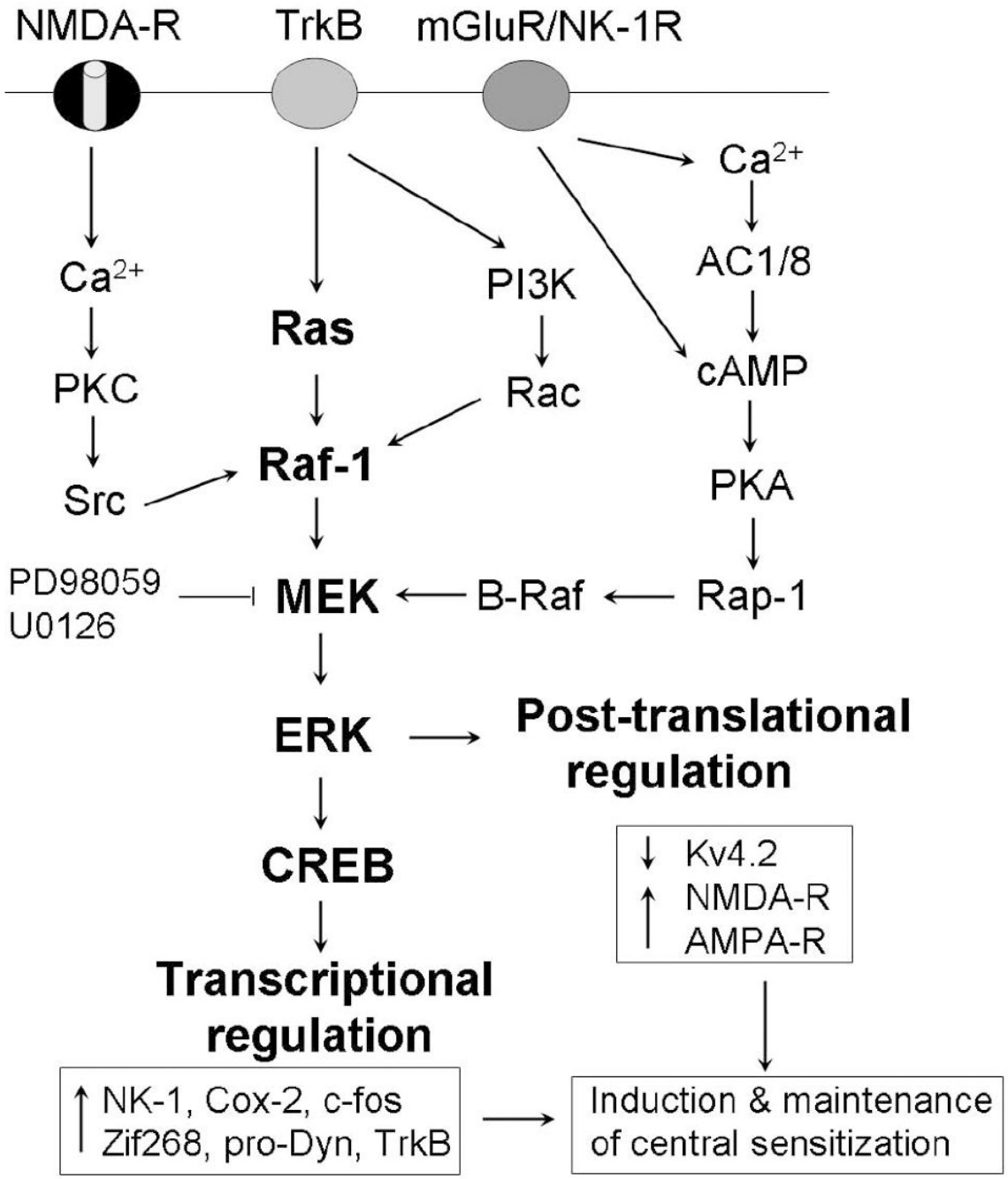

Figure 4.
Activation of the ERK pathway in SCDH neurons by various postsynaptic receptors and multiple protein kinases. Upon activation, ERK contributes to the induction and maintenance of central sensitization via post-translational and transcriptional regulation, respectively.

Table I

Multiple pain mediators and receptors that have been shown to activate p38 MAPK in the spinal cord.

Name of molecules	Regulation	References
Cytokines		
TNF-α	Positive	Svensson et al., 2005b
IL-1β	Positive	Sung et al., 2005
IL-4	Negative	Hao et al., 2006
IL-10	Negative	Zhou et al., 2008
Chemokines		
FKN (CX3CR1)	Positive	Zhuang et al., 2007
MCP-1 (CCR2)	Positive	Abaadie et al., 2003
ATP receptors		
P2X	Positive	Ikeda et al., 2007
P2Y12	Positive	Kobayashi et al., 2008
Other mediators		
NK-1 receptor	Positive	Svensson et al., 2003b
Inducible NOS	Positive	Tang et al., 2007
Cathepsin S	Positive	Clark et al., 2007
TLR-3	Positive	Obata et al., 2008
MMP-9	Positive	Kawasaki et al., 2008a