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Peripheral Formalin Injury Induces 2 Stages of Microglial Activation in the Spinal Cord

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Abstract: The formalin test produces 2 well-known acute phases of nociceptive behavior. Recently, we have shown that this same formalin test produces a third phase of nociceptive behavior consisting of prolonged thermal and mechanical hyperalgesia beginning days after formalin injection and lasting for at least 3 weeks. Here we investigated the activity of 3 MAPKs (p38, ERK and JNK) in the spinal dorsal horn following 5% formalin injection into rat hind paw. The p38 MAPK was rapidly activated in the spinal microglia minutes after injection and the activation persisted for 1 hour. In addition, this same injury induced a secondary increase of phospho-p38 expression in spinal microglia that was maximal 3 to 7 days postinjection. Intrathecal administration of p38 inhibitor SB203580 not only inhibited the early acute spontaneous nociceptive behaviors, but also inhibited the long-term formalin injury-induced mechanical hyperalgesia. Our results suggest that peripheral formalin injection induces 2 stages of microglial activation, and p38 activation in spinal microglia plays key roles in central pain modulation in formalin test respectively for the early acute phases and the late secondary long-term pain state as well.

***Perspective:** This article presents unique properties of spinal microglial activation in a pain animal model. This finding could potentially help clinicians to further understand the contributions of spinal microglia to acute and chronic pain state.*

***Key words:** Microglia, spinal cord, formalin, mitogen-activated protein kinases, p38.*

Pain hypersensitivity, ie, hyperalgesia and/or allodynia attributed to neural plasticity states in the dorsal root ganglion (DRG) and spinal cord, is a hallmark of neuropathic pain following peripheral inflammation and nerve lesions in humans. Recent studies implicate not only neurons, but glial cells (microglia and astrocytes) in the generation and maintenance of the pain hypersensitivity.[19,24,37,43,45,46] Both astrocytes and microglia are activated in the spinal cord in almost all animal pain models, including nerve injury, traumatic injury, inflammatory, and bone cancer pain models.[2,3,8,12,14,16,27,29,32,38] The enhanced nociceptive behaviors in these models could be reduced or blocked by drugs that disrupt glial activation.[15,20,24-26,31,39,45] While glial involvement in pain facilitation is now accepted, the mechanisms by which glia contribute to enhanced pain transmission is not fully understood. Several recent reports suggested that activation of mitogen-activated protein kinases (MAPKs) in glial cells is essential for the pathogenesis of exaggerated pain responses.[11,18,22]

MAPKs are a family of serine-threonine kinases that are part of the cellular signaling cascade activated by extracellular stresses or stimuli. These signaling molecules consist of extracellular signal-regulated kinase (ERK), p38, c-Jun N-terminal kinase (JNK), and ERK5. The involvement of ERK, p38, and JNK activation in neurons and glial cells in spinal cord induced by nerve injury or inflammation have been demonstrated recently,[19,37,43,49,50] with the notable exception of ERK5.[28] For example, ligation of the L5 spinal nerve resulted in activation (phosphorylation) of p38 in spinal microglia. This activation was required for the development of tactile allodynia that could be suppressed by intrathecal administration of the p38MAPK inhibitor SB203580.[19,43]

Subcutaneous formalin injection is widely used to study pain mechanisms and to evaluate the analgesic action of various endogenous and exogenous substances.

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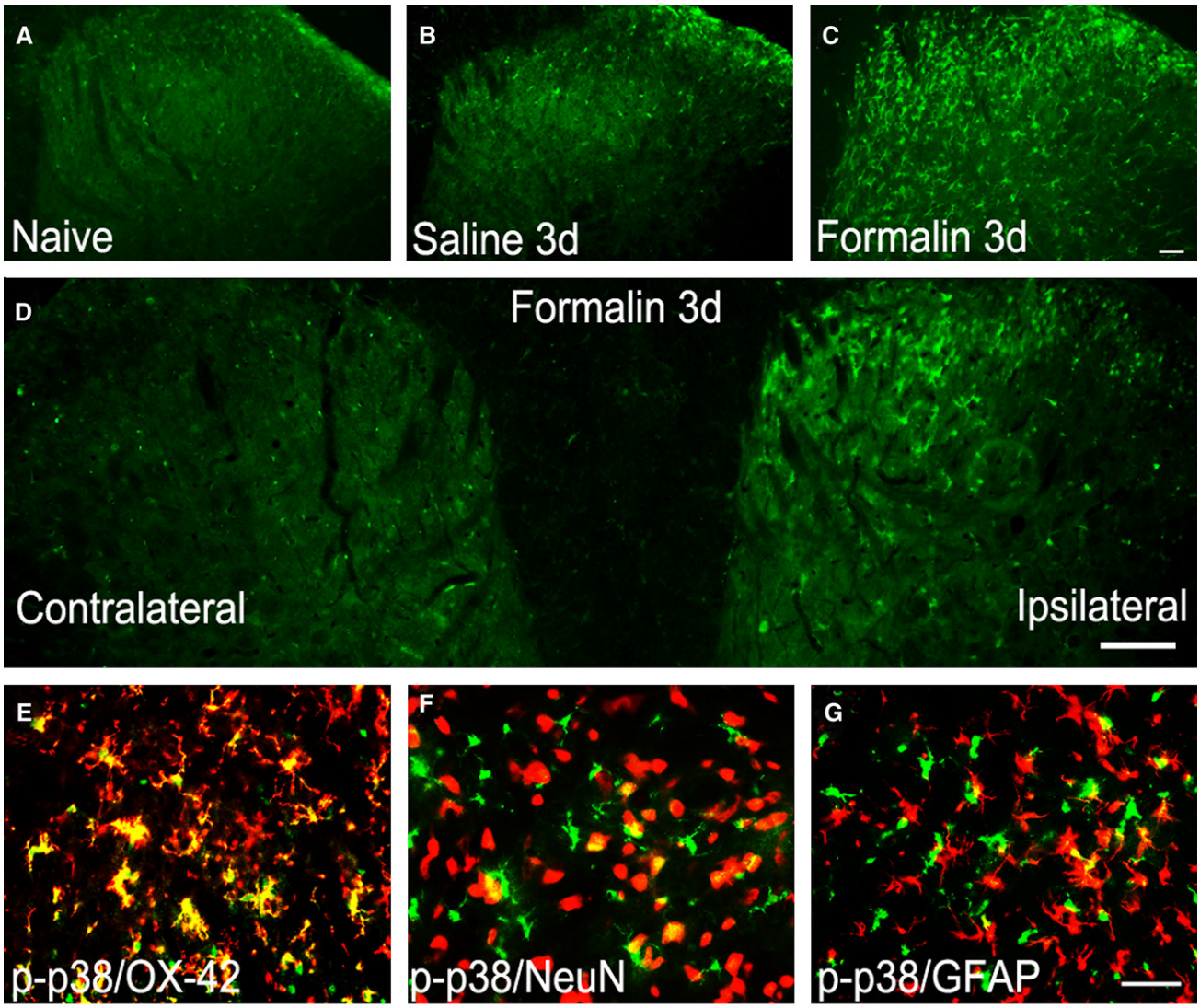
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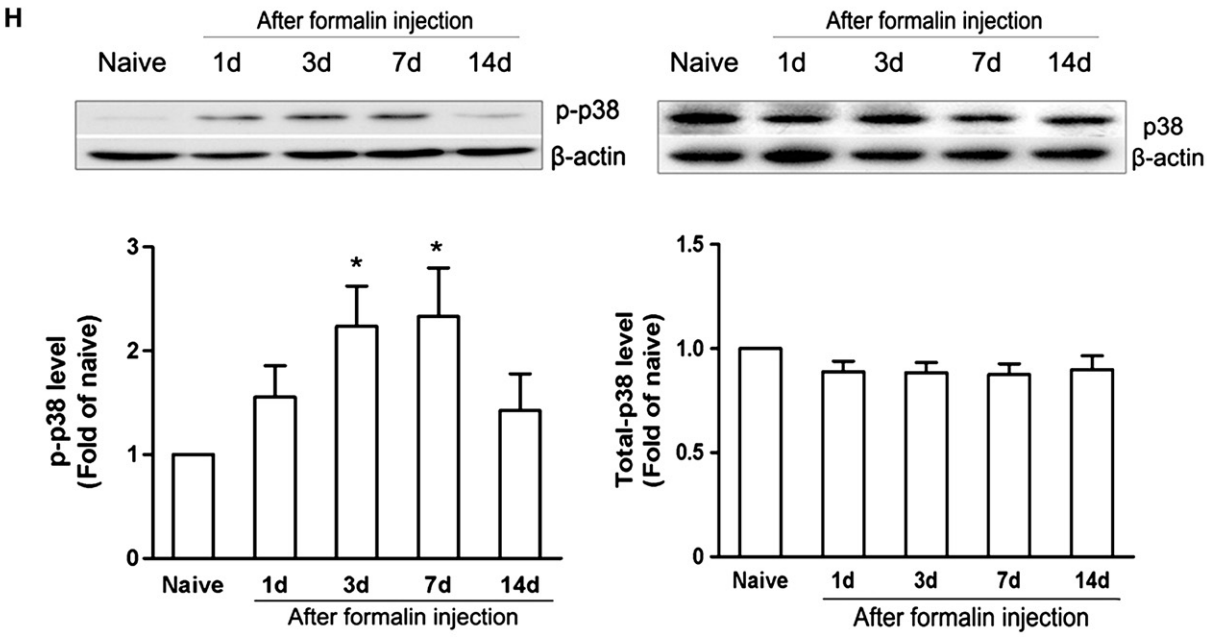
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3
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1
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Naive 1d 3d 7d 14d
After formalin injection
Total-p38 level (Fold of naive)
1.5
1.0
0.5
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Naive 1d 3d 7d 14d
After formalin injection

Injection of formalin into the rat's hind paw produces 2 well-known phases of nociceptive behavior. The first phase (acute phase) lasts about 5 minutes. After a short quiescent period, the second phase (tonic phase) begins and lasts approximately 40 minutes. As expected, formalin injection into the paw induced a rapid (5 minutes after injection) increase in phosphorylated p38 MAPK (p-p38) in spinal cord microglia, and pretreatment with p38 MAPK inhibitors suppressed the characteristic second (tonic phase) phase of nociceptive reflexes.[37] However, in addition to the 2 phases of spontaneous nociceptive behaviors, formalin-injected tissue injury produces a third phase of nociceptive behaviors characterized by secondary prolonged thermal and mechanical hyperalgesia beginning days after the injection and lasting for about 3 weeks.[9,34,40,44] This long-term time course was closely related to microglial activation in the spinal cord as determined by OX-42 labeling and morphological changes.[8] In the present study, we report that p38, but not ERK or JNK, was activated in microglia in the spinal dorsal horn days after injection and this late activation was maintained for weeks. Moreover, the activation of p38 was required for not only the early second phase of spontaneous nociceptive behaviors, but also for the third phase of the formalin-induced long-term mechanical hyperalgesia.

Methods

Animals and Treatments

Male adult Sprague-Dawley rats weighing 200 to 225 g (Vital River Laboratory Animal Technology Co. Ltd, Beijing) were used, and all protocols for the experiments were approved by the Animal Care and Use Committee of Peking University Health Science Center and certified that the care and use of animals conformed to applicable national/international guidelines. All rats were housed at temperature of 22 $\pm$ 1°C on a 12-hour light/dark cycle with free access to food and water. Experimental rats in the formalin model group received subcutaneous injections of 100 μL 5% formalin (diluted in 0.9% saline) into the plantar surface of the right hind paw. The control group rats were injected with 100 μL 0.9% saline instead of formalin or received no treatment. Survival times were 30 minutes, 60 minutes, 6 hours, 1 day, 3 days, 7 days, and 14 days postinjections, and lumbar spinal cord was taken for immunohistochemical and western blotting analysis.

Chronic lumbar intrathecal (it) catheters were implanted according to the procedure described by Størkson et al.[35] Briefly, under adequate anesthesia with sodium pentobarbital (40 mg/kg, ip), a polyethylene catheter (PE-10, 20 cm, Warner Instruments, Hamden, CT) was introduced in the subarachnoidal space via the L5/6 intervertebral space and advanced rostrally 3.0 to 3.5 cm in order to reach the lumbar enlargement. The catheter was sutured to the fascia, tunneled subcutaneously on the back of the rats, and its proximal end was externalized in the occipital region. Animals were allowed to recover for at least 5 days after implantation, and a rat that had signs of neural dysfunction (1 of 43) was removed from the study. The p38 inhibitor (SB203580; Calbiochem, La Jolla, CA) or vehicle was delivered intrathecally (with the same volume of 10 μL) and the catheter flushed with 12 μL of saline. SB203580 was dissolved in dimethylsulfoxide (DMSO), and diluted in 0.9% saline when used.

Behavioral Analysis

Acute Spontaneous Nociceptive Behavior

The animals were placed in a 30$\times$30$\times$30-cm clear plastic box with a mirror below the surface to allow an unobstructed view of the paws. To allow familiarization with surroundings, rats were habituated in the test chambers singly for 20 minutes for 3 days. On the test day, following 5% formalin injections, rats were returned to the observation chamber immediately, and flinching behaviors were monitored with blinded condition for 60 minutes. The flinches were recorded as the number of flinches for 5-minute periods and the total number of flinches during phase 1 (0–9 minutes) and phase 2 (10–60 minutes). SB203580 (10 μg, 30 μg – 6 rats) or vehicle (6% DMSO, diluted in saline – 6 rats—same concentrations of DMSO and saline as used for SB203580), was given 20 minutes prior to the formalin injection.

Secondary Chronic Mechanical Hyperalgesia

Animals received subcutaneous injections of 100 μL 5% formalin into the plantar surface of the right hind paw, 6 rats for each group. SB203580 (10 μg), or vehicle (2% DMSO) was first delivered intrathecally 20 minutes prior to the formalin injection, and repeated once a day at 10 AM until day 7. The mechanical threshold for nociceptive response was conducted as described previously,[33] and all tests were conducted before treatment and on day 1, 3, and 7 pre drug delivery under blind conditions. Briefly, the rat was habituated to standing on its hind paws and against the tester's gloved hand. The withdrawal threshold of the hind paw in response to mechanical stimulation was determined by using a hand-held force transducer (electronic anesthesiometer; IITC Life Science, Woodland Hills, CA) adapted with 0.5-mm diameter polypropylene rigid tip. The area tested was the

Figure 1. Peripheral formalin injection induced p38 activation in the lumbar spinal dorsal horn. **(A-C)**, Representative immunostaining images of p-p38 expression in the spinal dorsal horn from naïve, saline and formalin-injected group at day 3 after injection. Scale bar: 50 μm. **(D)**, p-p38 immunoreactivity increased in the ipsilateral side of the dorsal horn at day 3 after formalin injection. Scale bar: 100 μm. **(E-G)**, Merged images of double immunofluorescent labels of p-p38 (green) with markers of microglia (red, OX-42), neurons (red, NeuN), and astrocytes (red, GFAP), p-p38 was expressed in most microglia but rarely in neurons or astrocytes. Scale bars: 50 μm. **(H)**, Representative bands and quantification of Western blot analysis showed persistent increased p-p38 protein level in the lumbar spinal cord after formalin injection (left) while total p38 showed no increases (right). Quantification of p-p38 and total p38 level were normalized against a control protein, β-actin. *P < .05, 1-way ANOVA and Tukey post hoc test, compared with the naïve (uninjected) control, n = 4.

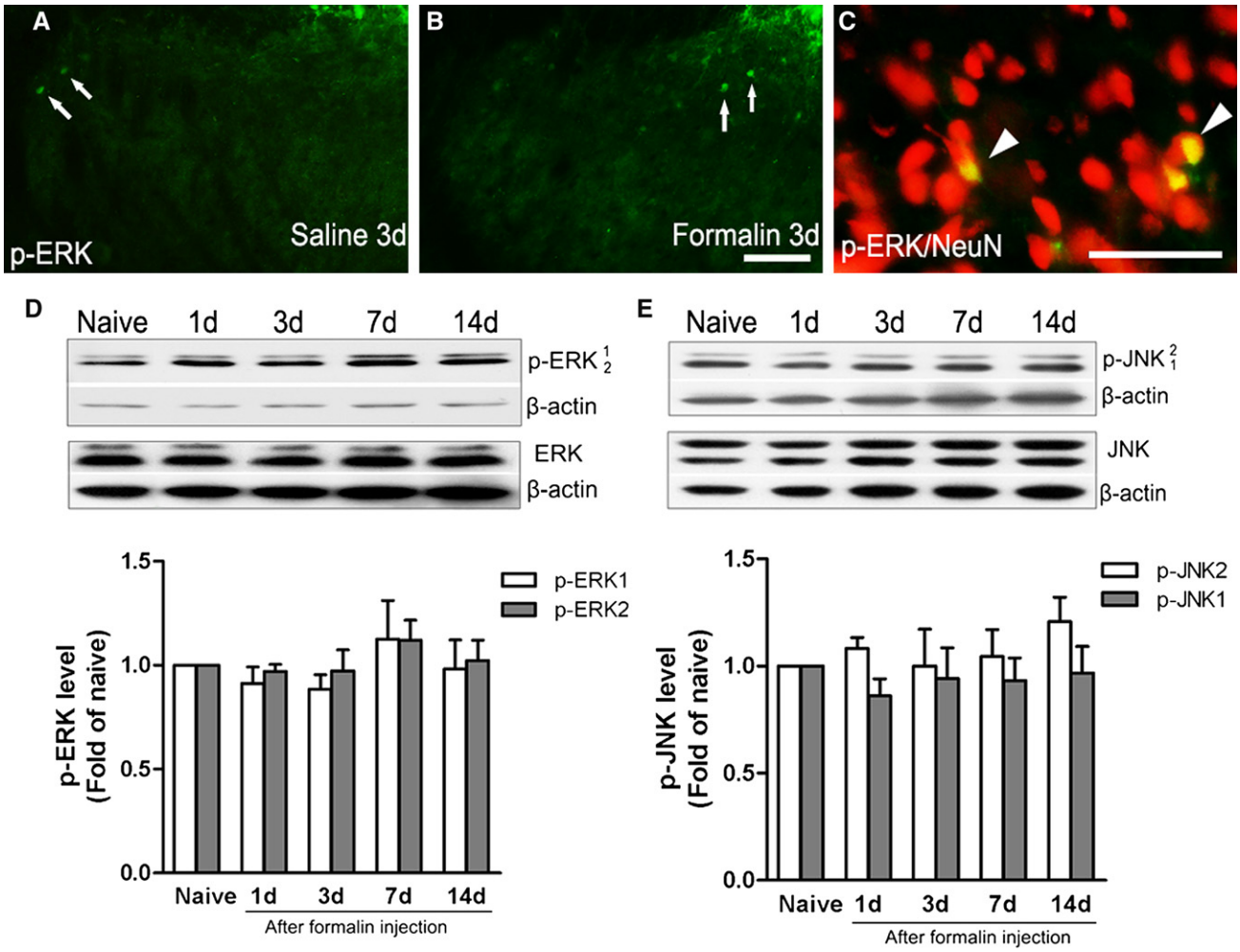

Figure 2. Peripheral formalin injection did not increase p-ERK and p-JNK expression. **(A, B)**, p-ERK immunoreactivity in the ipsilateral side of the spinal dorsal horn at day 3 from saline- and formalin-injected rats, and a very small number of p-ERK positive cells were seen (arrows). Scale bar: 50 μm. **(C)**, The double immunofluorescent label with NeuN indicated that p-ERK positive cells (arrowheads) were neurons. Scale bar: 50 μm. **(D, E)**, Representative bands and quantification of Western blot analysis showed no significant changes on p-ERK and p-JNK protein levels in the lumbar spinal cord after formalin injection. Quantification of p-ERK, total ERK, p-JNK, and total JNK protein levels were normalized against loading control β-actin. n = 4.

dorsal surface of right hind paw, between the third and fourth metatarsals. The investigator was trained to apply the tip perpendicular to the central area of the hind paw with a gradual increase in pressure. The force in grams needed to elicit clear paw withdrawal indicative of nociceptive response was recorded 4 times for each animal at 1-min intervals, and the average of the 4 values was used as the withdrawal threshold.

Immunohistochemistry

Animals (3 to 5 rats at each time point) were anesthetized with an overdose of pentobarbital sodium (100 mg/kg, ip) and euthanized by transcardiac perfusion with 250 mL body temperature 0.1 M phosphate-buffered saline (PBS, pH 7.4), followed by 300 mL ice-cold 4% paraformaldehyde/4% sucrose in 0.1 M PB, pH 7.4. After perfusion, the lumbar spinal cord (L4–5), about 4 mm long was removed, postfixed in 4% paraformaldehyde fixative for 4 hours, and then placed in a 30% sucrose solution (in 0.1 M PBS) overnight at 4°C. Thirty-micron thick spinal cord sections were transversely cut on a cryostat and successively transferred to 48 well-plates for free-floating immunohistochemical staining. The sections were blocked with 3% normal goat serum (NGS) and then incubated for 48 hours at 4°C in the primary antibody (anti-phospho-p38 1:200, anti-phospho-p44/42 (ERK) 1:200, anti-phospho-JNK 1:200; Cell Signaling, Beverly, MA). Binding sites were visualized with FITC-conjugated secondary antibody (1:200; Jackson ImmunoResearch, West Grove, PA).

For double immunofluorescence, tissues were incubated with a mixture of primary antibodies with monoclonal neuronal-specific nuclear protein (NeuN, neuronal marker, 1:5000; Chemicon, Temecula, CA), glial fibrillary acid protein (GFAP, astrocyte marker, 1:200; NeoMarkers, Fremont, CA) and OX-42 (CD11b, microglia marker, 1:200; Serotec, Indianapolis, IN). Following the incubation, spinal sections were washed and incubated for 2 hours at room temperature in a mixture of FITC- and TRITC-conjugated secondary antibodies (Jackson ImmunoResearch, West Grove, PA). The stained sections

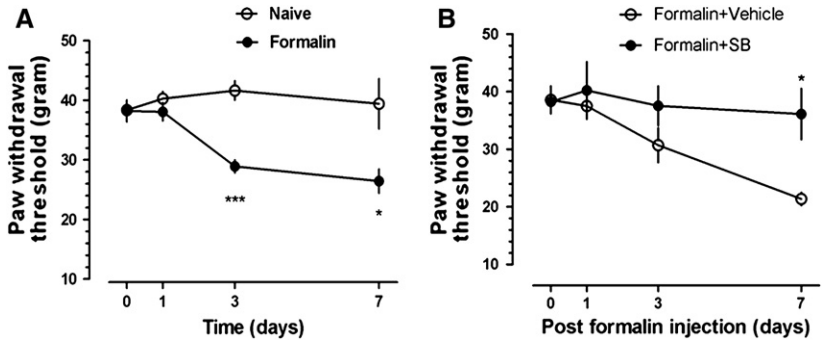

Figure 3. Effect of intrathecal administration of SB203580 (p38 inhibitor) on the formalin-induced secondary long-term mechanical hyperalgesia. **(A)**, The paw-withdrawal thresholds to mechanical stimulation were measured at day 0, 1, 3, 7 in naïve (n = 6) and formalin-injection rats (n = 6). Formalin injection resulted in secondary mechanical hyperalgesia as measured on day 3 and day 7. **(B)**, The decreased paw-withdrawal thresholds were reduced by SB203580 administration once a day for 7 days (n = 6), compared to the vehicle-treated group (n = 6). Each point represents the mean (in grams) ± SEM of paw-withdrawal threshold. *$P < .05$, ***$P < .001$, ANOVA for repeated measures and Student's t test (compared with the corresponding control group).

were examined with an Olympus (BX51, Tokyo, Japan) fluorescence microscope, and images were captured with a CCD spot camera. The image enhancement was performed by using Adobe Photoshop 10.0. In our experiments, replacement of primary antibody by normal serum or PBS resulted in no staining.

Western Blots

Rats (4 rats at each time point) were deeply anesthetized with sodium pentobarbital (100 mg/kg, ip), then decapitated. The spinal cord segments L4–5 (lumbar enlargement) ipsilateral to the injection were removed rapidly via hydroextrusion and homogenized in lysis buffer (20 mM Tris buffer, pH 7.6, containing 150 mM NaCl, 1% NP-40, 5% sodium deoxycholate, 1 mM EDTA, 2 mM sodium orthovanadate, 1 mM PMSF, phosphatase and protease inhibitor cocktail; Sigma, St. Louis, MO). The homogenate was centrifuged at 15,000 *g* for 45 minutes at 4°C. The protein concentration of tissue lysates was determined with a BCA Protein Assay Kit (Pierce, Rockford, IL). Twenty-μg aliquots were subjected to 12% SDS-PAGE, and proteins were transferred electrophoretically to PVDF filters (Millipore, Bedford, MA). After blocking with 5% nonfat milk in Tris-buffered saline (TBS) containing 0.1% Tween-20 for 1 hour in room temperature, the membranes were incubated with antibody to phospho-p38, phospho-p44/42 (ERK1/2), phospho-JNK, total p38, total p44/42 (ERK1/2), total JNK(1:1000, in 5% BSA; Cell Signaling, Beverly, MA) overnight at 4°C. After washing, the antibody-protein complexes were probed with HRP-conjugated secondary antibody (1:10000; Jackson), developed in ECL solution for 3 minutes, and exposed onto Kodak hyperfilms. The intensity of immunoreactive bands was quantified using NIH ImageJ 1.38 software, normalized to the density of internal control (β-actin), and expressed as fold changes as compared to control group.

Statistical Analysis

All data are presented as mean ± SEM. Statistical significance was calculated using Student's t-tests or 1-way ANOVA for western blot analysis or ANOVA for repeated measures for behavior test using SPSS software (version 11.5; SPSS Inc, Chicago, IL). Differences were considered to be significant when the critical value reached a level of $P < .05$.

Results

Intraplantar Formalin Injection Induced a Long-Lasting Activation of p38 MAPK in the Spinal Microglia

Few p-p38 immunoreactive cells were found in the spinal cord from naïve or saline-injected rats (Figs 1A, 1B). P-p38 protein level was almost undetectable before formalin injection by western blot. However, a marked increase in p-p38 labeled cells was observed in the medial portion of the ipsilateral dorsal horn 3 days after formalin injection (Figs 1C, 1D). The increased p-p38 was mostly found in microglia as can be seen in the double labeling (Fig 2E). A few neurons were labeled with p-p38 as well (Fig 1f) while astrocytes were mostly unlabeled (Fig 1G). The immunostaining result was confirmed by Western blots, which showed a significant increase in the amount of p-p38, but not total p38 protein 3 to 7 days after formalin injection (Fig 1H).

However, no significant change was observed in p-ERK or p-JNK as determined by immunohistochemistry or Western blot at the time points measured following formalin injection (Fig 2).

P38 Activation and Formalin-Evoked Secondary Chronic Mechanical Hyperalgesia

We have previously reported that formalin-injected tissue injury produces a third phase of nociceptive behaviors, both long-term thermal and mechanical hyperalgesia lasting for about 3 weeks.[9] In the present study, we tested the mechanical nociceptive threshold on the dorsal surface (formalin was injected into the plantar surface) stimulated by a 0.5-mm diameter polypropylene rigid tip. This demonstrated mechanical hyperalgesia at day 3 and day 7 following formalin injection (Fig 3A). Based on our immunohistochemistry and Western blot analysis, we

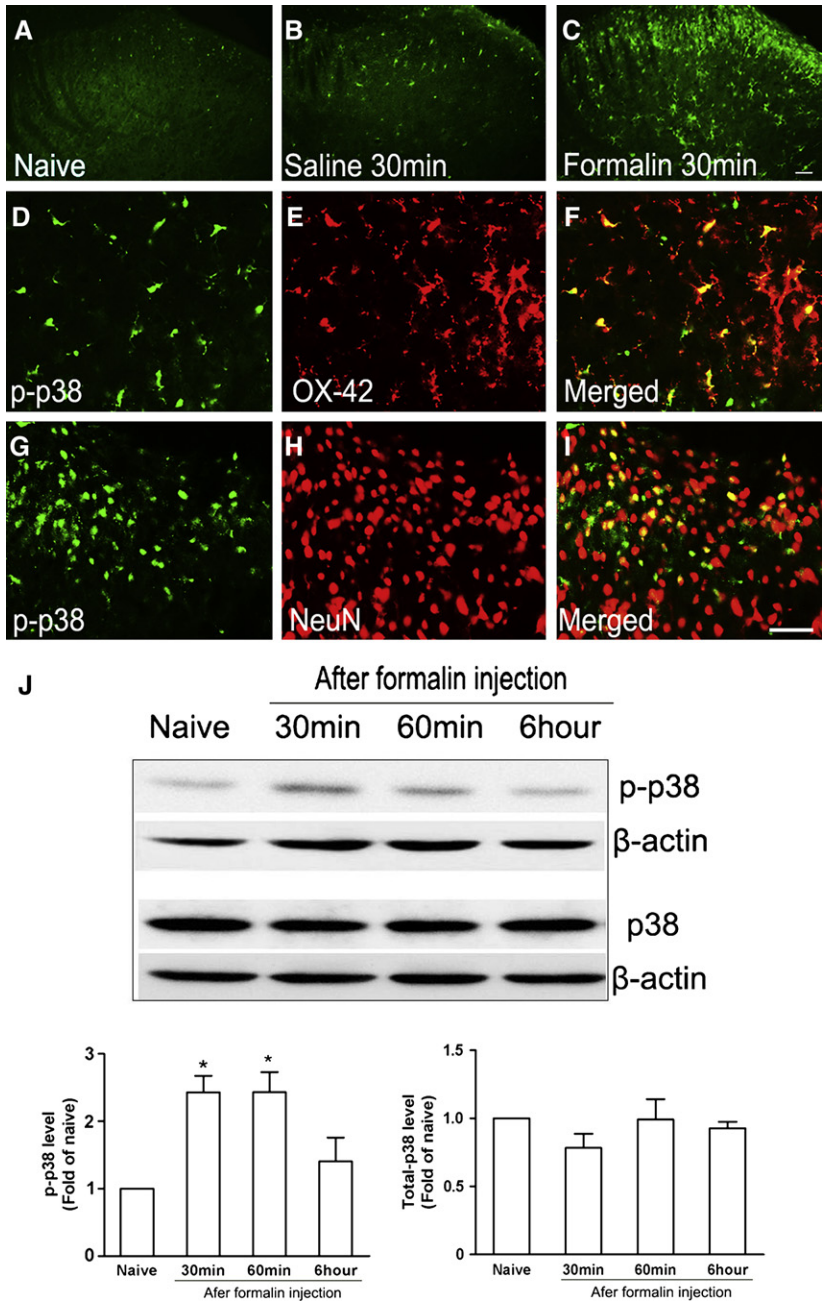

Figure 4. Peripheral formalin injection induced rapid and short-term p38 activation in the lumbar spinal dorsal horn. **(A-C)**, p-p38 immunoreactivity in the ipsilateral side of dorsal horn at 30 minutes postinjection from naïve, saline- and formalin-injected rats. Scale bars: 50 μm. **(D-F)**, Double immunostaining showed that p-p38 was mainly expressed in microglia. **(J-I)**, Double immunofluorescence indicated that there were also some neurons expressing p-p38 in the superficial layers of the dorsal horn (p-p38 colocalized with NeuN). Scale bars: 50 μm. **(J)**, Representative bands and quantification of Western blot analysis at 30 minutes, 60 minutes, and 6 hours after formalin injection showed a rapid increase of p-p38 at 30 minutes, then a decrease at 6 hours. Quantification of p-p38 and total p38 level were normalized against respective loading control β-actin. *P < .05, 1-way ANOVA and Tukey post hoc test, compared with the naïve control, n = 4.

intrathecally applied the p38 specific chemical inhibitor (SB203580) to investigate the contribution of p38 activation to this hyperalgesic behavior. We found that SB203580 had no effect on basal mechanical sensitivity (data not shown). We delivered SB203580 (10 μg) once a day for 7 days, with the first administration 20 minutes before the formalin injection. This treatment protocol significantly inhibited formalin-induced long-term mechanical hyperalgesia on day 7 as compared with the animals treated with vehicle (P < .05, Student's t test) (Fig 3B).

Intraplantar Formalin Injection Induced a Rapid Activation of p38 MAPK in the Spinal Microglia

To investigate whether there is an activation of p38 MAPK in the spinal dorsal horn in rats subjected to formalin injection during the normal time that spontaneous behaviors are recorded immediately after —60 minutes after formalin injection, we measured p-p38 expression by immunohistochemistry and western blot at early

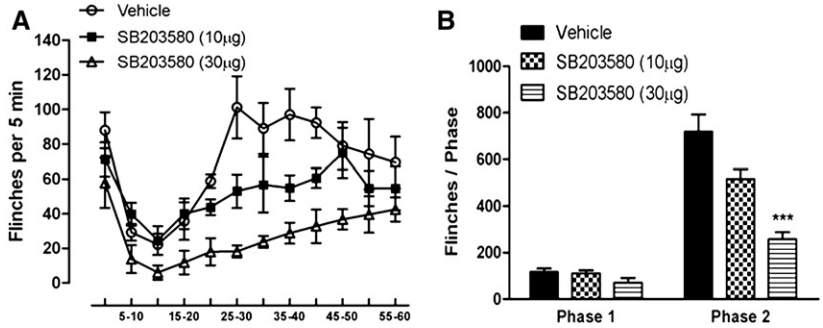

Figure 5. P38 inhibitor SB203580 reduced formalin-induced the early biphasic flinching behavior. **(A)**, Time courses of flinching behavior number from 3 different treatment groups following peripheral 5% formalin injection. Each point was the mean number of flinches ±SEM (n = 6) per 5-minute epoch. Animals pretreated with SB203580 (it) 10 μg and 30 μg showed fewer flinches compared with vehicle-treated animals. **(B)**, The bars showed total numbers of flinches during the 2 phases in rats treated with vehicle and different doses of SB203580. 30 μg SB203580 significantly suppressed phase II flinching behavior. ***$P < .001$, compared to vehicle group, n = 6, 1-way ANOVA, followed by a Tukey post hoc test.

time points. As shown in Fig 4 by immunohistochemistry, there was little constitutive expression of p-p38 in the spinal dorsal horn from naïve rats (Fig 4A), and at 30 minutes following saline injection an apparent, but nonsignificant increase of p-p38 labeling (Fig 4B). However, at 30 minutes after formalin injection, p-p38 was significantly upregulated (Fig 4C). Double labeling with OX-42 confirmed that most of the p-p38 positive cells were microglia (Figs 4D-4F), but some labeled cells located in the superficial layers were neurons (Figs 4G-4I). The increase was maintained at 1 hour postinjection, and declined at 6 hours, as measured by Western blot analysis (Fig 4J).

P38 Activation And Formalin-Evoked Early Biphasic Nociceptive Behavior

The 5% formalin-injected animals displayed classic early, biphasic spontaneous nociceptive behaviors immediately after injection, lasting for 1 hour. In this study, we quantified the number of flinching behaviors per 5 minutes within 1 hour in 3 different treatment groups, pretreated with SB203580 10μg, 30μg, and vehicle. As shown in Fig 5, pretreatment (−20 min) with SB203580 (it) showed a nonsignificant decreasing trend on phase 1 (0–9 min) flinching, but significantly and dose-responsively suppressed phase 2 (10–60 min) behaviors relative to vehicle control.

Discussion

While several previous reports have indicated the importance of p38 MAPK in enhancing pain, here we show that activation of p38 occurs in 2 stages. The first stage is quite rapid, increasing in spinal microglia tens of minutes after injection and lasting for over 1 hour, but less than 6 hours. In addition, the formalin model produces a secondary increase of p-p38 expression in spinal microglia, increasing maximally at 3 to 7 days postinjection. Intrathecal administration of the p38 inhibitor SB203580 not only reduces early acute spontaneous nociceptive behaviors of the formalin pain model, but also reverses the formalin injury-induced long-term mechanical hyperalgesia. Our results suggest that the 2 stages of microglial activation (p38 activation in microglia) play key roles in central pain modulation in formalin test, both for the early acute phases and the late long-term pain state as well.

Peripheral Formalin Injury Induces 2 Stages of Microglial Activation

Spinal cord microglia can be activated by peripheral inflammation and nerve injury. Microglial activation takes several forms, such as changes in morphology from ramified to amoeboid,[7] increase in the expression of microglial markers,[3,7,10] and increase in the number of microglia.[6] These changes usually take days to be demonstrated.[3,6,8,13,14,16,21,27] We have previously reported that peripheral formalin injection activated spinal microglia as observed by changes in morphology and increases in the expression of several immune markers (CD11b, CD45, and MHC class I) begining from 1 to 3 days after injection.[8,10] However, microglia have recently been shown to be activated at early times after peripheral injury or inflammation without morphological changes, as was characterized by increased p38 activation.[17,37] Svensson et al[37] reported that peripheral formalin injection could rapidly (within minutes) increase phosphorylation of p38 in microglia after formalin injection into the paw. This activation of p38 occurred before morphological changes. Pretreatment with p38 MAPK inhibitors could reduce the early phases of formalin-induced paw flinching behaviors and spinal neuronal c-fos expression.[37] In the present study, we confirmed that peripheral formalin injury induced early stage of spinal microglial activation indicated by phosphorylation of p38 in microglia within 1 hour and also showed a late stage of p38 MAPK activation 3 days to weeks after injection. These results suggest that microglia can be activated (particularly in the early stages 0 to 24 hours) without observable morphological changes, but only at later times (second stage, 3 days to weeks) acquire the morphological and immune marker changes as well as a second wave of p38 MAPK activation. Thus, microglia may undergo at least 2 distinct stages of activation on the basis of their morphological and immunological changes.[10]

Nerve injury induces spinal microglial activation with a series of sterotypical morphological changes and considerable increases in OX-42 labeling at days postinjury.[3,13,14,16,27] This contrasts with peripheral inflammation, which induces no or very moderate morphological changes and increases of OX-42 immunoreactivity in spinal microglia.[1,17,21,38,48] Peripheral formalin injection has long been used as a model of inflammation,[5,30,41] but the injection also produces nerve injury in the peripheral axons.[21,23,36] Thus, peripheral formalin injection induces 2 stages of spinal microglial p38 activation; an early stage associated with inflammation or immediate injury discharge and a late stage associated with nerve injury.

P38 Activation in Spinal Microglia is Required for Both Early Acute Phases and Late Secondary Chronic Pain State

Most studies have been using CD11b (as observed with OX-42) or Iba-1 as markers for microglial activation. These markers show profound changes after peripheral tissue injuries. But recently it has been accepted that phosphorylated MAPKs (eg, p-p38 or p-ERK) are functional markers that reflect the activation of microglia. Increased phosphorylation of p38 in microglia has been reported in a number of experimental models of inflammation and nerve injury-induced pain, and inhibition of spinal p38 activation reduced the behavioral sensitivity in these models.[17-19,37,43] In the present study, peripheral formalin injection induced 2 stages of spinal microglial p38 activation, and pretreatment with p38 inhibitor SB203580 dose-dependently reduced formalin-induced phase 2 flinching behavior numbers. Daily delivery of SB203580 also suppressed late long-term mechanical hyperalgesia. In our another study, intraperitoneal administration of minocycline (a putative microglial inhibitor) reduced spinal microglial p-p38 expression and prevented enhanced nociceptive behavior, but failed to reverse morphological and immune marker changes on microglia (Li K et al, J Neuroimmunol, in press). Thus, microglial activity indicated by the p-p38 expression, but not the late stage morphological and immune marker changes, has a critical role in the microglia-mediated pain mechanisms. Late-stage morphological changes, which in some conditions were not closely associated with pain behaviors,[2,42] while a "footprint" of nerve injury associated long-term microglial activation, are neither necessary nor sufficient for the pain-behavior enhancement.

P38 Activation in Spinal Dorsal Horn Neurons

Activation of p38 MAPK in spinal cord in neuropathic pain models was found exclusively in microglia, but not in neurons or astrocytes.[19,43] However, in other studies, p-p38 was also seen in a small population of neurons in laminae I and II when a carrageenan-induced pain model was used.[17] In the models of bee venom and incision-induced pain models, p38 activation was also found in neurons in the early phase of pain processes. The most prominent increase was in laminae I–II of the dorsal horn.[4,47] The number of p-p38-IR microglia was significantly increased from day 1, but the number of p-p38-IR neurons was significantly increased from 1 hour after bee-venom injection.[4] In our study, some p-p38 expressing neurons were also found in the superficial layer of the dorsal horn at an early time following formalin injection, at 30 and 60 minutes after injection. Altogether, the current evidence is that phosphorylation of p38 has a distinct role in generating pain sensitivity, and this kinase in spinal neurons may also contribute to pain mechanisms during the early phase of formalin injury-induced pain.

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