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Neonatal Bee Venom Exposure Induces Sensory Modality-Specific Enhancement of Nociceptive Response in Adult Rats

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Abstract

Objective. Previous studies have shown that inflammatory pain at the neonatal stage can produce long-term structural and functional changes in nociceptive pathways, resulting in altered pain perception in adulthood. However, the exact pattern of altered nociceptive response and associated neurochemical changes in the spinal cord in this process is unclear.

Method. In this study, we used an experimental paradigm in which each rat first received intraplantar bee venom (BV) or saline injection on postnatal day 1, 4, 7, 14, 21, or 28. This was followed 2 months later by a second intraplantar bee venom injection in the same rats to examine the difference in nociceptive responses.

Results. We found that neonatal inflammatory pain induced by the first BV injection significantly reduced baseline paw withdrawal mechanical threshold, but not baseline paw withdrawal thermal latency, when rats were examined 2 months from the first BV injection. Neonatal inflammatory pain also exacerbated mechanical, but not thermal, hyperalgesia in response to the second BV injection in these same rats. Rats exposed to neonatal inflammation also showed up-regulation of spinal NGF, TrkA receptor, BDNF, TrkB receptor, IL-1β, and COX-2 expression following the second BV injection, especially with prior BV exposure on postnatal day 21 or 28.

Conclusion. These results indicate that neonatal inflammation produces sensory modality-specific changes in nociceptive behavior and alters neurochemistry in the spinal cord of adult rats. These results also suggest that a prior history of inflammatory pain during the developmental period might have an impact on clinical pain in highly susceptible adult patients.

Key Words. Pain; Neonatal; Inflammation; Bee Venom; Long-Term Consequences

Introduction

Pain is a subjective experience that signals impending or actual injury. However, pain also induces metabolic and behavioral responses that can be detrimental in many aspects [1,2]. Recently, a number of studies have demonstrated that the early postnatal stage is a vulnerable period during which nociceptive stimulation may result in structural and functional reorganization of the sensory system, leading to changes in pain perception in adult life [3–7].

A large body of evidence indicates exacerbated nociceptive response can be elicited in adult rats exposed to an inflammatory insult during the neonatal period [3,5–10].
Ruda et al. reported that neonatal inflammatory pain has a significant effect on cellular responses of the spinal cord dorsal horn to subsequent inflammation in adulthood [3,11]. In addition, repeated colonic irritation during the neonatal period can produce chronic visceral hyperalgesia when these animals reach their mature age, a response that is thought to involve long-term changes in the nociceptive system [8].

Interestingly, one study has suggested that long-lasting neonatal inflammation differentially affects nociceptive responses later in life, depending on the type of subsequent noxious insult [12]. For example, hypalgesia has been observed in adult animals that suffered from neonatal inflammatory pain induced by a single application of carrageenan [9,13–15]. In contrast, abnormal nociceptive response was not observed in animals exposed to long-lasting inflammation produced by complete Freund’s adjuvant (CFA) during the critical developmental period [3,16,17]. Moreover, inflammatory pain induced by daily injection of carrageenan into the hindpaw for three weeks after birth resulted in basal hyperalgesia to noxious heat stimulus [9]. These previous studies suggest that the influence of neonatal inflammation on changes in nociceptive responses in adult rats may depend on the time point, as well as the extent, of neonatal inflammation. It has been suggested that animal models of nociception should include minimum interindividual and intermodel differences, as well as integration of both acute and persistent nociceptive responses [18,19]. In this regard, intraplantar injection of whole honeybee venom (BV) has been shown to produce long-term behavioral and neuronal changes in the spinal sensorimotor reflex circuitry [18,20–23]. This BV model is characterized by immediate spontaneous paw flinches lasting for more than 60 minutes, followed by 72–96 hours of heat and mechanical hypersensitivity [18,20–23].

In order to examine whether exposure to BV at the neonatal period would produce different patterns of nociceptive behavior and neurochemical changes in the spinal cord in the same rats at adulthood in response to a second BV exposure, we used an experimental paradigm in which each rat first received an intraplantar BV or saline injection on postnatal day 1, 4, 7, 14, 21, or 28 (P1, P4, P7, P14, P21, and P28), followed 2 months later by a second intraplantar BV injection. Behavioral changes after the second BV injection were examined to determine the influence of neonatal inflammation at various postnatal time points on baseline and evoked nociceptive response in adulthood, including paw withdrawal mechanical threshold (PWTm), paw withdrawal thermal latency (PWTt), and persistent spontaneous nociceptive behavior (hindpaw flinches). In addition, we examined the expression of nerve growth factor (NGF) and its receptor TrkA, brain-derived neurotrophic factor (BDNF) and its receptor TrkB, interleukin 1β (IL-1β), and the inducible, prostaglandin-producing, rate-limited enzyme cyclooxygenase 2 (COX-2) in the spinal cord of the same adult rats with prior BV exposure on P7, P14, P21, or P28.

**Methods**

**Animals**

The young Sprague Dawley rats used in the study were provided by the Laboratory Animal Center of the Fourth Military Medical University (FMMU). The present study was in compliance with the guidelines established by the International Association for the Study of Pain (IASP) [24]. The animal protocol was reviewed and approved by the FMMU’s Animal Care and Use Committee. All the animals were housed in plastic boxes at room temperature with food and water available ad libitum. A 12:12 light–dark cycle with lights on at 08:00 was maintained, and behavioral tests were performed between 09:00 and 18:00. Each rat was used only once, and it was sacrificed at the end of the experiment by intraperitoneal injection of an overdose of sodium pentobarbital (200 mg/kg).

**Experimental Protocol**

Twenty-four colonies of neonatal rats were divided into six age groups: P1, P4, P7, P14, P21, and P28. In order to avoid procedural bias in our experiments, we used pups from the same colony and divided them into different subgroups according to each postnatal time point (P1, P4, P7, P14, P21, and P28). A counterbalanced design was used to determine the group assignment, such that each neonatal group (P1, P4, etc.) consisted of two subgroups: 1) the BV subgroup and 2) the saline subgroup. As each neonatal group included four colonies of pups with 6–10 pups per group (i.e., 32–40 pups for each neonatal group), each subgroup included 16–20 pups. We examined the impact of neonatal BV-induced nociception (between P1 and P28) on nociceptive behavior in response to a second BV exposure in the same rats at a mature age [25]. The baseline behavioral response to thermal and mechanical stimulation was determined when these pups grew up (2 months from the first BV or saline injection). Then, both subgroups of rats (neonatal BV and saline) were given a single injection of BV solution. Their behavioral response was assessed after BV injection, including persistent spontaneous nociceptive behavior during the first hour after the BV injection as well as thermal (PWTt) and mechanical (PWTm) nociceptive response assessed at 2, 4, 8, 24, 48, 72, and 96 hours after the BV injection. After the last behavioral test, spinal cords of adult rats with prior BV exposure on P7, P14, P21 and P28 were harvested and used to examine the expression of NGF, TrkA receptor, BDNF, TrkB receptor, IL-1β, and COX-2 using immunohistochemistry (Figure 1).

**Intraplantar Injection**

Lyophilized BV was obtained from whole venom of Apis mellifera (0.2 mg BV diluted in 50 μl saline), BV (20 μl) or saline was injected subcutaneously into the plantar surface of the left hindpaw in neonatal rats (N = 24–36) [19,20,26]. The same rats received a second BV injection (0.4%, 50 μl) 2 months after the initial injection. BV was
injected using a 26-gauge, 5/8-inch needle while an experimenter gently held the rat’s hindpaw.

Measurement of Persistent Spontaneous Nociceptive Behavior

An acrylic test box (30 × 30 × 30 cm) with a transparent glass floor was placed on a supporting frame placed 50 cm above a table to allow the experimenter to observe hindpaw flinch. A rat was placed in the observation box for a 30-minute acclimation period before the intraplantar injection of BV. After injection, the rat was put back into the observation box, and the number of paw flinches every 5 minutes was counted for 1 hour [20]. The majority of behavioral responses are seen within 1 hour. Therefore, this was chosen as the cutoff time.

Measurement of Thermal and Mechanical Hyperalgesia

Thermal and mechanical hyperalgesia was assessed after spontaneous nociceptive behavior (hindpaw flinch) had disappeared, which typically occurred within two hours after the BV injection. Bilateral hindpaws of neonatal rats were examined both prior to and 2 hours after the first BV injection. For adult rats, thermal and mechanical hyperalgesia was examined at 2, 4, 8, 24, 48, 72, and 96 hours after the second BV injection for a time-course analysis.

Thermal Hyperalgesia

A rat was placed on the surface of a 2-mm-thick glass plate covered with the previously mentioned acrylic chamber. PWTL was measured using an RTY-3 radiant heat stimulator (Fenglan Instrument Factory, Xi’an, China), as described previously [20]. The radiant heat source was a high-intensity projector (100 W, 105 V) positioned under the glass floor directly beneath the target area of the hindpaw. Tests were repeated five times (interval time >10 minutes), and a mean PWTL was calculated after discarding the values from the first and second stimuli. A cutoff of 40 seconds was preset to avoid tissue injury.

Mechanical Hyperalgesia

Von Frey filaments with bending forces of 0.2, 0.4, 0.7, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 16.0, 20.0, 25.0, 30.0,

Figure 1 A flowchart illustrating the experimental design.

Figure 2 Effect of neonatal inflammation on baseline thermal and mechanical nociceptive threshold in adult rats. (A) When examined in maturity (2 months after the first BV exposure), rats receiving intraplantar BV injection before P28 showed no differences in baseline paw withdrawal thermal latency (PWTL) compared with those not receiving BV. (B) Those rats receiving intraplantar BV injection before P21 showed significantly decreased baseline paw withdrawal mechanical threshold (PWMT). Ipsil.Sal/BV = ipsilateral hindpaw of rats exposed neonatally to saline or BV; Contil.Sal/BV: contralateral hindpaw of rats exposed neonatally to saline or BV. * P < 0.05, ** P < 0.01 as compared with the saline group; N = 12–18.
35.0, 40.0, and 50.0 g were used. A rat was placed on a metal mesh floor covered with a Plexiglas chamber (20 × 20 × 25 cm). Von Frey filaments were applied, in ascending order of bending force, from underneath the metal mesh floor to each hindpaw. A single von Frey filament was applied 5 times (once every several seconds) to each testing site. The bending force sufficient to evoke paw withdrawal 50% of the time was recorded as PWMT [20].

**Immunohistochemistry**

Animals were deeply anesthetized with pentobarbital (50 mg/kg, i.p.) and perfused intracardially with 100 mL of 0.01-M phosphate-buffered saline (PBS, pH 7.4), followed by 500 mL of 4% paraformaldehyde and 15% of a saturated solution of picric acid in 0.1-M phosphate buffer (PB, pH 7.4). The lumbar spinal cord (segments L4 and L5) was removed on ice, postfixed in the same fixative for 12 hours, and cryoprotected in 30% sucrose in PB. Transverse sections of 30 μm were cut with a cryostat at −20°C and collected in PBS for immunohistochemical processing.

The ABC method was used to determine the immunoreactivity of NGF, TrkA receptor, BDNF, TrkB receptor, IL-1β, and COX-2. Tissue sections were incubated in 0.3% H2O2 in distilled water for 30 minutes at room temperature. After being blocked in 5% normal goat serum in PBS containing 0.3% Triton X-100 for 30 minutes, sections were incubated overnight at 4°C with primary antibodies to NGF (1:200; rabbit anti-rat, Santa Cruz Biotechnology, Santa Cruz, CA, USA), TrkA receptor (1:200; rabbit anti-rat, Santa Cruz), BDNF (1:200; rabbit anti-rat, Santa Cruz), TrkB receptor (1:200; rabbit anti-rat, Santa Cruz), IL-1β (1:200; rabbit anti-rat, Santa Cruz), or COX-2 (1:200; goat anti-rat, Santa Cruz). HRP–avidin–biotin antibody (1:200; goat anti-rabbit IgG or donkey anti-goat, Chemicon International, Temecula, CA, USA) was used. The integral optical density of positive staining was measured from five fields in each section for semiquantitative analysis.

**Statistical Analysis**

All results were expressed as mean ± SEM. Percentage change in thermal withdrawal latency (seconds) or mechanical withdrawal threshold (g) was calculated by using the following equation: postinjection thermal latency or mechanical threshold/baseline values × 100%. The data were then analyzed using repeated-measures two-way ANOVA (time vs group) followed by post hoc Fisher’s protected least significant differences test. The paired t-test (or the Wilcoxon signed-rank test), unpaired t-test (or the Mann–Whitney rank-sum test), and one-way ANOVA were used wherever appropriate. P values <0.05 were considered to be statistically significant.

**Figure 3** Effects of neonatal inflammation on hindpaw flinching in adult rats. A second intraplantar BV injection (0.4%, 50 μl) was given to all rats at 2 months after the first BV or saline injection. Those rats receiving the intraplantar BV injection at P4, P14, P21, and P28 showed an increased number of flinches. Arrows indicate the time point of BV administration. Sal-BV = rats receiving saline injection neonatally and then exposed to BV in adulthood. BV-BV = rats receiving BV neonatally and then again exposed to BV in adulthood. * P < 0.05, ** P < 0.01 as compared with the Sal-BV group; N = 8–10.
Results

Reduced Baseline PWMT, But Not PWTL, Before the Second BV Injection in Adult Rats

Two months from the first BV or saline injection, baseline PWTL and PWMT were examined before the second intraplantar BV injection. As shown in Figure 2, rats receiving intraplantar BV injection during the neonatal period between P1 and P21 showed no significant differences in the PWTL of the hindpaw that received the injection compared with their contralateral hindpaw or with rats receiving intraplantar saline injection during the neonatal period (P > 0.05; Figure 2A). In contrast, those rats receiving intraplantar BV injection between P4 and P21 showed significantly decreased baseline PWMT of stimulation with von Frey filaments as compared with the saline group (P < 0.01; Figure 2B). The results indicated that neonatal inflammation selectively altered the baseline mechanical nociceptive response, but not the thermal nociceptive response.

Exacerbated Spontaneous Nociceptive Behavior Following the Second BV Injection in Adult Rats

To examine spontaneous nociceptive behavior (hindpaw flinch) in response to the second BV injection at 2 months from the first BV or saline injection, intraplantar BV (0.4%, 50 μl) injection was given to all groups of rats following baseline nociceptive tests. The number of hindpaw flinches was recorded over a 1-hour period after the second BV injection.

As shown in Figure 3, those rats receiving the first intraplantar BV injection on P4, P14, P21, and P28 showed an increased total number of flinches within 1

Figure 4 Effects of neonatal inflammation on BV-induced paw withdrawal thermal latency (PWTL) in adult rats. A second intraplantar BV (0.4%, 50 μl) injection was given to all rats at 2 months from the first BV or saline injection. No differences were observed in paw withdrawal thermal latency in these adult rats regardless of whether they received intraplantar BV or saline at the neonatal stage. Arrows indicate the time point of BV administration. Sal-BV = rats receiving saline injection neonatally and then exposed to BV at adulthood. BV-BV = Rats receiving BV neonatally and then again exposed to BV at adulthood. * P < 0.05, ** P < 0.01 as compared with the Sal-BV group; N = 8–10.
hour after the second BV injection, as compared with those rats exposed to intraplantar saline injection at the same postnatal age ($P < 0.05$). Moreover, those rats receiving the first intraplantar BV injection on P4 and P14 showed an increased number of hindpaw flinches during the first 15 minutes after the second BV injection, whereas those rats exposed to the first BV injection on P21 and P28 demonstrated an increased number of hindpaw flinches beginning 15 minutes after the second BV injection and lasting for 45 minutes. The data indicate that neonatal inflammation influences spontaneous nociceptive behavior differently depending on the time point at which it occurs.

Reduced PWMT, But Not PWTL, Following the Second BV Injection in Adult Rats

To examine evoked nociceptive response to the second BV exposure in adult rats at 2 months from the first BV or saline injection, an intraplantar BV (0.4%, 50 μl) injection was given to all groups of rats after baseline nociceptive tests. Thermal (PWTL) and mechanical (PWMT) behavioral tests were carried out at 2, 4, 8, 24, 48, 72, or 96 hours after the second BV injection.

As shown in Figure 4, no significant differences were observed in PWTL between adult rats receiving intraplantar BV or saline at their neonatal stage ($P > 0.05$). In contrast, although PWMT was not significantly reduced after the second BV injection in those rats receiving the first BV injection before P21, PWMT was significantly reduced in those rats receiving the first BV injection on P21 and P28 (but not in those receiving saline on these days) ($P < 0.05$, Figure 5). The results indicate that neonatal inflammation had a different impact on nociceptive response in adult rats, depending on the type of stimulation (thermal vs mechanical), the type of behavioral test (paw flinch vs evoked response), and the postnatal time point at which BV-induced inflammation occurred.

![Figure 5](http://painmedicine.oxfordjournals.org/)

**Figure 5** Effects of neonatal inflammation on BV-induced paw withdrawal mechanical threshold (PWMT) in adult rats. A second intraplantar BV (0.4%, 50 μl) injection was given to all rats at 2 months from the first BV or saline injection. PWMT was almost absent in adult rats receiving first BV injection before P21; however, PWMT was significantly reduced in those rats receiving the first BV injection at P21 and beyond. Arrows indicate the time point of BV administration. Sal-BV = rats receiving saline injection neonatally and then exposed to BV as adults. BV-BV = rats receiving BV neonatally and then again exposed to BV as adults. * $P < 0.05$, ** $P < 0.01$ as compared with the Sal-BV group; N = 8–10.
Spinal NGF, TrkA, BDNF, TrkB, IL-1β, and COX-2 Expression Following the Second BV Injection in Adult Rats

In order to examine whether spinal neurochemical changes in response to the second BV exposure would differ in adult rats who had received neonatal BV or saline injection, we examined the spinal expression of NGF and its receptor TrkA, BDNF and its receptor TrkB, interleukin-1β (IL-1β), and cyclooxygenase-2 (COX-2). These neural substrates were chosen because they have been implicated in inflammation-related nociceptive processing in the spinal cord [27–33].

In the spinal cord dorsal horn of adult rats, the expression of both NGF/TrkA (Figure 6) and BDNF/TrkB (Figure 7) immunoreactivity was increased in rats receiving intraplantar BV on P7, P14, P21, and P28. In contrast, the expression of IL-1β and COX-2 in the spinal cord dorsal horn was increased only in those adult rats receiving intraplantar BV injection after P21 (Figure 8). Taken together, the results indicate that spinal expression of neurotrophins and their corresponding receptors, as well as IL-1β and COX-2, was up-regulated in adult rats receiving the first intraplantar BV exposure at certain time points during the neonatal period.

Discussion

Recent preclinical and clinical studies have shown that persistent nociception during the neonatal stage can alter pain perception in the mature stage [34–38]. In the present study, we examined whether neonatal inflammatory pain induced by intraplantar BV injection would alter behavioral responses to a second intraplantar BV injection at 2 months after the initial BV injection. We found that neonatal inflammatory pain enhanced mechanical (but not thermal) nociceptive response, as well as persistent spontaneous nociceptive behavior (hindpaw flinch), following the second intraplantar BV injection in adult rats. In addition, the spinal expression of neurotrophins, IL-1β, and COX-2 was up-regulated in the adult rats showing altered nociceptive response. These results indicate that neonatal

Figure 6 Effect of neonatal inflammation on the spinal expression of NGF and TrkA in adult rats. Compared with adult rats without neonatal inflammation, rats receiving the first BV injection on P7, P14, P21, and P28 showed up-regulated expression of NGF and TrkA immunoreactivity in the spinal cord dorsal horn. Scale bar: 200 μm. IOD = integral optical density. Sal-BV = rats receiving saline injection neonatally and then exposed to BV as adults. BV-BV = rats receiving BV neonatally and then again exposed to BV as adults. * P < 0.05, as compared with the Sal-BV group.
inflammation produces modality-specific changes of nociceptive behavior and alters neurochemical responses in the spinal cord of adult rats.

It has been controversial as to whether baseline nociceptive threshold would be changed in adult rats exposed to tissue injury during the neonatal period. Although inflammatory nociception can be induced by formalin [39], carrageenan [4,40], capsaicin, bee venom [26], or CFA [3], different chemical stimuli during the neonatal period may have different impacts on baseline nociceptive response in adult rats. For example, it has been shown that CFA injection into the hindpaw of newborn rats enhances nociceptive response to subsequent inflammation [12]. However, the basal response to thermal or mechanical stimuli during the neonatal period may have different impacts on baseline nociceptive response in adult rats. For example, it has been shown that CFA injection into the hindpaw of newborn rats enhances nociceptive response to subsequent inflammation [12].

Our results demonstrate that several factors may influence changes, or lack thereof, in baseline and evoked nociceptive responses in adult rats that have been exposed to neonatal inflammation. First, we found that there was a significant increase in both baseline and BV-evoked mechanical nociceptive response in adult rats exposed to intraplantar BV (but not in those exposed to saline injection) during their neonatal period. No differences in baseline or BV-evoked thermal nociceptive response had been demonstrated previously in these adult rats. Second, we also found that the time course of spontaneous nociceptive behavior (hindpaw flinch) differed in adult rats exposed to inflammatory nociception on P4 and P14 vs those exposed on P21 and P28. That is, if the first BV injection was on P4 or P14, early paw flinching was significantly greater (in the first 15 minutes). If the first BV injection was given on P21, increased flinching was delayed until 15–25 minutes after the second injection, and it was further delayed until 40–55 minutes after the second injection if the initial BV injection was given on P28. Third, we demonstrated neurochemical changes in the spinal cord, specifically in the levels of NGF, TrkA, BDNF, TrkB, IL-1β, and

**Figure 7** Effect of neonatal inflammation on the spinal expression of BDNF and TrkB in adult rats. Compared with adult rats without neonatal inflammation, rats receiving the first BV injection on P7, P14, P21, and P28 showed up-regulated expression of BDNF and TrkB in the spinal cord dorsal horn. Scale bar: 200 μm. IOD = integral optical density. Sal-BV = rats receiving saline injection neonatally and then exposed to BV as adults. BV-BV = rats receiving BV neonatally and then again exposed to BV as adults. * P < 0.05, as compared with the Sal-BV group.

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COX-2, in these same adult rats with behavioral changes. Therefore, sensory modality, postnatal time elapsed before neonatal inflammation, and neurochemical changes all appear to contribute to the altered nociceptive response in adult rats exposed to BV-induced neonatal inflammation. The differences between the findings from this study and those from previous studies [3,9,16,17] may be related to the different inflammatory agent (BV) used at the neonatal stage. Thus, our results suggest that preclinical studies regarding the influence of neonatal inflammation on nociceptive response in adult rats should take into consideration sensory modality, inflammatory agent, and time after birth of initial insult.

While the exact mechanisms of enhanced nociceptive response in adult rats previously exposed to neonatal inflammation remain unclear, several possibilities have been suggested in the literature. First, it is possible that neonatal inflammation may change the density of nociceptive primary afferents in the superficial dorsal horn laminae as well as their connections to nociceptive pathways [41]. Second, neonatal inflammation may alter cellular responses involving voltage-dependent calcium channels, nitric oxide, and the mGluR1/5-cAMP-dependent protein kinase A pathway [42]. Third, the maturity of nociceptive pathways at the time of neonatal inflammatory insult may determine the long-term effect in adult rats. For example, C-afferent fibers do not specifically innervate laminae I and II and Aβ-terminals do not predominantly terminate in laminae III and IV until P21 [7]. Fourth, the duration and effect of neonatal inflammation may be dependent on the type and amount of inflammatory agent used. Nonetheless, the current study provides additional data to the existing literature that may help explain the exact long-term impact of neonatal nociception [42].

As discussed earlier, neonatal peripheral inflammatory insult may result in alteration of development in nociceptive pathways. During the early postnatal period, neurotrophins play an important role in neural development and sensory nerve innervation [27]. This is consistent with the role of growth factors in the development and postnatal maintenance of sensory neurons [43,44]. In this study, we did not directly examine the impact of neurotrophins in the development of nociceptive pathways following neonatal
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inflammation because our primary goal was to evaluate behavioral changes in the rats. Nonetheless, we obtained spinal cord samples and examined neurochemical changes in the spinal cord, including changes in the levels of NGF, TrkA, BDNF, TrkB, IL-1β, and COX-2, in the adult rats with behavioral changes. Our results showed significant differences in neurochemical changes in adult rats exposed to the same BV injection, both with and without (saline control) neonatal inflammation.

Neurotrophins such as NGF and BDNF have been implicated in nociceptive processing in the adult nociceptive system [31,32]. Peripheral inflammation increases the expression of NGF and BDNF, mainly in dorsal root ganglion cells coexpressing substance P and/or calcitonin gene-related peptide. In addition, tissue inflammation induces expression of tyrosine kinase receptors such as TrkA and TrkB that respectively bind NGF and BDNF in the spinal cord dorsal horn [32,45,46]. Moreover, central COX-2 expression is induced by inflammation [47], and IL-1β, a major inducer of COX-2, is overexpressed in the spinal cord dorsal horn of rodents with arthritis [48]. It has been shown that behavioral changes induced by subcutaneous injection of BV into the rat’s hindpaw [18,26] may be mediated by both peripheral and spinal neural mechanisms [49]. As the expression of spinal NGF-TrkA, BDNF-TrkB, and IL-1β/COX-2 was enhanced in the adult rats showing exacerbated mechanical hyperalgesia, it is possible that these neural substrates may have played a role in this behavioral phenotype. Future studies should be considered to examine a causal relationship between these neurochemical changes and altered nociceptive response in adult rats.

In summary, the present study demonstrates that neonatal inflammation can substantially alter mechanical but not thermal nociceptive response in adult rats and that neurotrophins/receptors and IL-1β/COX-2 at the spinal level may have a role in this process. These results suggest that a prior history of inflammatory pain during the developmental period might have an impact on clinical pain in highly susceptible adult patients. Future investigations may further explore the role of spinal neurotrophins and cytokines in the development of neonatal nociceptive circuits and their influence on nociceptive response at both neonatal and adult stages.

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