Gabapentin alleviates facet-mediated pain in the rat through reduced neuronal hyperexcitability and astrocytic activation in the spinal cord

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Abstract

Although joint pain is common, its mechanism(s) remain undefined, with little known about the spinal neuronal responses that contribute to this type of pain. Afferent activity and sustained spinal neuronal hyperexcitability correlate to facet joint loading and the extent of behavioral sensitivity induced after painful facet injury, suggesting spinal neuronal plasticity is induced in association with facet-mediated pain. This study used a rat model of painful C6/C7 facet joint stretch, together with intrathecal administration of gabapentin, to investigate the effects of one aspect of spinal neuronal function on joint pain. Gabapentin or saline vehicle was given via lumbar puncture prior to and at 1 day after painful joint distraction. Mechanical hyperalgesia was measured in the forepaw for 7 days. Extracellular recordings of neuronal activity and astrocytic and microglial activation in the cervical spinal cord were evaluated at day 7. Gabapentin significantly ($p=0.0001$) attenuated mechanical hyperalgesia and the frequency of evoked neuronal firing also significantly decreased ($p<0.047$) with gabapentin treatment. Gabapentin also decreased ($p<0.04$) spinal GFAP expression. Although spinal Iba1 expression was doubled over sham, gabapentin did not reduce it. Facet joint-mediated pain appears to be sustained through spinal neuronal modifications that are also associated with astrocytic activation.

Keywords

neuronal hyperexcitability; facet joint; astrocyte; spinal cord; gabapentin

Introduction

The facet joint and its capsule have been reported to contribute to persistent pain.[3,4,29] Excessive stretching of the facet capsule beyond its physiologic range of motion can induce retraction balls and swelling in axons of the nerve fibers that innervate the facet capsule.[22] Those same joint loading conditions that produce excessive capsular stretch have been shown also to induce increased firing in the dorsal rootlets in the goat,[30] as well as sustained

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Disclosures
There are no conflicts of interests for any authors with any aspect of this study.

neuronal sensitivity in the spinal cord in the rat in association with behavioral sensitivity.[34] Painful transient facet joint loading also produces a sustained increase in the glutamate receptor and a reduction in the neuronal glutamate transporter in the spinal cord,[16] suggesting altered neuronal signaling in the spinal cord.[55,58] Despite growing speculation suggesting the involvement of spinal neuronal plasticity in facet-mediated pain, this hypothesis has not been tested.

Although gabapentin's specific mechanism of action is still unknown, it has been shown to be anti-hyperalgesic.[37,40] Studies have proposed that gabapentin acts on the $\alpha 2\delta$-1 subunit of voltage-dependent Ca^{2+} channels and prevents entry of extracellular Ca^{2+}, thereby reducing overall neuronal activity.[18,31] Anti-hyperalgesic effects of gabapentin have been demonstrated both clinically and in animal models.[8,26,41,42,45,51] For example, surgical studies suggest that pre-operative administration of gabapentin decreases postoperative pain scores and opioid analgesic requirements after mastectomy, spinal and otolaryngologic surgeries.[14,17,49] Further, spinal application of gabapentin before the induction of knee joint inflammation in the rat prevents the development of heat hyperalgesia.[29] Gabapentin also attenuates mechanical allodynia in the diabetic rat.[57] Collectively, these studies demonstrate the effectiveness of gabapentin in attenuating or abolishing pain in several different diverse models of pain, but no study has investigated whether gabapentin can attenuate facet-mediated pain from joint trauma.

These studies tested the hypothesis that spinal administration of gabapentin can mitigate facet-induced pain via reducing the hyperexcitability of the dorsal horn neurons in the spinal cord that is normally induced. As such, painful facet joint distraction was imposed using previously published methods[23–25,34] and gabapentin was administered intrathecally. Behavioral hypersensitivity in the forepaw was measured every other day until day 7 to evaluate the effects on pain symptoms. On day 7, electrophysiological recordings in the spinal dorsal horn were acquired after either gabapentin or vehicle treatment. Since painful facet joint loading has been shown to activate spinal glia[23,54,56] and those cells contribute to both the development and maintenance of neuropathic and inflammatory pain,[13,33,36,39,43,52,53] spinal glial activation was also evaluated at day 7.

Methods

Experiments were performed using male Holtzman rats (Harlan Sprague-Dawley; Indianapolis, ID) weighing 394±23 grams at the start of the study. Rats were housed under USDA- and AAALAC-compliant conditions with a 12-12 hour light-dark cycle and free access to food and water. All experimental procedures were IACUC-approved, and followed the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain.[61] Separate studies were performed to determine the effects of gabapentin on behavioral hypersensitivity and on cellular responses such as neuronal and glial activity in the spinal cord.

Joint Distraction

Surgical methods to impose painful facet joint distraction in the rat were similar to those described in previous studies.[23–25,35] Briefly, under inhalation anesthesia (4% isoflurane for induction, 2.5% for maintenance), the C6/C7 facet joints were exposed and isolated bilaterally by surgical procedures. The C6 and C7 posterior spinous processes were each isolated and rigidly attached to a customized loading device using microforceps. The joint was manually distracted using a micrometer to displace the C6 facet 0.7 mm while the C7 facet remained stationary.[23] Prior to distraction, polystyrene markers (diameter=0.17±0.01 mm) (Spherotech Inc.; Libertyville, IL) were placed at the center of each of the C6 and C7 laminae and were used to track the bony motions as a measurement of the magnitude of

applied joint distraction.[23,24] Sham surgeries were also performed as surgical controls in which there was attachment to the device but no distraction. After loading, wounds were closed using 3-0 polyester suture and surgical staples. Surgical procedures lasted for no more than 60 minutes from induction of anesthesia until wound closure. After which, rats were recovered in room air and monitored throughout the recovery period.

Gabapentin Treatment & Administration

Both injury and sham groups were separately administered either intrathecal gabapentin or vehicle via lumbar puncture injection. Pilot dose-response studies were performed to identify the optimal dosing paradigm for gabapentin, based on studies in the literature in other models of knee osteoarthritis and spinal nerve injury.[2,6,32,60] Accordingly, gabapentin (4.2 μmol in 30 μL of sterile saline) was delivered via lumbar puncture at 90 minutes prior to, and then again 1 day after, either injury (*injury-GBP*; n=12) or sham (*sham-GBP*; n=4) surgery. Separate groups of rats received vehicle treatment (30 μL of sterile saline) after injury (*injury-vehicle*; n=12) or sham (*sham-vehicle*; n=4) under the same dosing paradigms. All intrathecal treatments were performed in the space between L4 and L5 using a 25G needle under inhalation isoflurane. A Student's t-test was used to compare the magnitudes of vertebral distraction between *injury-GBP* and *injury-vehicle* to test that the same injury severity was imposed in both of the injury groups.

Behavioral Assessment

A subset of rats was evaluated for behavioral hypersensitivity (*injury-GBP*; n=6; *injury-vehicle*; n=6; *sham-GBP*; n=4; *sham-vehicle*; n=4). Rats were evaluated for bilateral mechanical hyperalgesia in the forepaws on postoperative days 1, 3, 5, and 7. Following behavioral testing on day 1, lumbar puncture was performed to administer either gabapentin or vehicle treatment. Mechanical hyperalgesia was also measured prior to surgery, as baseline values and to serve as each rat's own control. The testing procedures are customary and have been detailed in previous reports,[16,24] using a series of logarithmically-increasing von Frey filaments. Briefly, each filament was applied five times before progressing to the next higher filament having a greater strength, and the response threshold was taken as the first filament to elicit a positive response. A positive response was identified by emphatic lifting of the paw. Each testing session consisted of three rounds, and was performed on each forepaw separately. Because the injury is applied to the bilateral facet joints simultaneously, with little-to-no motion off the midline, and the resulting behavioral and spinal responses do not show a sidedness,[10,15,16,23–25,34,54] behavioral responses were averaged across both sides. Mechanical hyperalgesia was compared across all groups (*injury-GBP*, *injury-vehicle*, *sham-GBP*, *sham-vehicle*) using a repeated-measures ANOVA following by a one-way ANOVA with Bonferroni correction comparing groups on each day.

For the electrophysiological study, a subset of rats (*injury-GBP* n=6; *injury-vehicle* n=6, *sham* n=6) was used to evaluate neuronal excitability in the spinal cord. For those groups, mechanical hyperalgesia was measured only at baseline and on days 1 and 7, in order to confirm the onset and persistence of sensitivity. A repeated-measures ANOVA was used to compare the differences in hyperalgesia at days 0 (baseline), 1, and 7 between this study and the responses from the rats in the behavioral study above, for each group (*injury-GBP*, *injury-vehicle, sham*).

Electrophysiological Recordings in the Spinal Cord

In order to determine the effects of gabapentin treatment on spinal neuronal excitability, extracellular electrophysiological recordings were acquired in the deeper laminae (IV-VI) of the C6-C7 spinal dorsal horn at day 7 after facet joint distraction, for the *injury-GBP* (n=6), *injury-vehicle* (n=6), and *sham* (n=6) groups, using previously published methods.[35]

Measurements were made in those laminae, since that region of the spinal cord contains multi-receptive, wide dynamic range neurons that modulate central sensitization in many chronic pain states and exhibits increased neuronal firing after joint distraction in this same painful facet injury model.[9,20,35,50] At day 7 after the initial injury or sham procedures, anesthesia was induced using sodium pentobarbital (45 mg/kg, i.p.) and supplementary doses were given as needed (5–10 mg/kg, i.p.). A bilateral dorsal laminectomy and dural resection at the C6 and C7 spinal levels were performed to expose the spinal cord. The spinal cord was bathed in 37°C mineral oil for the duration of the recordings. Following the surgical preparation, the rat was immobilized in a stereotaxic frame using ear bars and a vertebral clamp at T2 to stabilize the cervical spine. The forepaw was inverted and secured to the platform to expose the plantar surface for mechanical stimulation during recording. Core temperature was monitored and maintained at 35–37°C using a heating plate with a temperature controller and isolated rectal probe (Physitemp Instruments, Inc.; Clifton, NJ).

Sensory afferents were identified by lowering the electrode (400–1000 μm) below the pial surface of the spinal cord using a micropositioner (Narishige; Tokyo, JP), while lightly brushing the plantar surface of the forepaw with a cotton swab.[20,34] A neuron was identified if spikes were distinguishable from the background activity during the brushing.[50] Once an evoked potential was identified, the receptive field of the neuron was marked at the forepaw location that evoked the response, and a stimulation protocol was performed that included light brushing and a series of non-noxious and noxious von Frey filaments.[34] Prior to performing the stimulation protocol, 30 seconds of baseline activity was recorded at each probe location and taken as the unstimulated response. Following that baseline period, stimulation with 10 consecutive light brush strokes was applied at the targeted location on the forepaw using a cotton swab. Four logarithmically-spaced filament strengths that included the non-noxious (1.4 and 4 g) and noxious (10 and 26 g) filaments that are used in behavioral assessment were applied. For each of the four filament strengths, five stimulations were applied for 1 second each, at approximately 1 second apart. At least 30 seconds were allowed between stimuli to prevent windup of mechanically sensitive neurons. All von Frey filaments were mounted to a load cell (SMT S-Type Model; Interface Inc.; Scottsdale, AZ) to synchronize the application of the mechanical stimulus with the acquisition of the extracellular recordings.

Extracellular voltage potentials were continuously recorded using a carbon fiber electrode (<5 μm tip; Kation Scientific, Inc.; Minneapolis, MN). Signals were amplified with a gain of 1000 and a passband filter between 300 Hz and 3000 Hz. The amplified signal was processed with a 60 Hz noise eliminator (Hum Bug; Quest Scientific; North Vancouver, BC), digitally stored at 25 kHz (CED; Cambridge, UK), and monitored with a speaker for audio feedback (A-M Systems; Calsborg, WA). Voltage recordings during the stimulation protocol of each neuron were spike-sorted using Spike 2 software (CED; Cambridge, UK) to ensure that only the firing of a single unit was measured from each recording. The total number of spikes during the period of light brushing was counted for each neuron. For von Frey filament stimuli, spikes were counted if action potentials were recorded either during the stimulation period or the rest period immediately following stimulation. Each filament had a total of five sets of spike counts because each filament was applied five times. The baseline firing rate measured in the 30-second period prior to stimulation was subtracted from the spike counts for the brush and each von Frey stimulus to yield only the evoked spikes;[34] the spike counts were log-transformed due to a positive skew. Residuals from the statistical models were plotted after the transformation to confirm a normal distribution. Neurons were classified as spontaneously firing if spikes were recorded during a 2-second period immediately before the application of the stimulation protocol.[34] Neurons were classified as wide dynamic range or low threshold neurons based on their response to a noxious pinch administered after the completion of the stimulation protocol.[34] Neurons with

evoked spikes during the noxious stimulus were considered wide dynamic range, while those that did not respond to pinch were classified as low threshold neurons.

All statistical analyses were performed using JMP8 (SAS Institute Inc.; Cary, NC). Evoked activity in response to the brush was tested between groups using a mixed-effect ANOVA with neurons nested within rats and rats nested within groups,[34] because both fixed (treatment group) and random (rats) effects were present in the model. Post-hoc Tukey HSD analysis tested the differences between groups. A mixed-effect ANOVA with the same levels of nesting was used to analyze differences between groups, von Frey stimulation magnitudes, stimulus order, and their interactions. The number of spontaneously firing neurons and the number of wide dynamic range neurons in each group were compared using Pearson's chi-square tests. All statistical tests were performed with $\alpha=0.05$, and all values are expressed as mean ± standard error (S.E.).

GFAP and Iba1 Expression in the Spinal Cord

Expression of glial fibrillary acidic protein (GFAP) and ionized calcium binding adaptor molecule 1 (Iba1) in the spinal dorsal horn were quantified by immunohistochemistry at day 7. As such, rats were perfused with 250 ml of PBS followed by 250 ml of 4% paraformaldehyde in PBS (pH 7.4). After perfusion, the cervical spinal cord was exposed by laminectomy, the C6 segment of the cervical spinal cord was harvested, and tissue was post-fixed in 4% paraformaldehyde at 4°C for 14–18 hours. Tissue was transferred to 30% sucrose/PBS and stored for 5–7 days at 4°C. Samples were freeze-mounted with OCT medium (Fisher Scientific; Waltham, MA) for cryosectioning.

Thin (16 μm) axial C6 spinal cord sections were mounted onto APES-coded slides for immunohistochemical labeling. For each rat, 5–6 axial sections spanning the rostral and caudal regions of the C6 cord were collected to ensure unbiased sampling. Polyclonal antibodies to GFAP (Millipore; Billerica, MA) and Iba1 (Wako Chemicals USA; Richmond, VA) were individually used as markers of astrocytic and microglial activation, respectively. Slides were blocked with normal goat serum (Vector Labs; Burlingame, CA) for 2 hours followed by incubation in a primary antibody solution for GFAP (1:1000), or Iba1 (1:1000) overnight. Sections were then treated with the secondary antibody containing an Alexa 546 conjugated anti-mouse antibody (Invitrogen; Carlsbad, CA) to label GFAP (1:1000) and Alexa 488 goat anti-rabbit secondary antibody (1:500; Invitrogen; Carlsbad, CA) to label Iba1.

Quantification of GFAP or Iba1 reactivity in the spinal cord was performed using densitometry.[21,23] Each sample was imaged at 10X using a Carl Zeiss LSM 510 microscope (Carl Zeiss LLC; Thornwood, NY). Images were cropped to include several regions of interest in the dorsal horn, including the superficial laminae and the deeper laminae.[22] Densitometry was performed using a customized MATLAB code to quantify the percentage of pixels above a defined threshold for staining in normal tissue.[1,39] Measurement for each protein for each spinal cord region was averaged and compared among *injury-GBP*, *injury-vehicle*, *sham-GBP*, *sham-vehicle* using an one-way ANOVA.

Results

All groups receiving a facet joint distraction underwent the same degree of joint injury, regardless of whether they received treatment or not. The mean vertebral distraction for the *injury-GBP* group was 0.66±0.08 mm, and was not different from the mean distraction measured for the *injury-vehicle* group (0.69±0.07 mm). Similarly, the distractions were closely aligned with a direction along the long-axis of the spine, with only 3.0±3.4° off the midline. Gabapentin treatment reduced behavioral hypersensitivity after distraction and

nearly abolished it (Fig. 1). There was no difference in the baseline unoperated withdrawal threshold between any groups. Thresholds for eliciting a paw withdrawal also were not significantly different between the right and left forepaws in any rat; as such, the data from each side were averaged for each rat. Response thresholds for *injury-GBP* and *sham-vehicle* were unchanged from their corresponding baseline (uninjured) values. In addition, the withdrawal threshold for the *injury-vehicle* group was significantly lower (p=0.0001) than each of the *injury-GBP,* the *sham-vehicle* and the *sham-GBP* groups (Fig. 1). On each day, *injury-vehicle* exhibited a significantly lower response threshold than that for *injury-GBP* (p<0.001) or for *sham-GBP* (p<0.01). Of note, there was no difference in response thresholds for *injury-GBP*, *sham-vehicle*, and *sham-GBP* throughout the testing period (Fig. 1). In addition, the behavioral responses produced in the groups (*injury-GBP*, *injury-vehicle, sham*) used for the measurement of spinal neuronal excitability were not different from their corresponding groups (data not shown).

A total of 101 neurons were recorded at an average depth of 627 ± 167 µm from the pial surface of the spinal cord. Twenty-four neurons were recorded at 555 ± 179 µm for the *injury-GBP* group, 44 neurons were recorded in the *injury-vehicle* group at a comparable depth of 648 ± 166 µm, and 33 neurons were recorded in the *sham* group at a depth of 642 ± 169 µm. In general, gabapentin treatment decreased the neuronal firing in the deeper laminae of the spinal cord but firing was not decreased back to sham levels (Fig. 2). Firing was not different between any groups for light brushing. Neuronal firing in response to most of the von Frey stimuli was reduced in the *injury-GBP* group compared to the *injury-vehicle* group, but was still greater in the *injury-GBP* group compared to the *sham* group (Fig. 2). Specifically, the spike counts evoked by light brushing of the forepaw in the receptive field of each neuron were not different between the *injury-vehicle* (85 ± 8 spikes/10 brushes), *injury-GBP* (73 ± 10 spikes/10 brushes) and *sham* groups (61 ± 6 spikes/10 brushes) at day 7 (Fig. 2). The 26 g filament evoked an average of 112 ± 12 spikes over the five applications in the *injury-vehicle* group, which was significantly greater (p<0.03) than the number of spikes evoked in the *injury-GBP* group (79 ± 14 spikes) and the *sham* group (63 ± 11 spikes) by the same stimulus train (Fig. 2). Evoked firing was significantly different between all three of the groups for stimulation with the 10 g (p<0.04) and 4 g (p<0.04) filaments (Fig. 2). For the 1.4g filament, evoked firing in both the *injury-vehicle* (25 ± 6 spikes) and *injury-GBP* (15 ± 3 spikes) groups was significantly greater (p<0.03) than the *sham* group (4 ± 1 spikes) (Fig. 2).

Spontaneous firing in the spinal cord was altered following the intrathecal gabapentin treatment. The number of spontaneously firing neurons (presented as a percentage of total neurons in each group) was 36% (16 of 44 neurons) in the *injury-vehicle* group, which was a significantly greater percentage of spontaneously firing neurons than in the *injury-GBP* group (17%; 4 of 24 neurons; p=0.048), and in the *sham* group (6%; 2 of 33 neurons; p=0.002) (Fig. 2C). The *injury-vehicle* group also had more neurons classified as wide dynamic range neurons (84%) than were detected in the *injury-GBP* (71%) and *sham* (67%) groups, but these differences were not significant.

Gabapentin treatment significantly reduced astrocytic activation in the superficial lamina, but not in the deeper lamina (Fig. 3). Specifically, spinal GFAP expression at day 7 after treatment in the *injury-vehicle* group in both regions of the spinal cord was significantly (p<0.01) elevated above that expressed in both the *sham-GBP* and *sham-vehicle* groups (Fig. 3). In addition, *injury-vehicle* exhibited significantly elevated (p<0.001) GFAP expression in the superficial laminae compared to expression in those regions of *injury-GBP;* but, this relationship was not observed in the deeper laminae (Fig. 3). There was no difference in GFAP expression between the *sham-GBP* and the *sham-vehicle* groups, in either the superficial or the deeper laminae of the spinal cord.

In contrast to the astrocytic activation, microglial activation in the spinal cord at day 7 was not modified in either the superficial or deeper laminae after gabapentin treatment (Fig. 4). Iab1 expression was not different between the *injury-GBP* and *injury-vehicle* groups in either region of the spinal cord that was probed (Fig. 4). However, Iba1 expression in both of the *injury-GBP* and *injury-vehicle* groups was significantly higher (p<0.01) than the expression levels in the *sham-GBP* and *sham-vehicle* groups (Fig. 4). Further, no significant difference was detected between *sham-GBP* and *sham-vehicle* in Iba1 expression for either spinal region measured.

Discussion

This study is the first to demonstrate that early intrathecal administration of gabapentin modulates neuronal hyperexcitability and astrocytic activation in the spinal cord in association with a reduction in pain (Figs. 1–3). Specifically, results showed that gabapentin reduced the frequency of neuronal firing in the spinal dorsal horn in association with attenuating behavioral hypersensitivity at day 7 (Figs. 1 and 2). Interestingly, it reduced the firing rates evoked by von Frey stimulations using filaments above 4 g (4–26 g), but did not return the responses to sham levels (Fig. 2). Responses to the 1.4g filament were not reduced by gabapentin treatment; however, that filament strength has previously been shown to be non-noxious for stimulation to the forepaw, whereas the other filaments used in this study elicit noxious responses in normal rats.[21,23] Further, since the 1.4 g filament likely activates only low-threshold mechanoreceptors, the electrophysiological findings from our current study suggest that gabapentin may attenuate behavioral hypersensitivity by reducing the firing rate of those high-threshold afferents that are sensitive to noxious stimulation. Consistent with the current findings, another study also demonstrated that a decrease in the amplitude of calcium transients evoked by depolarization of the membrane was only evident in the nociceptive small neurons, but not in the non-nociceptive large diameter neurons of the DRG.[38] Although the specific action(s) of gabapentin on neuronal sub-populations requires further work, results from our study demonstrate that gabapentin reduced the percentage of spontaneously firing neurons and wide dynamic range (WDR) neurons after injury to *sham* levels (Fig. 2). Spontaneous firing and the development of sensitivity to a broader range of non-noxious and noxious stimuli could contribute to hyperexcitability in the dorsal horn.[35] Preventing the development of spontaneous firing and a phenotypic shift to WDR neurons in the dorsal horn may contribute to the elimination of behavioral sensitivity that is evident with the gabapentin treatment (Fig. 1). The generation of neuronal after-discharge following mechanical stimuli has been observed after this painful facet joint injury;[34] although such an outcome may provide additional insight in to the effects of gabapentin on reducing behavioral sensitivity in this model, the design of the current study does not enable such evaluations.

Gabapentin is believed to act by binding to the α2δ-1 subunit of the voltage-gated calcium channel.[11,47,51] Previous work in this same joint injury model showed that hyperexcitability of neurons in the spinal dorsal horn is associated with facet-mediated pain.[34] As such, it was hypothesized that by limiting the activity of calcium channels or by binding to one of the calcium channel subunits, the neuronal excitability may also be sufficiently decreased to produce a reduction in hyperalgesia. Indeed, overall the sensitivity after injury was reduced with gabapentin treatment (Fig. 1). However, the small group size of the sham groups and the averaging of the right and left paw responses weakens the strength of the statistical relationships in behavioral outcomes for the current study. Of note, binding of gabapentin to the subunit of calcium channels may be optimized if delivered to the site of injury prior to the onset of the neurons becoming hyperexcitable (for this model, between 6 and 24 hours after injury).[10] Since treatment in the current study is given via lumbar puncture and requires time for its travel to the cervical region, the current study administered gabapentin

before the facet joint injury. However, this gabapentin treatment timing has limited clinical relevance, since pre-injury treatment is not possible. In addition, neither expression of voltage-gated calcium channels nor calcium transients were specifically evaluated in this study, so the action of gabapentin in reducing spinal and behavioral sensitivity is not known. Future studies assaying the different aspects of neuronal function in the afferents and spinal cord would more specifically define the specific mechanisms of joint pain.

Since the deeper laminae of the spinal dorsal horn contain mostly wide dynamic range neurons[5] and our study mostly made measurements in laminae IV-VI, which contains both nociceptive and non-nociceptive neurons, this study is unable to detect whether gabapentin treatment modified the responses of nociceptive (Aδ and C) or mechanoreceptive (Aβ) fibers in the superficial laminae. In carrageenan-induced pain in the rat, gabapentin reduced the C fiber-evoked phase II pain, but not the acute pain evoked by Aβ fiber activity in the spinal dorsal horn.[46] Further, gabapentin also reduced Aδ fiber-evoked excitatory postsynaptic currents after carregeenan injection, in isolated neurons prepared from the spinal cord.[27] Those findings together with the current data, suggest that gabapentin may mitigate the behavioral hypersensitivity in our painful facet joint model by acting specifically on nociceptive fibers. In a goat model, both Aδ and C fibers were identified in the facet joint capsule and were activated in response to magnitudes of joint distraction analogous to those used in this study.[7] In fact, previous work with our model of joint distraction also suggest that those mechanoreceptors and nociceptors may act as 'sensors' to transmit pain signals from the periphery to the central nervous system; mechanical allodynia produced by facet distraction was eliminated following transection of the facet capsule and the neuronal stress response in the DRG was activated after a painful distraction.[15,56] Nociceptive fibers also project to the superficial laminae of the dorsal horn,[5,59] which exhibited a greater decrease in astrocytic activation following gabapentin treatment than in the deeper laminae (Fig. 3). However, this study did not record the neuronal activity by electrophysiology in the superficial laminae. Such work would provide further insight in to the effects of gabapentin specifically on nociceptors and shed light on the mechanism(s) by which nociceptors contribute to the maintenance of facet-mediated pain.

Although gabapentin reduced astrocytic activation in the superficial laminae, it did not modulate spinal microglial activation in either of the spinal regions probed (Figs. 3 and 4). This observation is in contrast to a report of reduced spinal microglial activation in association with reduced allodynia after intrathecal gabapentin in streptozotocin (STZ)-diabetic rats.[57] This discrepancy may be due to the putative roles of microglia and astrocytes in the two different pain models. Specifically, spinal microglial activation has been shown to contribute to behavioral hypersensitivity in STZ-diabetic pain,[12,48] whereas astrocytic activation is not directly linked to pain symptoms.[57] In contrast, after painful facet joint injury, astrocytes have been implicated as having an integral role in the maintenance of pain, while microglia activation has not been evident.[23] Collectively, these spinal glial outcomes in mechanically-induced joint pain suggest that the effects of gabapentin on spinal glia may depend on the specific responses of these cells in the different pain models and the timing of the intervention. In fact, we have evidence that spinal neuronal hyperexcitability is established between 6 hours and 1 day after painful joint distraction.[10] Repeated administration of intrathecal gabapentin was given in the lumbar region prior to and at 1 day after injury, which may correspond to the time at which these changes could be prevented. Nonetheless, based on current study, it can be hypothesized that gabapentin may reduce behavioral hypersensitivity and neuronal hyperexcitability independent of modulating spinal microglia, but having effects that directly or indirectly prevent astrocytic activation. It is possible that the lack of microglial changes may also be due to the lumbar administration of gabapentin and evaluation of *cervical* spinal responses. Conversely, the reduced astrocytic activation could itself be due to the decreased neuronal hyperexcitability.

In conclusion, this study demonstrates that early intrathecal administration of gabapentin can eliminate the development of behavioral hypersensitivity that is elicited by painful facet joint distraction, and also reduces neuronal hyperexcitability in the spinal cord at day 7. Spinal astrocytic activation is decreased in the superficial laminae with gabapentin, while microglial activation was not changed. This further suggests that astrocytic responses may be more involved in this painful injury condition. Overall, results from this study provide evidence that spinal neuronal dysfunction, astrocytic activation, and/or their interaction may be responsible for facet joint-induced pain.

Acknowledgments

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Perspective

Intrathecal gabapentin treatment was used to investigate behavioral, neuronal and glial response in a rat model of painful C6/C7 facet joint stretch. Gabapentin attenuated mechanical hyperalgesia, reduced evoked neuronal firing and decreased spinal astrocytic activation. This study supports facet joint pain being sustained through spinal neuronal andastrocytic activation.
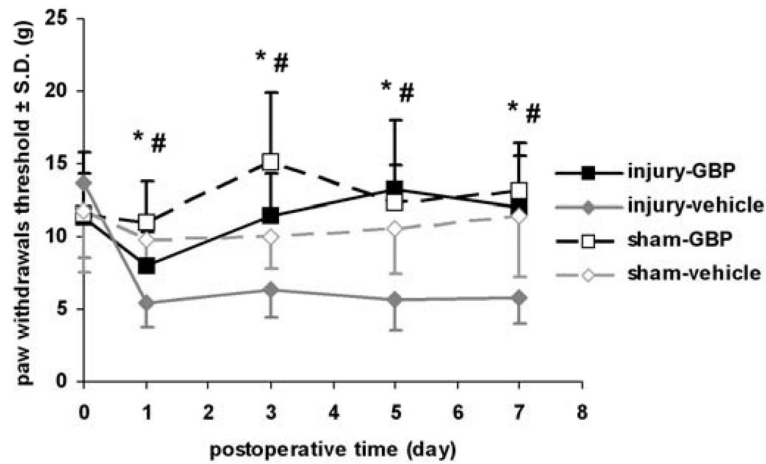
Fig. 1.
The response threshold to von Frey filament stimulation in the forepaw is significantly (p=0.0001) lower for *injury-vehicle* compared to each of *injury-GBP, sham-GBP* and *sham-vehicle*. On each postoperative day, the response threshold for the *injury-vehicle* group is significantly lower (p=0.0001) than its corresponding baseline control values, while the response threshold after *sham-vehicle* is not different from its baseline. Additionally, *injury-vehicle* exhibits a significantly lower response threshold than that for the *injury-GBP* (*p<0.001) and for *sham-GBP* groups (#p<0.01) on days 1, 3, 5, and 7. There is no difference between *injury-GBP*, *sham-GBP*, and *sham-vehicle* throughout the testing period.
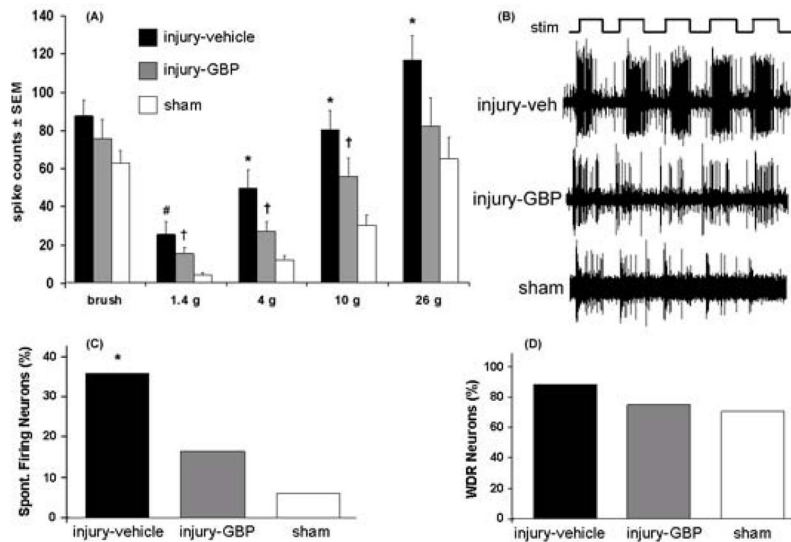
Fig. 2.
Electrophysiological responses in the spinal dorsal horn. **(A)** The total number of evoked spikes is not different between groups in response to brush. The *injury-vehicle* group has significantly greater firing than the *sham* group across all von Frey stimuli. For the 4, 10, and 26 g von Frey filaments, the number of evoked spikes is significantly reduced for the *injury-GBP* group compared to the *injury-vehicle* group. For 1.4, 4, and 10 g von Frey filaments though, the *injury-GBP* group is still significantly greater than the *sham* group. The asterisk (*) denotes significant differences (p<0.047) between *injury-vehicle* and both *injury-GBP* and *sham*; the pound sign (#) denotes a significant difference (p=0.0001) between *injury-vehicle* and *sham* and the dagger (†) denotes significant differences (p<0.036) between *injury-GBP* and *sham*. **(B)** Representative extracellular recordings for *injury-vehicle*, *injury-GBP*, and *sham* groups during the application of a 26 g von Frey filament (stim). **(C)** The percentage of spontaneously firing neurons is significantly decreased for *injury-GBP* (*p=0.048) and *sham* (*p=0.002) relative to *injury-vehicle*. **(D)** The percentage of wide dynamic range (WDR) neurons is lower in the *injury-GBP* and *sham* groups than the *injury-vehicle*.
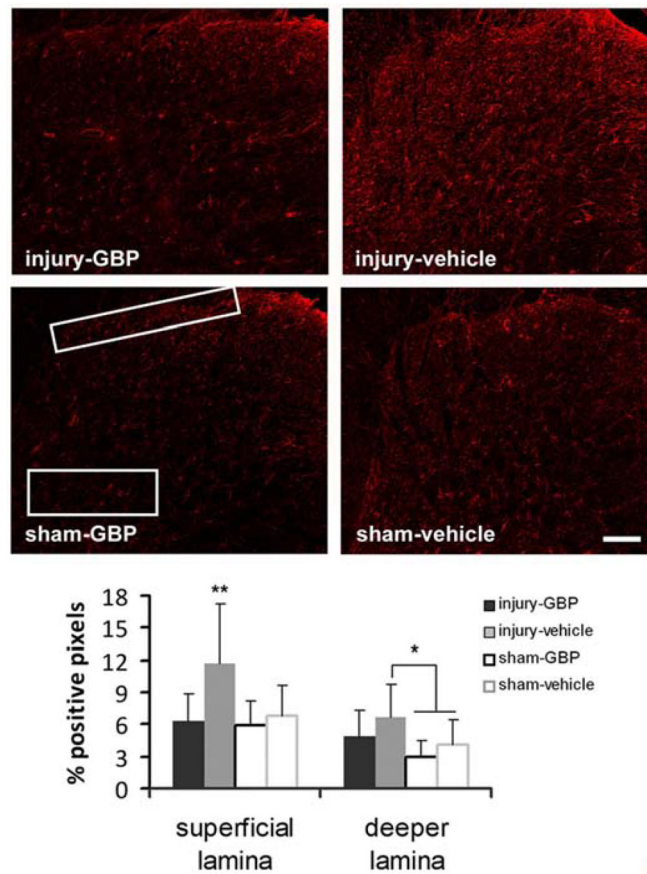
Fig. 3.
Spinal GFAP expression in the superficial and deep laminae of the dorsal horn after injury or sham with either gabapentin (*injury-GBP*, *sham-GBP*) or vehicle (*injury-vehicle*, *sham-vehicle*) treatment. In the superficial lamina, GFAP is significantly elevated (**p<0.001) in *injury-vehicle* above all other groups (*injury-GBP*, *sham-GBP*, *sham-vehicle*). In contrast, the increase in *injury-vehicle* is only significantly higher (*p<0.01) than *sham-GBP* and *sham-vehicle* in the deeper lamina. There is no difference between *injury-GBP*, *sham-GBP*, and *sham-vehicle* in either region. The scale bar (100 μm) applies to all panels.
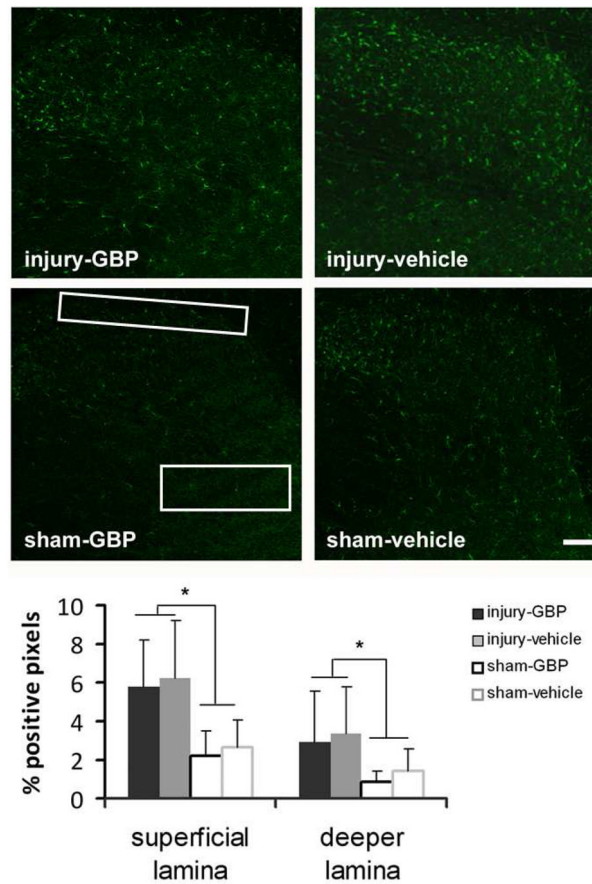
Fig. 4.
Spinal Iba1 expression in the superficial and deep laminae of the dorsal horn after injury or sham procedures is unchanged with gabapentin (*injury-GBP*, *sham-GBP*) treatment compared to vehicle (*injury-vehicle*, *sham-vehicle*). Iba1 expression at day 7 exhibits the same trend in both dorsal horn regions, with no significant difference between *injury-GBP* and *injury-vehicle* in either the superficial or the deeper laminae. There is a significant difference (*p<0.01) between those groups and both *sham-GBP* and *sham-vehicle*. The scale bar (100 µm) applies to all panels.