

Spinal neuronal plasticity is evident within 1 day after a painful cervical facet joint injury

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HIGHLIGHTS

- Excessive cervical facet capsular ligament stretch induces persistent hyperalgesia.
- It is unclear when spinal modifications are induced after painful joint injury.
- Dorsal horn hyperexcitability develops between 6 and 24 h after facet injury.
- The development of mechanical hyperalgesia parallels spinal hyperexcitability.

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ABSTRACT

Excessive stretch of the cervical facet capsular ligament induces persistent pain and spinal plasticity at later time points. Yet, it is not known when such spinal modifications are initiated following this painful injury. This study investigates the development of hyperalgesia and neuronal hyperexcitability in the spinal cord after a facet joint injury. Behavioral sensitivity was measured in a model of painful C6/C7 facet joint injury in the rat, and neuronal hyperexcitability in the spinal cord was evaluated at 6 h and 1 day after injury or a sham procedure, in separate groups. Extracellular recordings of C6/C7 dorsal horn neuronal activity (229 neurons) were used to quantify spontaneous and evoked firing. Rats exhibited no change in sensitivity to mechanical stimulation of the forepaw at 6 h, but did exhibit increased sensitivity at 1 day after injury ($p = 0.012$). At 6 h, both spontaneous neuronal activity and firing evoked by light brushing, pinch, and von Frey filaments (1.4–26 g) applied at the forepaw were not different between sham and injury. At 1 day, spontaneous firing was noted in a greater number of neurons after injury than sham ($p < 0.04$). Evoked firing was also increased 1 day after injury compared to normal and sham ($p < 0.03$). Dorsal horn hyperexcitability and increased spontaneous firing developed between 6 and 24 h after painful facet injury, suggesting that the development of hyperalgesia parallels dorsal horn hyperexcitability following mechanical facet joint injury, and these spinal mechanisms are initiated as early as 1 day after injury.

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1. Introduction

Chronic pain affects an estimated 1/3 of adults in the United States, costing \$635 billion annually [29]. In particular, chronic facet joint-mediated pain is prevalent in the lumbar and cervical spines [21,30]. The cervical facet joints are reported to be a source of pain in 54–60% of individuals with chronic pain from whiplash [19,21]. Although many cadaveric studies report abnormal motions of the cervical facet joints and capsule stretch during whiplash [1,26,33],

the physiologic sequelae leading to chronic pain from that injury remain unclear.

The facet capsular ligament is innervated by proprioceptive and nociceptive mechanoreceptors with projections largely to the same spinal level as the facet from which they originate [2,23,25]. During facet capsule stretch, capsule afferents increase their firing rates with increases in applied capsule strain [2,20]. Increased peripheral neuronal input to the spinal cord can be sufficient to produce long-lasting central sensitization via increased excitability of dorsal horn spinal neurons, leading to hyperalgesia (increased pain sensitivity) and allodynia (painful response to normally non-noxious stimuli) that can be initiated as early as minutes or hours after the insult [13,28,34,35]. Central sensitization often involves wide dynamic range (WDR) neurons, a subpopulation of second-order cells found in the deep laminae (III–VI) of the dorsal horn [3,4].

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Although central sensitization has been studied in several models of knee inflammation and temporomandibular joint pain [10,14,22], such models do not use mechanical injury. Transient mechanical facet joint trauma induces sustained behavioral sensitivity and neuronal hyperexcitability by 7 days [17,27]. Yet, it is not known how early such putative spinal changes develop after a mechanical facet injury and how the timing of the onset of spinal involvement relates to pain.

This study investigated the development of hyperalgesia and neuronal hyperexcitability in the spinal cord at either 6 h or 1 day after a painful cervical facet capsule stretch. Extracellular potentials were recorded from the deep laminae of the dorsal horn at those times to assess spontaneous firing and evoked responses to peripheral mechanical stimuli. Because hyperalgesia is evident 1 day after facet injury [17], we hypothesized that dorsal horn neuronal firing also would increase by that time.

2. Methods

All experimental procedures were approved by the University of Pennsylvania IACUC and carried out under the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain [37]. Male Holtzman rats (350–464 g) were anesthetized with isoflurane (4% induction, 2–3% maintenance). A bilateral distraction of the C6/C7 facet joint was administered using a custom loading device as previously described [16]. The C6 vertebra was distracted 0.7 mm rostrally to stretch the facet capsule across the joint ($n=10$ rats). A separate sham group ($n=10$) underwent surgical procedures identical to the stretch group, including mounting on the loading device, but no joint distraction. Post-operative handling was the same for all groups; after recovering from anesthesia rats were returned to their cages with free access to food and water until behavioral and electrophysiological testing at either 6 h ($n=4$ injury, $n=4$ sham) or 1 day ($n=6$ injury, $n=6$ sham). Normal rats ($n=6$) were included for both assays as an additional control group.

Behavioral sensitivity in both forepaws was assessed preoperatively and at 6 h or 1 day after injury. A series of von Frey filaments (1.4–26 g) was applied in increasing order to the left and right forepaw to determine the filament at which a rat responded by withdrawing, licking, or shaking the forepaw [17].

Immediately after behavioral testing at the designated time point (6 h or 1 day), rats were anesthetized with sodium pentobarbital (45 mg/kg, i.p.) and given supplementary doses (5–10 mg/kg, i.p.) as needed. The spinal cord was exposed bilaterally at C6/C7 and bathed in 37 °C mineral oil. Rats were immobilized on a stereotaxic frame and core temperature was maintained at 35–37 °C.

Extracellular potentials were recorded using a glass-insulated tungsten electrode (FHC), and signals were digitally sampled at 25 kHz (Micro1401; CED). A micropositioner lowered the electrode to the deep laminae (III–VI) of the C6 and C7 dorsal horn; these laminae include WDR neurons that play a role in central sensitization [3,4,31]. Mechanosensitive neurons were identified by brushing the plantar surface of the forepaw. Stimuli were then applied to the forepaw at 30 s intervals, including a 2-s baseline period before each stimulus, 10 s of light brushing, 5 consecutive 1-s stimulations at 1-s intervals with von Frey filaments (1.4 g, 4 g, 10 g, 26 g), and 10 s of noxious pinch by a 60 g vascular clip. After recordings were complete, rats were given an overdose of sodium pentobarbital (>100 mg/kg) to terminate the non-survival procedure.

Voltage recordings from each neuron were spike-sorted using Spike2 (CED) to evaluate individual neurons. Baseline spontaneous activity was established by totaling the number of spikes in the 2-s baseline period. For brush and pinch stimuli, evoked spikes were summed over the 10 s of continuous stimulation. For von Frey

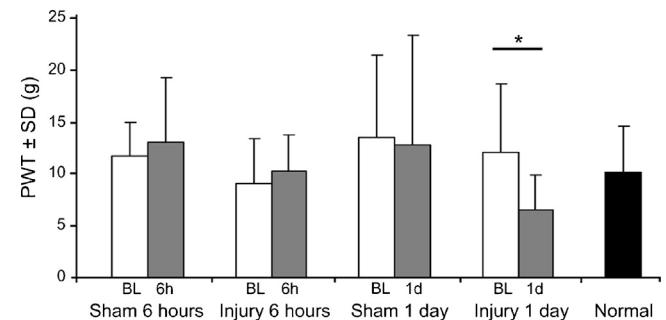


Fig. 1. Paw withdrawal threshold (PWT) at baseline (BL) prior to surgery, and at either 6 h or 1 day. The PWT at BL for each group was not different from normal. At 6 h, PWT was not different from baseline in either group. PWT was significantly reduced ($p=0.012$) from baseline at 1 day after injury but was unchanged at that time point in sham.

stimuli, each filament had a total of 5 spike counts since each was applied to the forepaw 5 times. Baseline spike rates were subtracted for each stimulus to isolate the evoked response, and spike counts were log-transformed due to a positive skew in the distribution of spike totals.

Statistical analyses were performed using JMP9 (SAS) with $\alpha=0.05$. A repeated-measures ANOVA compared withdrawal thresholds between groups at each time point. Pearson's Chi-square test evaluated differences in the number of spontaneously firing cells in each group. Evoked responses were compared between sham, injury, and normal rats using an ANOVA with post hoc Tukey's HSD test. Neurons having evoked responses with studentized residuals greater than 2 were excluded from statistical analysis. Behavioral data are presented as mean \pm SD and spike counts as mean \pm SEM.

3. Results

Paw withdrawal threshold (PWT) at 6 h was not changed from baseline (BL) for the injury group (9.0 ± 4.3 g BL; 10.2 ± 3.4 g at 6 h) or the sham group (11.7 ± 3.2 g BL; 12.9 ± 6.2 g at 6 h) (Fig. 1). At 1 day, the sham group (13.4 ± 7.9 g) also was not changed from BL (12.8 ± 10.4 g); however, the PWT was significantly reduced ($p=0.012$) at 1 day after facet capsule injury (12.0 ± 6.6 g BL; 6.5 ± 3.3 g at 1 day) (Fig. 1). The baseline PWT for each group was not different from the PWT (10.2 ± 4.4 g) measured in the normal rats (Fig. 1).

A total of 229 neurons (46 ± 13 neurons/group) were recorded at an average depth of 636 ± 170 μ m (Table 1), which includes laminae III–VI in the rat spinal cord [31]. Of the 229 neurons, 31 neurons were removed from analysis because their evoked responses had studentized residuals greater than 2 (6 ± 4 neurons/group). Two groups of cells were classified based on spontaneous activity during the baseline period – quiescent neurons with no baseline firing (Fig. 2a) and active neurons with baseline firing greater than 1 spike/s (Fig. 2b). Eighty percent of neurons in the normal group had no spontaneous firing and 5% were active. At 6 h, the numbers of quiescent neurons (68% after injury, 64% after sham) and active

Table 1

Total number of neurons recorded for each level of spinal cord.

	C6	C7	Group total	Average depth
Injury, 6H	22	19	41	633 ± 167 μ m
Sham, 6H	25	11	36	656 ± 175 μ m
Injury, D1	29	12	41	652 ± 152 μ m
Sham, D1	27	15	42	607 ± 170 μ m
Normal	33	36	69	646 ± 174 μ m
Totals	136	93	229	636 ± 170 μ m

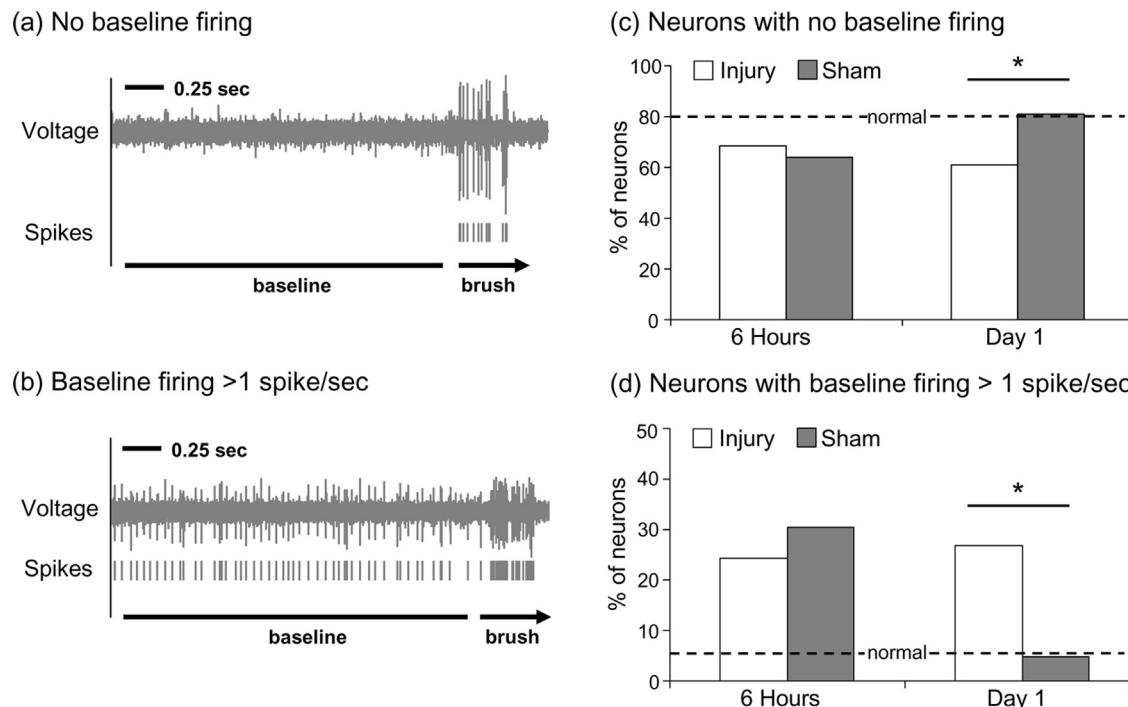


Fig. 2. Spontaneous firing in the spinal dorsal horn. Example recorded voltages from neurons with (a) no baseline firing and (b) baseline firing greater than 1 spike/s. (c) The number of quiescent neurons (presented as percent of total neurons) was unchanged at 6 h relative to sham at that time point, but at 1 day was significantly lower (* $p=0.038$) after injury compared to sham. (d) The number of active neurons exhibited a similar trend with a significantly greater number of neurons (* $p=0.006$) at 1 day after injury. Percentages of cells in the normal group are shown (dotted line) for (c) and (d).

neurons (24% after injury, 31% after sham) were not different from each other (Fig. 2). At 1 day after injury the numbers of quiescent neurons (61% after injury, 81% after sham, $p=0.04$) and active neurons (27% after injury, 5% after sham, $p=0.006$) were significantly different (Fig. 2).

Overall, evoked firing was elevated at 6 h and 1 day after injury, but firing returned to normal levels in the sham group at 1 day (Fig. 3). The evoked responses for light brushing and noxious pinch were not different between the 6 h sham (107 ± 15 spikes for brush, 78 ± 15 spikes for pinch), 6 h injury (91 ± 12 spikes, 79 ± 11 spikes), or 1 day injury (89 ± 8 spikes, 77 ± 15 spikes) groups, and each was elevated over normal (48 ± 4 spikes, 29 ± 5 spikes) for both brush ($p<0.003$) and pinch ($p<0.0001$) (Fig. 3a). The evoked response for the injury group at 1 day also was significantly higher than sham at 1 day (59 ± 7 spikes, 32 ± 5 spikes) for brush ($p=0.003$) and pinch ($p=0.01$) (Fig. 3a). Evoked responses for the 4 g, 10 g, and 26 g von Frey filaments were not different between either of the groups at 6 h or the injury group at 1 day. However, for the 4 g, 10 g, and 26 g filaments, each of the 6 h sham, 6 h injury, and 1 day injury groups exhibited significantly elevated firing over both 1 day sham ($p<0.004$) and normal ($p<0.02$) (Fig. 3b). For the 1.4 g filament, only the 6 h sham and 1 day injury groups demonstrated evoked firing that was greater than 1 day after sham ($p<0.03$) and normal responses ($p<0.007$) (Fig. 3b). There was no difference between normal firing and 1 day sham responses for any stimulus.

horn neurons at 1 day was detected as an increase in both spontaneous and evoked firing (Figs. 2 and 3). These results suggest that neuronal plasticity indicative of central sensitization develops early after facet joint injury and plays a role in the development of the

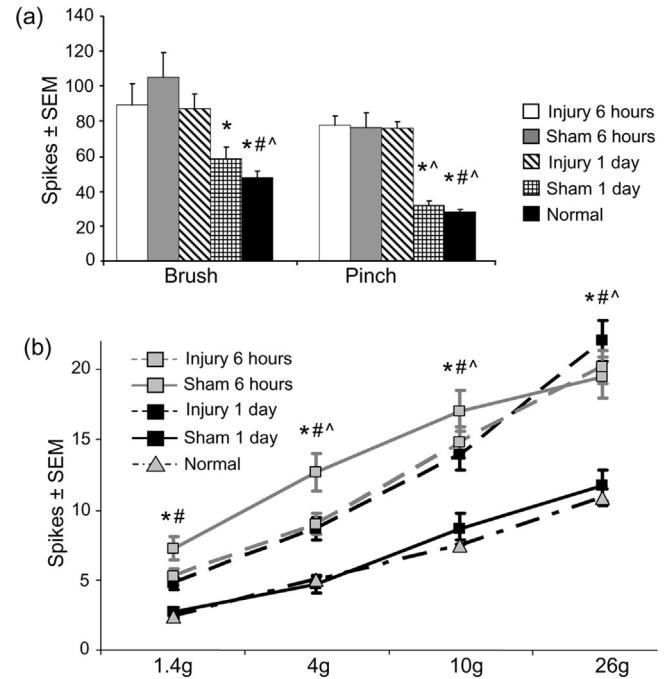


Fig. 3. Evoked firing in the spinal dorsal horn. (a) Normal rats and those at 1 day after sham exhibited significantly lower evoked responses to light brush and noxious pinch than those at 1 day after injury (* $p<0.014$), while only normal rats were significantly lower than those at 6 h after sham (# $p<0.0008$) and 6 h after injury (# $p<0.004$). (b) In response to von Frey filament stimuli, normal rats and those at 1 day after sham exhibited significantly less evoked firing than those at 1 day after injury (* $p<0.03$), 6 h after sham (# $p<0.0006$), and 6 h after injury (# $p<0.017$).

4. Discussion

Neuronal hyperexcitability in the dorsal horn develops within 1 day after a mechanical facet joint injury, corresponding to the development of behavioral sensitivity (Figs. 1–3). Although neuronal hyperexcitability was evident at 1 day after painful facet joint injury, neither behavioral nor neuronal responses were different from sham at 6 h after the injury. The hyperexcitability in dorsal

behavioral sensitivity that persists at later time points in this model [17,27].

There were no behavioral or electrophysiological differences between the injury and sham groups at 6 h. However, at 6 h, 31% of neurons in sham rats and 24% in injured rats exhibited spontaneous firing greater than 1 spike/s, similar to those percentages found at 1 day after injury (27%) (Fig. 2). Both evoked and spontaneous firing were elevated over normal for both injury and sham at 6 h, suggesting that afferent activity may be acutely potentiated by the surgical procedure without causing secondary behavioral sensitivity. However, the neurons largely return to normal activity levels over the following hours in sham controls while spontaneous activity and hyperexcitability persist after injury (Figs. 2 and 3). Although it is possible that the studies performed at 6 h may be affected by interactions between the anesthetics used for the initial surgery and electrophysiology procedures, the sham and normal groups control for these effects.

The development of mechanical hyperalgesia parallels the development of persistent spinal neuronal hyperexcitability. At 1 day after a painful injury, there was a significant decrease in PWT (Fig. 1), which is consistent with other studies of behavioral sensitivity after painful facet capsule stretch [17,32]. In fact, here PWT was reduced to below the baseline 10–15 g threshold in normal rats, suggesting the recruitment of typically non-nociceptive fibers and WDR neurons for nociception [15]. Consistent with clinical reports, mechanical hyperalgesia in this study was measured in the forepaw, a site remote from the injury but with afferents that terminate in the same dorsal horn regions as those from the C6/C7 facet joint [5,11]. Secondary hyperalgesia is commonly observed in centrally-mediated pain after whiplash and in central sensitization resulting from the potentiation of dorsal horn neurons that receive somatosensory and nociceptive input from both the primary injury and secondary sites [15].

Spontaneous activity in primary afferents is a potential mechanism for the initiation and long-term maintenance of central sensitization [6,15]. A greater percentage of dorsal horn neurons (27%) exhibited spontaneous firing greater than 1 spike/s at 1 day after painful injury compared to sham (5%) and normal (5%) (Fig. 2). This finding is supported by reports that up to 1/3 of primary sensory neurons develop spontaneous activity between 6 and 30 h after peripheral nerve transection, in conjunction with the onset of tactile allodynia [6,7,24]. Although spontaneous A δ - and C-fiber nociceptor activity and low-threshold A β -fibers are proposed to drive central sensitization after injury [6,8,18], better classification of the primary neurons is necessary to determine which dorsal horn populations are involved in the hyperexcitability observed here. Of note, this painful facet joint model is a ligamentous injury, not an explicit nerve injury, though axonal swelling indicative of secondary axotomy has been noted in a caprine model after similar facet capsule stretch [12]. Nonetheless, taken together with the literature, the increased spontaneous activity in the spinal cord at 1 day after painful facet injury suggests that the etiology of pain and central sensitization following mechanical joint injury may be similar to peripheral neuropathic injuries.

Evoked activity was recorded from the same neurons in which spontaneous activity was quantified (Fig. 3). There was a significantly greater evoked response to light brush and the 4 g von Frey filament 1 day after injury, indicating allodynia since both are non-noxious stimuli in normal rats. These results, in conjunction with the increased spontaneous activity, suggest that low-threshold A β -fibers may have a role in the development of spinal hypersensitivity following facet injury. The evoked responses to noxious stimuli also were significantly greater 1 day after injury (Fig. 3). The consistent increase in excitability across both non-noxious and noxious stimuli further supports the involvement of WDR neurons in the development of central sensitization after facet injury,

because WDR neurons receive input from both types of afferents. The increases in evoked activity following both stimuli are consistent with the hyperexcitability observed later at 7 days after this facet capsule injury [27], suggesting that the mechanisms of neuronal plasticity associated with long-term development of central sensitization begin between 6 and 24 h after facet injury. One potential mechanism may involve PKC ϵ , a downstream regulator of the glutamatergic system, which is upregulated in the dorsal horn 1 day after this injury [9,36] and also regulates NMDA receptor potentiation, which is a key step in the induction and maintenance of sensitization [15].

This study suggests that dorsal horn hyperexcitability, a sign of central sensitization, develops very early after a persistent painful facet capsule injury, paralleling the onset of behavioral hypersensitivity. These findings suggest there is a critical and short time between injury and the development of persistent elevated spinal responses. This has importance in the design of more effective acute treatments for traumatic joint injuries, and provides a foundation for future studies to identify key regulators in pain following traumatic joint injury.

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