Mechanical sensitization of cutaneous sensory fibers in the spared nerve injury mouse model

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Abstract

Background: The spared nerve injury (SNI) model of neuropathic pain produces robust and reproducible behavioral mechanical hypersensitivity. Although this rodent model of neuropathic pain has been well established and widely used, peripheral mechanisms underlying this phenotype remain incompletely understood. Here we investigated the role of cutaneous sensory fibers in the maintenance of mechanical hyperalgesia in mice post-SNI.

Findings: SNI produced robust, long-lasting behavioral mechanical hypersensitivity compared to sham and naïve controls beginning by post-operative day (POD) 1 and continuing through at least POD 180. We performed teased fiber recordings on single cutaneous fibers from the spared sural nerve using ex vivo skin-nerve preparations. Recordings were made between POD 16–42 after SNI or sham surgery. Aδ-mechanoreceptors (AM) and C fibers, many of which are nociceptors, from SNI mice fired significantly more action potentials in response to suprathreshold mechanical stimulation than did fibers from either sham or naïve control mice. However, there was no increase in spontaneous activity.

Conclusions: To our knowledge, this is the first study evaluating the contribution of primary afferent fibers in the SNI model. These data suggest that enhanced suprathreshold firing in AM and C fibers may play a role in the marked, persistent mechanical hypersensitivity observed in this model. These results may provide insight into mechanisms underlying neuropathic pain in humans.

Keywords: Neuropathic, Nociceptor, Sensory neuron, C fiber, A fiber, Hyperalgesia, Mechanotransduction
and thereby, may contribute to the maintenance of mechanical hypersensitivity after SNI.

**SNI mice exhibit long-lasting behavioral mechanical hypersensitivity**

As previously reported, SNI mice exhibited pronounced hypersensitivity to mechanical stimuli compared to sham and naïve animals beginning by post-operative day (POD) 1 and continuing for at least 6 months post-surgery (Figure 1). The dynamic component of the Light Touch Behavioral Assay [14] was used as a control to ensure adequate denervation of the tibial territory post-SNI injury. SNI mice showed significant tibial desensitization, measured by percent response to a puffed cotton swab applied to the tibial territory of the glabrous skin, from POD 1–42 (Figure 1A, p < 0.005), which was expected because of transection of the tibial nerve and subsequent denervation of the skin territory. However, by POD 49, sensation in the tibial area began to return (Figure 1A). In sural nerve-targeted behavioral testing, SNI mice showed a significant decrease in paw withdrawal threshold by POD 1 through POD 180 (Figure 1B, p < 0.005) and exhibited a significantly higher percent response to the suprathreshold 3.31 mN monofilament from POD 1–49 (Figure 1C, p < 0.005). Locomotor activity of the mice did not differ between groups (data not shown). Complete transection of the tibial and common peroneal nerves was validated post-mortem. Overall, these results parallel those found in rat [1] and previously shown in mouse [3,9].

**Aδ and C fibers from SNI mice exhibit enhanced mechanical firing**

Sensory afferent sensitization is known to contribute to mechanical hypersensitivity observed in diabetic and chemotherapy-induced neuropathies [15,16], and has been shown to contribute to hypersensitivity observed in other models of nerve injury [10-12]. To assess the contribution of cutaneous sensory afferents to SNI-induced mechanical hypersensitivity, we performed ex vivo teased fiber recordings on the spared sural nerve. Fibers from SNI animals exhibited enhanced suprathreshold firing compared to controls. Specifically, Aδ-mechanoreceptor (AM) fibers fired an average of 22% more action potentials across all forces compared to sham or naïve mice, and C fibers exhibited 24% more action potentials across all forces compared to sham and naïve mice (AM: Figure 2A, p < 0.01; C: Figure 2C, p < 0.05). Post hoc comparison showed no differences at individual forces for either AM or C fibers (Figure 2). There were no differences in mechanical thresholds or conduction velocity of any fiber subtype across treatment groups (Table 1). There was no difference in the percentage of Aβ, Aδ, and C fibers encountered in preparations from the different surgical groups (Figure 3A, p > 0.05). There was also no

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**Figure 1 SNI mice exhibit prominent behavioral hypersensitivity.**

A) In response to dynamic stroke of a puffed cotton swab, SNI mice show significant desensitization of the tibial territory beginning POD 1 and continuing through POD 42 compared to sham or naïve animals (**p<0.005**). At POD 49 SNI mice regain sensation in the tibial territory, in that SNI mice still show some sensitization compared to naïve animals (*p<0.05) but not sham animals (p>0.05). Treatments were compared across time using a repeated measure 2-way ANOVA with Tukey’s post hoc comparisons.

B) SNI mice show a significant decrease in the 50% mechanical withdrawal threshold beginning POD 1 and continuing through POD 49 compared to sham or naïve mice (**p<0.005**). Furthermore, SNI mice continue to show a significant decrease in mechanical withdrawal threshold at POD 90 compared to sham (**p<0.005**) and at POD 180 compared to sham (*p<0.05*). Treatments were compared across time using a 2-way ANOVA with Bonferroni post hoc tests. SNI and sham treatments were compared at POD 90 and POD 180 using Mann–Whitney U tests.

C) In response to repeated stimulus with a 3.31 mN monofilament, SNI mice exhibit prominent hypersensitivity beginning POD 1 and continuing through at least POD 49 compared to sham (***p<0.005**). Treatments were compared across time using a 2-way ANOVA with Bonferroni post hoc tests. Error bars for all three graphs indicate S.E.M.
There was an overall difference in the distribution of Aβ fibers among SNI, sham and naïve mice (Figure 3C, p < 0.05). There was a decrease in the distribution of slowly adapting (SA) A-beta fibers from SNI animals compared to sham (Figure 3C, p< 0.5), although no difference was observed between SNI and naive animals (Figure 3C, p> 0.5). We also measured spontaneous activity because spontaneous activity in primary afferent fibers accompanies other nerve injury animal models including SNL and CCI [10-13,17-19]. There was no difference in the percentage of AM or C fibers that exhibited spontaneous activity in SNI versus sham or naïve groups (Figure 4, p > 0.05). Furthermore, preliminary analysis of Aβ fibers from SNI preparations also does

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**Figure 2** Aδ-Mechanoreceptor and C fibers in SNI mice exhibit enhanced mechanical firing. Using *ex vivo* skin nerve recordings, 10 sec mechanical stimuli ranging 5-200 mN were applied to the receptive field of each fiber using a 0.8 mm probe. All recordings were performed on the sural nerve and its innervating territory. A) Aδ-Mechanoreceptor (AM) fibers fired an average 22% more action potentials per second across all forces compared to sham or naïve animals (***p<0.001). B) Examples of AM fiber action potentials evoked by sustained mechanical stimuli in naïve, sham and SNI treatment groups. C) C fibers fired an average 24% more action potentials per second across all forces compared to sham and 28% more compared to naïve animals (**p<0.01). D) Examples of C fiber action potentials evoked by sustained mechanical stimuli in naïve, sham and SNI treatment groups. Number of action potentials fired per second was compared across all forces and treatment groups using a 2-way ANOVA with Bonferroni post hoc comparisons. Error bars indicate SEM.

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**Table 1 Summary of fiber properties in Naive, Sham and SNI mice**

| Fiber type | Genotype | n     | Median von frey threshold (mN) | Lower quartile | Upper quartile | Mean conduction velocity (m/s) ±SEM |
|------------|----------|-------|--------------------------------|----------------|--------------|-----------------------------------|
| Aδ-Mechanoreceptor | Naive    | 24    | 6.82                           | 6.82           | 13.88        | 4.30 ± 0.41                      |
|             | Sham     | 25    | 6.82                           | 5.41           | 11.70        | 5.33 ± 0.62                      |
|             | SNI      | 20    | 6.82                           | 4.00           | 11.70        | 3.95 ± 0.71                      |
| D-hair     | Naive    | 5     | 0.66                           | 0.66           | 1.15         | 4.80 ± 0.86                      |
|             | Sham     | 7     | 0.66                           | 0.27           | 0.66         | 4.93 ± 0.80                      |
|             | SNI      | 7     | 0.27                           | 0.23           | 1.63         | 5.57 ± 0.54                      |
| C          | Naive    | 11    | 6.82                           | 6.82           | 11.70        | 0.66 ± 0.10                      |
|             | Sham     | 17    | 11.70                          | 5.41           | 14.60        | 0.68 ± 0.06                      |
|             | SNI      | 13    | 6.82                           | 4.00           | 11.70        | 0.65 ± 0.08                      |
| RA-Aβ      | Naive    | 7     | 1.63                           | 0.66           | 4.00         | 11.60 ± 1.05                     |
|             | Sham     | 10    | 0.66                           | 0.66           | 1.63         | 13.99 ± 1.38                     |
|             | SNI      | 17    | 1.63                           | 0.66           | 1.63         | 12.85 ± 0.58                     |
| SA-Aβ      | Naive    | 8     | 1.63                           | 1.63           | 3.41         | 13.47 ± 1.46                     |
|             | Sham     | 13    | 1.63                           | .63            | 2.81         | 14.49 ± 1.33                     |
|             | SNI      | 11    | 1.63                           | 0.66           | 4.00         | 14.76 ± 1.15                     |
not suggest increased spontaneous firing, as has been observed in SNL or CCI (data not shown). There was also no difference in the frequency of spontaneous action potential firing in SNI versus controls for any fiber type (data not shown). In dividing groups into early and late stages post SNI, there was no significant difference in the spontaneous activity of fibers recorded at POD 16–21 compared to those at POD 37–42 for Aβ, Aδ, or C fibers (data not shown).

To our knowledge, this is the first study to assess sensitization of primary afferent fibers in SNI. Our results suggest that enhanced suprathreshold firing in AM and C fibers may contribute to the robust behavioral mechanical hypersensitivity that occurs in the SNI model of neuropathic pain. Sensitized nociceptors might contribute to SNI-induced behavioral hypersensitivity either directly through increased suprathreshold firing in response to external stimuli, or indirectly by driving central sensitization [10,11]. Previous nerve injury studies that used the SNL and CCI models of nerve injury suggest that Wallerian Degeneration of injured nerves drives sensitization of adjacent intact afferent fibers [10-12]. However, unlike CCI and SNL, Wallerian Degeneration is not a major factor in the SNI model of neuropathic pain as SNI involves minimal co-mingling of intact and injured afferent fibers [1]. Therefore, a different mechanism(s) likely drives the afferent mechanical sensitization observed in this model. One potential mechanism is paracrine signaling between injured and intact cell bodies within the dorsal root ganglia (DRG), a level where co-mingling...
occurs. A previous study has shown an increase in macrophage infiltration, expression of inflammatory mediators such as IL-6 and TNF-α, and expression of neurotrophins BDNF and NGF in the DRG after sciatic nerve injury [20], and these may be key factors driving afferent sensitization in the SNI model [1]. Alternatively or in addition, at the peripheral terminals, collateral sprouting of intact sensory afferent terminals into the denervated skin territory of the transected nerves has been shown in other neuropathic pain models [21,22], and may also occur and contribute to sensitization in SNI.

It has been shown that degeneration of injured fibers induces spontaneous activity in nearby uninjured primary afferent fibers [23]. Furthermore, previous studies have shown that increased spontaneous activity in A and C fibers can contribute to sensitization after nerve injury in other models of neuropathic pain [10-13,17-19]. However, we did not observe more spontaneous activity in either A or C fibers post-SNI. One explanation for the absence of spontaneous activity may be that ectopic discharge rates change over time after injury. Previous studies on injured primary afferent fibers show that there is a higher frequency of spontaneous activity early after nerve injury (POD 1–3) and less activity in late stages (POD 11–14) [17,18]. Furthermore, studies on uninjured afferents, which show spontaneous activity, have been performed primarily at early stages after nerve injury [13,23]. Thus, our recordings at later stages (POD 16–42) after injury may have occurred after SNI-induced spontaneous activity subsided. Another likely explanation is that spontaneous activity may not be present in the SNI model due to minimal co-mingling of injured and adjacent fibers. Previous reports of spontaneous activity after nerve injury have been recorded from nerve injury models that involve considerable Wallerian Degeneration and extensive co-mingling of intact and injured axons [10-12]. Sensitizing compounds associated with Wallerian Degeneration, such as TNF-α, have been shown to sensitize primary afferent fibers [24]. However, in the absence of co-mingling of intact and injured axons distal to the site of lesion, and the minimal degeneration of injured fibers proximal to the lesion, these compounds may not affect the intact peripheral afferent fibers in the SNI model.

Conclusions
These results may provide insight into the mechanisms underlying neuropathic pain in humans with traumatic peripheral nerve injury. Our results show an increase in suprathreshold firing in Aδ-mechanoreceptor (AM) and C fibers, suggesting that enhanced primary afferent drive may contribute to nerve injury-induced hypersensitivity, and peripheral afferent fibers may be targets for pharmacological treatment of neuropathic pain.
to assess spontaneous activity. Next a feedback-controlled mechanical stimulator was used to deliver increasing sustained mechanical forces (5-200 mN) for 10 sec each with 1 min recovery period between stimuli. Action potentials were recorded and analyzed using Lab Chart Data Acquisition Software (AD Instruments, Colorado Springs, CO).

Data analysis
All data sets were compared between SNI, sham, and naïve groups. Behavioral Data: Percent response to light touch was analyzed across time using repeated measures two-way ANOVA with Tukey’s post hoc test. Mechanical withdrawal thresholds and the percent response to a 3.31 mN monofilament were compared across time (Baseline 1 through POD 49) using a 2-way ANOVA with Bonferroni post hoc analysis. Withdrawal thresholds for SNI and sham were compared POD 90 and POD 180 with Mann Whitney U tests. Skin-Nerve Data: Each fiber type was analyzed for: 1) number of action potentials fired across mechanical forces using a two-way ANOVA with Bonferroni post hoc comparisons, 2) conduction velocity using a one-way ANOVA with Tukey’s multiple comparisons, 3) von Frey thresholds using Kruskal-Wallis with Dunn’s multiple comparisons, and 4) percent spontaneous fibers using Chi-square analysis. Column statistics of each fiber type were analyzed to compare the sum of the average number of action potentials fired across all forces. Percent distribution of the fiber types was compared using Chi-square analysis and Fisher’s exact post hoc test. Data analysis was completed using Prism 6 Software (GraphPad, La Jolla, CA).

Abbreviations
SNI: Spared nerve injury; CCI: Chronic constriction injury; SNL: Spinal nerve ligation; POD: Post, operative day; AM: AS-Mechanoreceptor; DRG: Dorsal root ganglia.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
All authors read and approved the final manuscript. AS and CO conducted the experiments and analyzed the data. AS and CS designed the study and wrote the manuscript.

Acknowledgements
This work was completed with support from the National Institute of Health grants NS040538 and NS070711 to C.L.S.

Received: 29 July 2013 Accepted: 22 November 2013 Published: 29 November 2013

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doi:10.1186/1744-8069-9-61

Cite this article as: Smith et al.: Mechanical sensitization of cutaneous sensory fibers in the spared nerve injury mouse model. Molecular Pain 2013 9:61.