Distinct Membrane Effects of Spinal Nerve Ligation on Injured and Adjacent Dorsal Root Ganglion Neurons in Rats

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Background: Painful peripheral nerve injury results in disordered sensory neuron function that contributes to the pathogenesis of neuropathic pain. However, the relative roles of neurons with transected axons versus intact adjacent neurons have not been resolved. An essential first step is identification of electrophysiologic changes in these two neuronal populations after partial nerve damage.

Methods: Twenty days after spinal nerve ligation (SNL), intracellular recordings were obtained from axotomized fifth lumbar (L5) dorsal root ganglion neurons and adjacent, intact L4 neurons, as well as from control neurons and others subjected to sham-SNL surgery.

Results: Pronounced electrophysiologic changes were seen only in L5 neurons after SNL. Both Aα/β and Aδ neurons showed increased action potential duration, decreased afterhyperpolarization amplitude and duration, and decreased current threshold for action potential initiation. Aα/β neurons showed resting membrane potential depolarization, and increased repetitive firing during sustained depolarization developed in Aδ neurons. The afterhyperpolarization duration in neurons with C fibers shortened after axotomy. In contrast to the axotomized L5 neurons, neighboring L4 neurons showed no changes in action potential duration, afterhyperpolarization dimensions, or excitability after SNL. Depolarization rate (dv/dt) increased after SNL in L4 Aα/β and Aδ neurons but decreased in L5 neurons. Time-dependent rectification during hyperpolarizing current injection (sag) was greater after SNL in Aα/β L4 neurons compared with L5. Sham-SNL surgery produced only a decreased input resistance in Aα/β neurons and a decreased conduction velocity in medium-sized cells. In the L5 ganglion after axotomy, a novel set of neurons, consisting of 24% of the myelinated population, exhibited long action potential durations despite myelinated neuron conduction velocities, particularly depolarized resting membrane potential, low depolarization rate, and absence of sag.

Conclusions: These findings indicate that nerve injury–induced electrical instability is restricted to axotomized neurons and is absent in adjacent intact neurons.

THE processes generating pain after peripheral nerve injury are mostly unresolved, including the site, timing, and specific cellular events responsible for clinical findings in painful neuropathic conditions. Although pain is unavoidably subjective and cannot be directly ascertained in animal studies, various models of peripheral nerve injury have yielded abundant observations of physiologic and anatomical defects. A current challenge is to define the relative importance of the diverse changes in sensory pathways induced by neural inflammation, ischemia, and axotomy. Abnormal function that potentially contributes to the emergence of pain behavior in experimental animals is seen in the spinal cord dorsal horn, supraspinal sites, and descending modulatory pathways. The primary sensory neurons themselves develop novel connectivity, shifts in receptor sensitivity and neurotransmitter expression, and selective cell death. In addition, injury is accompanied by altered membrane function due to modified channel expression and performance, which has the potential to directly increase neuronal excitability (reviewed in Millan). The individual contributions of these various pathologic events to generating pain after nerve injury have not been clearly defined.

Amplified responsiveness to cutaneous stimuli is a hallmark of clinical and experimental neuropathic pain. Therefore, animal models necessarily entail incomplete nerve injury that retains fibers connected to their peripheral receptive fields as well as axotomized fibers. As a critical first step in identifying which injury-induced processes generate pain, it is essential to distinguish the relative roles of intact and axotomized fibers. For the subset of neurons that have complete peripheral axotomy, previous reports have shown development of ectopic activity at the site of nerve injury and proximally at the neuronal somata in the dorsal root ganglia (DRGs), due possibly to altered currents through voltage-gated sodium channels, calcium channels, and potassium channels. Spontaneous activity may directly account for spontaneous pain or paresthesias in human subjects or spontaneous pain behavior in animals. However, to explain increased reactivity to sensory stimuli, it is hypothesized that spontaneous activity originating in transected
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fibers induces heightened responsiveness in secondary sensory neurons of the dorsal horn. Alternatively, recent findings increasingly support a pathogenic role for intact neurons whose axons share a peripheral nerve with degenerating distal fiber segments from axotomized neighboring DRG neurons. Such surviving neurons may themselves demonstrate enhanced excitability.10,11

After spinal nerve ligation (SNL),12 axotomized and intact neurons are segregated anatomically at the level of the dorsal roots and DRGs. Almost all the neurons of the L5 DRG are axotomized after transection of the ventral primary ramus of the L5 spinal nerve, which leaves intact only the small dorsal primary ramus. The large majority of L4 neurons merge with the degenerating L5 axons very proximally at the level of the L6 transverse process, because the other L4 branches to the femoral and obtrurator nerves are small.13 Using the SNL model, this study was designed to compare excitability changes among the relatively pure population of axotomized neurons from the L5 DRG and the intact neurons from L4 that share the sciatic nerve. Because these two sets of neurons are exposed to dissimilar pathogenic processes, we hypothesize that the resulting electrophysiologic modifications will be dissimilar. Sham surgery and the local inflammation it creates may also alter certain aspects of sensory behavior,14 so in addition, we examined electrophysiologic consequences of sham-SNL surgery compared with control neurons from animals subjected only to skin incision. Further, different neuronal types were compared to determine the relative importance of changes in Aβ, Aδ, and C-type neurons. Some of the current data have been published in abstract form.15

Materials and Methods

Studies were performed on tissue from 107 male Sprague-Dawley rats (weight, 150–250 g) obtained from Charles River Laboratories Inc. (Wilmington, MA) after approval from the Medical College of Wisconsin Animal Resource Center in Milwaukee, Wisconsin.

Animal Preparation

Animals were prepared with one of three kinds of surgery. SNL (n = 51 rats) was performed during halothane anesthesia similarly to the previously described method of Kim and Chung.12 Briefly, after exposure of the right paravertebral region, the sixth lumbar transverse process was removed, and the ventral rami of the right L5 and L6 spinal nerves were ligated with 6-0 silk thread and cut distal to the ligature. In contrast to the originally described method, paraspinous muscles and the adjacent articular process were not removed. In other animals, sham surgery was performed identically, including exposure and gentle lifting of the L5 and L6 nerves, but without passage of ligatures or transection of the nerves (sham-SNL, n = 30 rats). In both the sham and actual SNL surgery, care was taken not to manipulate or expose the L4 spinal nerve. Other rats had only anesthesia and lumbar skin incision (n = 26 rats). After surgery, the rats were returned to the animal colony, where they were kept in individual cages under normal housing conditions.

Behavioral Testing

Animals were familiarized with the testing environment for 4 h on the day before the first sensory evaluation. Our sensory testing paradigm was validated by previous study of 150 rats.16 Briefly, hind paws were stimulated in random order with a 22-gauge spinal needle applied with pressure adequate to indent but not penetrate the plantar skin. The probability of a hyperalgesia-type response that included licking, grooming, or sustained elevation of the paw was averaged from the responses on the 3rd, 8th, and 15th days after surgery, and the difference in probability between right and left was determined.

Tissue Preparation

Right L4 and L5 ganglia were removed 20.5 ± 0.5 days after surgery. Rats were anesthetized with halothane (2% in oxygen) by spontaneous ventilation. A laminectomy was performed up to the second thoracic level while the surgical field was bathed with oxygenated artificial cerebrospinal fluid (128 mM NaCl, 3.5 mM KCl, 1.2 mM MgCl₂, 2.3 mM CaCl₂, 1.2 mM NaH₂PO₄, 24.0 mM NaHCO₃, 11.0 mM glucose). The L4 and L5 ganglia and attached dorsal roots were removed, and the connective tissue capsule was dissected away from the ganglion under 15× magnification. DRGs were transferred to a recording chamber and bathed with 35°C artificial cerebrospinal fluid at a pH of 7.35, controlled by aeration of the solution with carbon dioxide.

The proximal cut end of the dorsal root was placed on a pair of platinum wire stimulating electrodes. DRG neurons were viewed using an upright microscope equipped with differential interference contrast optics and infrared illumination. Neuronal soma diameter was determined as the average of the maximum and minimum diameters using a calibrated video image, with the focal plane adjusted to reveal the maximum somatic area.

Electrophysiologic Recording

Recordings for this study were obtained from excised intact ganglia, which avoids phenotypic shifts associated with culture of dissociated cells17 and eliminates consideration of anesthetic and surgical stress that may affect recordings from intact animals.18,19 We recorded intracellularly with microelectrodes, which dialyze the cytosol less than patch electrodes that may significantly alter or eliminate specific membrane currents.20 Micro
Electrodes were fashioned from borosilicate glass (1 mm OD, 0.5 mm ID, with Omega fiber; FHC, Bowdoinham, ME) using a programmable micropipette puller (P-97; Sutter Instruments, Novato, CA). Stable recordings were achieved with microelectrode resistances of 60–90 MΩ when filled with 2M potassium acetate, which minimally alters intracellular ion levels. Recordings were obtained predominantly from neurons in the two outermost cell layers of the dorsal medial aspect of the DRG. This does not introduce a selection bias because there is no clustering of DRG neuronal subtypes, and neurons projecting the plantar skin of the hind paw are found scattered throughout L4 and L5 without somatotopic organization. Neurons were impaled under direct vision by contacting the membrane with the microelectrode and then advancing an additional 1–2 μm or, in some cases, applying an oscillating current to the microelectrode.

For most protocols, such as determination of action potential (AP) characteristics during axonal stimulation, somatic membrane potential (V_m) traces were recorded using an active bridge amplifier (Axoclamp 2B; Axon Instruments, Foster City, CA). However, during protocols in which current was injected through the microelectrode, voltage error was minimized using discontinuous current clamp with a switching rate of 2 kHz, while monitoring for complete settling of electrode potential between sampling. Currents were filtered at 10 kHz (bridge mode) and 1 kHz (discontinuous current clamp mode) and then digitized at 10–40 kHz (Digidata 1322A and Axograph 4.7; Axon Instruments) for data acquisition and analysis. Typical noise levels measured as SD of the resting membrane potential (RMP) using the bridge mode were 0.2 mV, and those using the discontinuous current clamp mode were 0.4–0.6 mV; events such as afterdepolarizations were readily identified with amplitudes of 1 and 2 mV, respectively. Somatic APs were evoked by dorsal root stimulation with square-wave pulses 0.06 ms in duration, using supramaximal stimulation intensity (up to 90 mA). None of the measured parameters were affected by the magnitude of axonal stimulation beyond threshold. Conduction velocity (CV) was calculated by dividing the distance between stimulation and recording sites by the conduction latency. Average dorsal root lengths were 19.3 ± 3.5 mm for L4 and 22.4 ± 2.9 mm for L5. This single stimulation site technique has been shown to accurately determine dorsal root CV for fibers conducting up to 14 m/s but to underestimate CV for faster fibers. Neurons were excluded from analysis (6%) if they lacked an RMP equal to or more negative than -40 mV and an AP amplitude of 50 mV or greater. The overshoot of the AP to a potential above 0 mV was not used as a criterion because this may be absent in healthy...
cells. A total of 539 neurons were included in electrophysiologic analysis.

Measured electrophysiologic parameters are depicted in figure 1A. RMP was determined after stable recording was achieved, typically after 2 min. AP amplitude was measured from RMP to the AP peak. AP duration was determined at a voltage 5% from RMP to the AP peak (AP95), to eliminate the contribution from uncertain determination of the moment of AP initiation. Findings of the study were comparable when the AP duration was measured at the base or midpoint between RMP and peak (data not shown). The differentiated AP waveform (fig. 1B) was used to measure peak positive dV/dt of the ascending limb of the AP. The presence of a hump or inflection on the descending limb of the AP was determined by examination of the differentiated trace (fig. 1C). Afterhyperpolarization amplitude was measured from RMP to the most hyperpolarized level of the afterhyperpolarization. Duration of the afterhyperpolarization was measured at points representing 50% and 80% recovery of the afterhyperpolarization back to RMP (AHP50 and AHP80, respectively; findings were comparable, so AHP80 data are not shown). Afterhyperpolarization magnitude was also expressed as area under the curve, approximated as the product of afterhyperpolarization amplitude and AHP80 divided by 2.

Neurons were classified according to cell types based on CV and AP duration because this tends to segregate neurons with specific receptive field properties. Neurons with a dorsal root CV less than 1.5 m/s were considered C type, neurons with a CV greater than 15 m/s were considered Aα/β type, and neurons with a CV greater than 1.5 m/s but less than 10 m/s were considered Aδ type. For neurons with a CV between 10 and 15 m/s, long AP duration was used to classify the cells as Aδ types, as described in figure 1D.

We examined time-dependent rectification (sag) of the voltage response to a hyperpolarizing voltage step induced by a sustained (100-ms) 1.2-nA hyperpolarizing current injection, measured as the fraction return of the potential back to RMP. We did not standardize the RMP before current injection because our goal was to identify the contribution of this current to membrane events under the natural conditions of each cell. Input resistance (Rin) was determined from the maximum voltage step generated by a 0.5-nA, 100-ms hyperpolarizing current injected through the microelectrode while recording in discontinuous current clamp mode. This method may somewhat underestimate Rin because of involvement of rectifying currents.

Three direct measures of excitability of DRG neurons were examined. Spontaneous AP occurrence was measured during an interval of 40 s. Rheobase was determined as the minimal current in a gradually increasing series of depolarizing 200-ms pulses that produced an AP (fig. 2). The pattern of impulse generation was determined during current injection steps of at least twice rheobase, at which neurons either produced single APs (fig. 2A) or fired repetitively (fig. 2B).

**Data Analysis and Statistics**

Data from different neuronal types (Aα/β, Aδ, or C) were analyzed separately. For each type, recordings were combined according to type of surgery (SNL, sham-SNL, or skin incision) and DRG level (L4 or L5) into four study groups (control, sham, SNL L4, and SNL L5) as follows. For Aα/β- and Aδ-type neurons, the control group combines recordings from the L4 and L5 ganglia after skin incision surgery plus recordings from the L4 DRG after sham-SNL surgery, because there were no differences among them (data not shown). The sham group for Aα/β and Aδ neurons thus includes only data from the L5 ganglion after sham-SNL surgery. There were fewer C-type recordings because of difficulty in impalement and sustained recording from these cells. Therefore, recordings from L5 DRG neurons after sham-SNL surgery, which showed no changes compared with those from animals having skin incision surgery, were included in the control group for C-type neurons. For all neuron types, data from the L4 and L5 after SNL surgery were analyzed as separate groups.

Data were analyzed using Axograph 4.7 (Axon Instruments), Excel (Microsoft Co., Redmond, WA), and Statistica 6.0 (StatSoft, Tulsa, OK) software. Continuous data are expressed as mean ± SEM. Unless otherwise stated, main effects were tested by analysis of variance.
When a significant main effect was identified, post hoc comparisons between groups were tested conservatively by Bonferroni test. Further nonparametric analysis was performed for parameters that were not normally distributed (Shapiro-Wilk W test) using Kruskal-Wallis analysis of variance by ranks, which in all cases showed comparable results. Therefore, parametric analysis of variance is reported for all \( A/\beta \) and \( A/\delta \) parameters, which is also justified by the large sample sizes, according to the central limit theorem. Significance testing of continuous data for C-type neurons was performed with Kruskal-Wallis analysis of variance because of small sample sizes. Nominal data, such as rates of occurrence of electrophysiologic features, were evaluated by cross-tabulation with significance tested by maximum-likelihood chi-square. When a main effect was significant, four planned post hoc comparisons (each group against control as well as SNL L4 against SNL L5) were tested by Fisher exact test. Because there is no standardized method for correcting for multiple Fisher exact tests, significance for these tests is reported at both the \( P < 0.05 \) and \( P < 0.01 \) levels. Otherwise, significance levels were set at 0.05. In cases in which post hoc analysis shows values for SNL L4 and SNL L5 to be significantly different but neither is significantly different from control, we interpret this to mean that the neurons in the two ganglia are affected dissimilarly by SNL, without attributing this specifically to a change in either group.

**Results**

**Behavioral Testing**

Surgery altered the probability of a hyperalgesia response that included licking, grooming, and sustained withdrawal from nociceptive stimulation (main effect \( P < 0.001 \)), such that the ipsilateral response rate was significantly higher in rats after SNL (0.29 ± 0.05) than after sham-SNL surgery (0.11 ± 0.04; \( P = 0.01 \)) or skin incision alone (−0.01 ± 0.01; \( P < 0.01 \)). Tested this way, sham-SNL pain behavior was not significantly different from animals after skin incision. Consistent with our previous study, hyperalgesic responses were not seen contralateral to SNL or sham-SNL.

**Passive Membrane Properties**

The RMP of \( A/\beta \) neurons was depolarized by axotomy but was unchanged in the SNL L4 group. There was a significant main effect of injury on RMP in \( A/\delta \) neurons, but no group had a significant difference from
control by post hoc analysis, and there was no effect in C-type neurons (fig. 3A). $R_{\text{in}}$ in the SNL L4 group was decreased compared to the SNL L5 group for both $A\alpha/\beta$- and $A\delta$-type neurons (fig. 3B). In addition, $A\alpha/\beta$ neurons in the sham group showed a decrease in $R_{\text{in}}$ compared with control.

**Action Potential Measures**

Distinct effects of injury were evident in AP measures. After SNL, AP amplitude for $A\delta$ neurons was diminished from L5 compared with other groups (fig. 4A), and that for $A\alpha/\beta$ neurons was less in the SNL L5 group compared with sham and SNL L4. SNL substantially increased AP duration of SNL L5 $A\alpha/\beta$ and $A\delta$ neurons compared with other groups (fig. 4B). In contrast, AP duration in L4 neurons was unaffected by injury of the adjacent spinal nerve, and sham-SNL similarly did not influence this measure. The rate of depolarization of the ascending limb of the AP (dV/dT) was accelerated in SNL L4 neurons but slowed in SNL L5 neurons compared with both control and each other in $A\alpha/\beta$ neurons. $A\delta$ neurons showed a similar relative effect between L4 and L5 after SNL (fig. 4C). No change was demonstrated in any of these measures for C-type neurons.

**Fig. 4. Effect of injury on action potential (AP) dimensions of dorsal root ganglion neurons.** Mean ± SEM for AP amplitude (A), AP duration at 95% return from peak to resting membrane potential (B), and dV/dT of the ascending limb of the AP (C). Symbols are described in the legend to figure 3.
Afterhyperpolarization and Afterdepolarization

The afterhyperpolarization strongly influences the bursting behavior of sensory neurons.\(^\text{30,31}\) Measures of afterhyperpolarization, including amplitude (figs. 5 and 6A), duration at 50% recovery (fig. 6B), and afterhyperpolarization area (fig. 6C), were diminished only in the axotomized SNL L5 neurons. These effects were evident in all neuronal types but were particularly marked in A\(\delta\) neurons. No changes in afterhyperpolarization measures were seen in the SNL L4 or sham groups.

Sensory neurons may occasionally develop a period of membrane depolarization after the descending limb of the AP (fig. 7). Afterdepolarization is often observed in patch clamp studies of dissociated neurons and is attributed to Ca\(^{2+}\) activation of Cl\(^{–}\) currents,\(^\text{32}\) reactivation of inward current through Na\(^{+}\) channels,\(^\text{33}\) or low-voltage-activated Ca\(^{2+}\) channels.\(^\text{34}\) In our experiments, afterdepolarizations were rare after APs triggered by axonal stimulation (19 of 381 A\(\alpha/\beta\), 3 of 135 A\(\delta\), and 1 of 50 C-type neurons, all surgical groups combined; average amplitude, 1.73 ± 0.46 mV), and their frequency was not affected by injury. We recorded from one control neuron that fired repetitively after a single axonal stimulation. This cell showed an afterdepolarization after axonally triggered APs, consistent with the observation of Amir \textit{et al.}\(^\text{35}\) that afterdepolarizations support such afterdischarge. A depolarizing phase after the AP, often appearing as an aborted AP, occurred more commonly after APs produced by sustained intracellular current injection (176 of 301 A\(\alpha/\beta\), 66 of 113 A\(\delta\), and 7 of 40 C-type neurons, all surgical groups combined; fig. 2B), but these also showed no influence of injury.

Hyperpolarization-induced Currents

Dorsal root ganglion neurons variably exhibit the phenomenon of voltage sag, in which initial hyperpolarization from current injection is followed by a depolarization toward RMP that supports excitability. Axotomized visceral sensory neurons show a loss in this time-dependent inward rectification.\(^\text{35}\) Although in this study we did not characterize the channel conveying the depolarizing current, the dominant source is the hyperpolarization-activated inward current (I\(_{\text{H}}\)), which depolarizes RMP, causes burst firing, and amplifies subthreshold membrane oscillations.\(^\text{36}\) We examined the sag ratio (fig. 8A) and found an influence of injury (main effect \(P = 0.009\)) in which values for L5 A\(\alpha/\beta\) neurons after SNL (0.14 ± 0.01) were depressed compared with L4 neurons (0.23 ± 0.02; fig. 8B). A comparable finding was reached by an alternative analysis considering the number of neurons that had a membrane voltage sag of 0.10 or more. Specifically, sag was present by this criterion in 36 of 48 L4 A\(\alpha/\beta\) neurons (75%) but in only 43 of 82 L5 neurons (52%) after SNL (\(P < 0.02\), two-tailed Fisher exact test) and in 79 of 119 control neurons (66%). This could in part be attributable to the greater R\(_{\text{m}}\) of L5 SNL neurons, which would cause an increased hyperpolarizing stimulus, although there is no correlation of sag ratio with the size of the hyperpolarizing step (data not shown) for the large depolarizing stimuli used here. The somewhat depolarized RMP of L5 neurons compared with L4 neurons after SNL might contribute to diminished sag by bringing RMP closer to the reversal potential of I\(_{\text{H}}\). A\(\delta\) neurons showed no effect of injury on sag, and sag was not detected in C-type neurons. Consistent with a report that I\(_{\text{H}}\) supports repetitive firing during membrane depolarization,\(^\text{37}\) we found that the average sag ratio for all neurons that fired repetitively during sustained depolarization (0.20 ± 0.02, \(n = 65\)) was greater than for neurons that fired singly (0.14 ± 0.01, \(n = 233\); \(P = 0.004\) by Student \(t\) test).

After termination of a period of hyperpolarizing current injection, membrane potential may rebound in a depolarizing direction that overshoots above RMP and may even result in an AP (anode break spike; fig. 8C). This overshoot is due to inward tail currents from I\(_{\text{H}}\)\(^\text{38}\) or to reprimed low-voltage-activated Ca\(^{2+}\) current and tetrodotoxin-resistant Na\(^{+}\) current associated with the Na\(_{\alpha}1.9\) isomorph.\(^\text{39}\) We identified \(V\(_{\text{m}}\)\) overshoot in myelinated neurons (59 of 309 A\(\alpha/\beta\), 21 of 113 A\(\delta\), and 0 of 39 C-type neurons, all surgical groups combined), but this was not affected by injury. Anode break spikes were observed only rarely in myelinated neurons and possibly decreased by injury (7 of 206 in A-type neurons from control and sham groups combined, 0 of 78 from SNL L4,

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Fig. 5. Axotomy diminishes membrane afterhyperpolarization (AHP) amplitude and duration. Sample traces show afterhyperpolarizations after axonal stimulation in two A\(\delta\)-type neurons from the same animal after spinal nerve ligation, one from the fourth lumbar dorsal root ganglion (A) and the other from the fifth lumbar ganglion (B). The initial deflection of each trace is the stimulation artifact. The dotted line indicates the resting membrane potential for each neuron.
and 1 of 138 from SNL L5; main effect $P = 0.04$ by chi-square).

**Excitability**

Spontaneous activity (SA) after injury has been noted with a widely variable incidence in previous studies. Quantification of teased fiber methods suffers from inability to determine the number of axons in a recorded bundle, but even somatic recordings show rates varying from none\(^4\) to 15%.\(^4\) Because the range of reported rates rarely includes frequencies less than 0.2 Hz and typical rates are much higher,\(^4\) the observation interval we used is in excess of the time necessary to identify most spontaneous activity. The only SA we identified by this criterion was seen immediately after impalement (8 of 203 control neurons [3.9%], 4 of 86 sham [4.7%], 5 of 114 SNL L4 [4.4%], 0 of 171 SNL L5) and did not persist past the 2-min settling time. The average RMP for these neurons with initial SA was $\pm 44.9 \pm 10.8$ mV, which is depolarized compared with all other neurons, and all neurons showing SA subsequently became inexcitable by axonal or somatic electrical stimulation. These observations suggest that the only SA observed here was probably associated with electrode injury.

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**Fig. 6. Effect of injury on afterhyperpolarization (AHP) dimensions of dorsal root ganglion neurons.** Mean ± SEM for afterhyperpolarization amplitude (A), afterhyperpolarization duration at 50% return to the resting membrane potential (B), and afterhyperpolarization area (C). Symbols are described in the legend to figure 3.

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tion is compromised by peripheral nerve injury, but nerve ligation, demonstrating an afterdepolarization (arrow) following an action potential induced by axonal stimulation.

Rheobase represents the depolarizing current injection necessary to trigger an AP (fig. 2). Our measurements revealed that axotomy of the SNL L5 neurons significantly reduces their rheobase compared with other groups. The effect was evident in both Aα/β and Aδ neurons, but especially the latter (fig. 9). There was no change in the neurons of the other groups that were not directly injured by axotomy.

Only a minority of Aα/β sensory neurons generate trains of APs during continued depolarization (fig. 2B), and repetitive firing is especially uncommon among Aδ and C-type sensory neurons. Adaptation to depolarization is compromised by peripheral nerve injury, but SNL effects have not been studied in nondissociated sensory neurons. We found an increased probability of repetitive firing in Aδ neurons of the SNL L5 group compared with SNL L4 and control neurons, representing decreased adaptation (table 1). In contrast, Aα/β neurons of the L5 DRG responded to axotomy with increased adaptation to sustained depolarization. Independent of cell type or injury group, the duration of the after hyperpolarization strongly regulates discharge pattern (fig. 10), because 3 of 75 cells (4%) with an AHP80 of greater than 24 ms fired repetitively, compared with 67 of 240 cells (28%) with a shorter AHP80 (P < 0.0001, Fisher exact test). The different effect of axotomy on the firing pattern of Aα/β versus Aδ neurons may be due in part to the proportionately greater decrease in afterhyperpolarization duration of Aδ neurons, as well as a significant injury effect decreasing \( I_{H} \) in axotomized Aα/β neurons.

Neuronal Phenotypes

Neurons in the sham and SNL L4 groups showed a reciprocal relation between CV and AP duration comparable to neurons from controls (fig. 11) and to that reported by others for uninjured sensory neurons. However, the pattern for axotomized L5 neurons was clearly different (fig. 11C), revealing a distinct group of cells (40 of the total 167 A-type L5 neurons) that have an AP duration greater than 2 ms but a CV greater than 1.5 m/s. This contrasts with only 7 neurons having these features among the 136 A-type neurons of all other groups (chi-square \( P < 0.001 \)). The SNL L5 neurons with this distinct electrophysiologic phenotype also show a depolarized RMP (−56.0 ± 1.5 mV), a very slow upstroke on the AP (119.3 ± 19.4 V/s), and no sag (0.02 ± 0.02). These neurons showed repetitive firing during current injection in only 1 of 13 tested and did not differ in \( R_m \) or rheobase when compared with Aδ SNL L5 neurons that have APs more brief than 2 ms. To determine whether these A-type neurons with abnormally long AP duration accounted for all the differences between the L5 SNL group and other groups, analysis was repeated excluding them from calculations and statistical testing. However, this did not eliminate any previously identified effects of axotomy on any measured parameters, indicating that the modifications in this aberrant group of neurons after axotomy are in addition to a generalized shift in electrophysiologic parameters for the entire axotomized cell population.

Recordings from intact animals have revealed that certain electrophysiologic features are more common in neurons with a nociceptive functional modality, including slow CV, overshooting AP, long AP duration, slow upstroke of the ascending limb of the AP, and inflection of the descending limb of the AP. In the current study, these features were examined to determine whether injury alters electrophysiologic phenotype. Comparison of CV histograms (fig. 12A) reveals that SNL caused a shift to slower CVs in L5 that was significantly greater than in L4, and no effect of sham surgery was seen. Axotomized L5 neurons lost the most rapidly conducting neurons, as has been reported by others after peripheral axotomy. This shift in CVs may have resulted either from a disproportionate loss of large, fast conducting neurons after injury or from a slowing of conduction for surviving neurons within a size group. We therefore examined changes of CV for neurons categorized by size (table 2), which showed that injury-dependent slowing is evident even within specified size groups. Specifically, mean CV of large neurons (diameter ≥ 40 \( \mu m \)) was decreased in SNL L5, whereas CV in medium-sized cells (diameter 30–40 \( \mu m \)) was reduced in both the L4 and L5 neurons after SNL, as well as L5 neurons after sham-SNL. Neuronal types assigned by CV are broadly associated with sensory modality, such that most neurons responsive only to nociceptive stimulation have axons conducting in the Aδ- and C-fiber range. Aδ- and C-type neurons were overabundant in all injury groups compared with the control group, and L5 ganglia after SNL surgery had more such neurons than the L4 ganglia (table 3).

Fig. 7. Membrane afterdepolarization (ADP) may follow action potentials. Sample trace from an Aδ L5 neuron after peripheral nerve ligation, demonstrating an afterdepolarization (arrow) following an action potential induced by axonal stimulation.
We also determined whether nerve injury alters the frequency of other features indicative of nociceptive modality. Djouhri and Lawson\textsuperscript{25} have reported that overshoot of the AP into the positive range is significantly greater in nociceptors than low-threshold units. In our data (table 3), the frequency of an overshoot of 10 mV or greater increased in L5 after sham-SNL and in both L4 and L5 after SNL. An inflection or hump on the descending limb of the AP, which is also a marker that specifies nociceptive modality\textsuperscript{48,49} was observed with increased frequency after sham-SNL and especially after axotomy (table 3). This is congruent with previous intracellular recordings after peripheral axotomy\textsuperscript{40,47} but contrasts with studies of dissociated neurons.\textsuperscript{46} Increased AP duration is a further trait of nociceptors\textsuperscript{48,49} that was increased in axotomized A-type neurons in the SNL L5 group, as noted above (fig. 4B). Together, these findings show that injury, especially axotomy, produces a general shift in electrophysiologic phenotype toward features that have been associated with nociceptors in uninjured neurons. Analysis restricted to medium-sized cells (data not shown) produced comparable findings, which demonstrates that the observed shift in electrophysiologic features is not due to either a selective loss of cells of a particular size or selection bias in the recordings.

**Cell Size**

To our knowledge, neuronal size distribution in non-fixed intact rat DRGs has not previously been examined. To determine the effect of injury on size measured in our model and to provide a basis for comparing cell size categories in this study with others, an inclusive count was made of the diameter of all cells within two cell layers of the surface and in the area sampled for recording. Five ganglia were examined for each of L4 and L5 after both skin incision surgery and SNL (total n = 1,698...
neurons). Because there were no differences between L4 and L5 after skin incision operation, these were combined as a control group. The mean neuronal diameter for L5 after SNL (33.49 ± 0.52 μm) was significantly less than that of the controls (36.09 ± 0.36 μm; \( P = 0.002 \)), whereas the mean diameter of L4 neurons after SNL (35.12 ± 0.54 μm) was not significantly different from that of the controls. The size histogram for control ganglia (fig. 12B) shows a distribution skewed toward small cells, consistent with the pattern shown in fixed histologic preparations.23 Studies of fixed tissue typically show a peak frequency at 20 μm diameter, which is smaller than we observed. Histologic technique may affect cell size, however, and the sizes determined by us in unfixed tissue closely match neuronal dimensions in resin embedded tissue.51 After SNL, the histogram for L5 neurons showed a decreased representation of large cells (Kolmogorov-Smirnov \( P < 0.001 \); fig. 12B), similar to changes reported by others.52 Because there is a preferential loss of small, dark neurons rather than large cells after SNL,53 the shift in cell sizes must be attributable to the reduction of individual cell volumes also noted after SNL.53 The distribution of L4 neuron sizes from SNL animals was not different from controls.

**Discussion**

The findings of this study indicate that abnormal electrophysiologic function in hyperalgesic nerve injury is found mainly in the axotomized rather than intact sensory neuron population. Specifically, injury-induced increase in excitability, including depolarization of RMP, decreased afterhyperpolarization and rheobase, and repetitive firing during depolarization, is pronounced after direct injury by axotomy of L5 neurons but is absent in adjacent L4 neurons after SNL.

**Axotomized Neurons Exhibit Multiple Alterations in Membrane Function**

Our recordings from directly injured neurons in the SNL L5 group reveal an ensemble of membrane changes, including depolarization of the RMP, broadening of the AP waveform, and diminished afterhyperpolarization dimensions, that generally confirm previous reports.35,40,41,47,54 We further examined two measures of membrane excitability that highlight the role of axotomized DRG neurons in amplified sensory behavior. Rheobase models the responsiveness of the neuron to generator currents such as may arise from natural stimulation. As others have found,40,41,54 we observed that axonal ligation and section triggers a substantial decrease in current threshold for stimulation, especially in Aβ-type neurons. Because this is not accounted for by a significant increase of Rin above control, the increased responsiveness is due to increased intrinsic membrane excitability.

Total afferent traffic is further influenced by the pattern of AP generation during sustained stimulation. Others26,46 have demonstrated that only 4–33% of myelinated sensory neurons from control animals can be made to fire repetitively despite prolonged depolarization. We show that axotomy results in a neuron type–specific alteration in firing pattern, in which SNL makes Aα/β...
neurons from the L5 ganglion less likely to fire repetitively, whereas A\(\delta\) putative nociceptors are sensitized and become more prone to burst excitation. Greater repetitive firing in A\(\delta\) neurons is at least in part due to a decrease in afterhyperpolarization commonly observed after injury\(^47,55\) and is confirmed in our observations.

\(\alpha/\beta\) neurons, however, developed less burst firing despite also having diminished afterhyperpolarization, so additional factors must regulate burst firing.\(^56\) Significant slowing of AP depolarization indicates a substantial disruption of voltage-gated sodium currents in axotomized \(\alpha/\beta\) neurons, which may contribute to loss of burst

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**Fig. 10.** Long afterhyperpolarization (AHP) prevents burst firing of dorsal root ganglion neurons. Neurons from all injury groups are shown together because there were no distinctions between groups. Only 3 of 75 neurons with an afterhyperpolarization duration (measured at 80% return to resting membrane potential) of greater than 25 ms generated multiple action potentials (APs) during sustained somatic depolarization (200 ms), whereas 67 of 240 neurons with an afterhyperpolarization duration less than 25 ms fired repetitively \((P < 0.0001)\).

**Fig. 11.** A novel type of dorsal root ganglion neurons with long action potential (AP) duration and moderate conduction velocity (CV) is evident after direct neuronal injury by spinal nerve ligation (SNL). AP duration at 95% return to resting membrane potential (APd95) versus CV scatterplots of control neurons \((A, \bigcirc)\), neurons from the sham group \((B, \bigcirc)\), neurons from SNL fourth lumbar \((L4)\) group \((B, \bullet)\), and neurons from the SNL fifth lumbar \((L5)\) group \((C, \bullet)\). The vertical line indicates 1.5 m/s CV, the horizontal line indicates 2 ms AP duration, and the shaded area includes cells with CV and AP duration exceeding these levels. Also shown are representative traces for a control neuron \((D)\) and two SNL L5 neurons \((E)\).
firing. Also, decreased excitability of Aα/β neurons may stem from loss of time-dependent rectification (sag), a phenomenon that supports repetitive firing in sensory neurons.37 Although direct measurement of \( I_H \) in dissociated neurons has showed either a decrease57 or an increase58 after peripheral axotomy, our microelectrode findings in nondissociated ganglia are consistent with depressed channel expression after axotomy.58 Alternatively, reduced \( I_H \) may reflect the \( Ca^{2+} \) sensitivity of this current59 in the context of the decreased inward \( Ca^{2+} \) flux of injured sensory neurons.8

The importance of SA to the generation of neuropathic pain is highly controversial. We found that SA is rare or absent in all injury groups. Using techniques that record from nondissociated neuronal somata, various studies report an incidence of SA after axonal injury that ranges from 0 to as high as 15%,33,40,41,43,46,54 although the highest rates are in preparations with a 5% frequency of SA before injury. A limitation of our in vitro preparation is that it lacks any contribution from diffusable agents, such as circulating catecholamines, and thus reveals only intrinsic activity. Also, higher rates in some studies may be due to technical factors, e.g., greater membrane injury during impalement in the absence of imaging. In the study of Ma et al.,54 a lower calcium concentration of 1.2 mM bathing the ganglion may have accentuated SA, and a longer observation period of 3 min may have identified SA missed by our 40-s search. However, the physiologic relevance to downstream neuronal plasticity of median rates of SA as low as 1/min60 is hard to interpret. Processes contributing to increased nociceptive transmission may require much higher rates,51,62 but sensitiza-

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**Fig. 12.** (A) Injury slows conduction velocity of dorsal root ganglion neurons (Kolmogorov-Smirnov test, main effect \( P < 0.025 \)). Compared with control neurons (○), the population histogram of conduction velocity was shifted to slower speed after spinal nerve ligation (SNL) in L4 neurons (●; \( P < 0.05 \)) and L5 neurons (○; \( P < 0.001 \)) but not in neurons of the sham group (□). Neurons from the SNL L5 group have slower conduction velocities than those from the SNL L4 group (\( P < 0.025 \)). (B) L5 neurons directly injured by SNL show a diminished proportion of large-diameter neurons and increased proportion of intermediate-sized neurons. The distribution of neuronal size is significantly different between control neurons (○, \( n = 1,026 \)) and SNL L5 neurons (●, \( n = 267; P < 0.001, \) Kolmogorov-Smirnov test). There was no difference in size distribution between control neurons and SNL L4 neurons (●, \( n = 408 \)).
tion of dorsal horn neurons might result from convergent stimulation by many afferent fibers even firing at very low rates.

Altered Ion Currents May Underlie Axotomy-Induced Changes in Membrane Function

The electrophysiologic events measured in this study result from a complex interplay of various voltage- and Ca\(^{2+}\)-sensitive currents, so the disruption caused by axonal injury may have multiple components. After axotomy, there is decreased expression of tetrodotoxin-resistant sodium channels Na\(_V\)1.8 (SNS)\(^6\) and Na\(_V\)1.9 (NaN)\(^6\) but renewed expression of the embryonic tetrodotoxin-sensitive Na\(_V\)1.3 (brain type III).\(^6\) A net decrease in sodium current may explain decreased AP amplitude, dV/dT, and CV noted in L5 after SNL, while increased Na\(_V\)1.3 may contribute to burst firing because of its rapidly repriming recovery kinetics.\(^6\) Loss of outward current through K\(^+\) channels after axonal injury\(^9\) likewise favors increased neuronal activation.

The shoulder on the descending limb of the AP is supported by inward tetrodotoxin-resistant Na\(^+\) currents and high-voltage-activated Ca\(^{2+}\) currents.\(^8\) Because both tetrodotoxin-resistant I\(_{Na}\) and high-voltage-activated I\(_{Ca}\)\(^8\) are reduced after axonal injury, these changes do not directly explain increased AP inflection or duration. However, delayed repolarization may result from reduced activation of Ca\(^{2+}\)-dependent K\(^+\) currents, prolonging the AP and accentuating the shoulder on the descending limb of the AP.\(^5\) This interpretation is consistent with our observation of diminished afterhyperpolarization, which also indicates loss of Ca\(^{2+}\)-dependent K\(^+\) currents.

### Table 2. Conduction Velocity (m/s) after Injury of DRG Neurons in Different Size Groups

<table>
<thead>
<tr>
<th>Size Group (Main Effect P)</th>
<th>Control</th>
<th>Sham</th>
<th>SNL L4</th>
<th>SNL L5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large (0.005)</td>
<td>20.9 ± 0.6 (104)</td>
<td>18.2 ± 0.9 (53)</td>
<td>20.0 ± 1.0 (46)</td>
<td>17.7 ± 0.7* (68)</td>
</tr>
<tr>
<td>Medium (&lt; 0.001)</td>
<td>21.7 ± 1.0 (44)</td>
<td>16.3 ± 1.7* (19)</td>
<td>16.0 ± 1.8* (21)</td>
<td>15.0 ± 1.0* (46)</td>
</tr>
<tr>
<td>Small (0.19)</td>
<td>13.2 ± 2.2 (24)</td>
<td>7.5 ± 2.0 (11)</td>
<td>13.9 ± 2.8 (17)</td>
<td>10.4 ± 1.1 (49)</td>
</tr>
</tbody>
</table>

Numbers indicate average ± SEM (number of neurons) for dorsal root ganglion (DRG) neurons from control rats, DRG neurons from rats after sham nerve injury, neurons from the fourth lumbar (L4) DRG after spinal nerve ligation (SNL) injury, and neurons from the fifth lumbar (L5) DRG after SNL. Significant main was effect tested by analysis of variance with post hoc comparisons using the Bonferroni test at \(P \leq 0.05\); different from control * \(P \leq 0.01\); different from SNL4 ‡ \(P \leq 0.01\); different from SNL4 ‡ \(P \leq 0.05\), different from control * \(P \leq 0.01\). AP = action potential.

Adjacent L4 Neurons Show Changes That Contrast with Axotomized L5 Neurons

The injury model used for this study involves ligation and section of the L5 spinal nerve, whereas the L4 spinal nerve is not directly injured. Therefore, we did not expect comparable electrophysiologic changes in the neurons of the two ganglia. The parameters most affected by axotomy, namely RMP, AP duration, and afterhyperpolarization dimensions, are not altered in L4 neurons. Important changes of excitability observed in axotomized L5 neurons are not evident in the L4 neurons. Furthermore, divergent changes in L4 and L5 are observed in the rate of AP upstroke, voltage sag during hyperpolarization, and \(R_{\text{m}}\). Together, these findings clearly demonstrate a distinct effect of SNL on axotomized and intact fibers of the sciatic nerve. Ma et al.\(^5\) have reported that SNL affects intact L4 neurons the same as axotomized L5 neurons. Differences in their study include lack of an unoperated or skin incision control group, and categorization of units by somatic size, which groups neurons with broad and overlapping ranges of CVs.\(^6\) Also, their study examined neurons within 3–7 days after injury rather than at 3 weeks as in our study, perhaps indicating that there is an early phase of injury response in the L4 neurons that resolves by the third week.

After transection of the L5 spinal nerve, inflammatory cells accumulate in the L4 ganglion,\(^6\) and L4 neurons develop increased sensitivity to tumor necrosis factor\(^7\) and express calcitonin gene–related peptide\(^7\) and the vanilloid receptor TRPV1.\(^7\) Other models reveal increased expression of substance P\(^7\) and the P2x3 ATP receptor\(^7\) in spared sensory neurons. These phenotypic changes in L4 neurons may augment afferent traffic by

### Table 3. Frequency of Electrophysiologic Features That Indicate Nociceptive Sensory Modality of DRG Neurons

<table>
<thead>
<tr>
<th>Parameter (Main Effect P)</th>
<th>Control</th>
<th>Sham</th>
<th>SNL L4</th>
<th>SNL L5</th>
</tr>
</thead>
<tbody>
<tr>
<td>A or C neuronal type (&lt; 0.001)</td>
<td>18/171 (11)</td>
<td>19/81 (23)†</td>
<td>17/82 (21)*</td>
<td>62/161 (39)†§</td>
</tr>
<tr>
<td>AP overshoot ≥ 10 mV (&lt; 0.001)</td>
<td>76/171 (44)</td>
<td>57/81 (70)†</td>
<td>57/82 (70)†</td>
<td>94/161 (58)†‡</td>
</tr>
<tr>
<td>Inflection or hump (&lt; 0.001)</td>
<td>58/171 (34)</td>
<td>43/81 (53)*</td>
<td>35/82 (43)</td>
<td>111/161 (69)†‡</td>
</tr>
</tbody>
</table>

Numbers indicate neurons positive for the feature/total neurons (%) for dorsal root ganglion (DRG) neurons from control rats, DRG neurons from rats after sham nerve injury, neurons from the fourth lumbar (L4) DRG after spinal nerve ligation (SNL) injury, and neurons from the fifth lumbar (L5) DRG after SNL. Significant main was effect tested by cross-tabulation chi-square; post hoc comparisons used the Fisher exact test: different from control * \(P \leq 0.05\), † \(P \leq 0.01\); different from SNL4 ‡ \(P \leq 0.05\), § \(P \leq 0.01\).
increased sensory transduction and sensitization of dorsal horn neurons and may be associated with the increase of certain electrophysiologic features of nociceptors that we observed, including an overrepresentation of Aδ- and C-type neurons and AP overshoot. Because the current study did not examine agonist responses or patterns of connectivity of sensory neurons in the dorsal horn, a role for the spared neurons in the generation of neuropathic pain is not excluded by our finding of unchanged electrical excitability of L4 neurons.

After SNL, Na+,L,8 channel expression is increased or unchanged in small L4 neurons, but is decreased in the axotomized neurons of L5.10,63 These opposite changes may account for the increase of dV/dt that we observed in L4 neurons despite depression in L5.

Sham Surgery Only Modestly Alters Sensory Neuronal Function

Behavioral changes representative of pain may occur after sham-SNL surgery,14 which we found causes increased Rm in L5 Aα/β neurons and increased frequency of AP inflection, large AP overshoot, and C and Aδ neuronal types. Humoral stress response19 and inflammatory cell invasion76 may affect DRGs even in the absence of axotomy. We infer that inflammation adjacent to the nerves may also contribute to electrophysiologic and behavioral changes seen after SNL. Ma et al.54 did not identify any effects of sham-SNL, but their analysis compared pooled data from L4 and L5 ganglia after sham-SNL to the range of control values in previously published studies, which may have obscured changes specific to the L5 neurons after sham surgery.

Injury Induces a Shift in Properties of A-type Neurons toward a Nociceptor-like Phenotype

Neurons of the DRG are a heterogeneous population with diverse sensory modalities, anatomic targets of central termini, and expression of peptides and receptors. Our data show that injury, particularly axotomy, expands the population of sensory neurons with electrophysiologic features that typify uninjured nociceptors (slow CV, overshooting AP, long AP duration, and inflection25,28,48,49). However, it is unknown whether this is accompanied by a shift in central connectivity or whether such markers continue to identify a nociceptive sensory modality after injury. Although Ma et al.54 determined that inflection, large AP amplitude, and long afterhyperpolarization duration were still characteristic of nociceptive neurons even after SNL and sham surgery, their sample did not include L5 neurons in which receptive fields could not be examined.

This shift in the frequency of electrophysiologic attributes may result from either selective elimination of nonnociceptors from the cell population or new expression of traits in neurons previously lacking them. Consistent with the first explanation, cell loss of up to 22% by 15 days after L5 ligation has been reported.55 Also, we observed a deficit of neurons with large diameters typical of nonnociceptors. However, this may have resulted from neuronal shrinkage,55 and other studies demonstrate a preferential loss of nociceptive C-type rather than A-type neurons after SNL.53,77 So available data do not support selective elimination of nonnociceptors.

The alternative hypothesis of a phenotypic change, in which cells develop new attributes, is well documented in sensory neurons after injury or inflammation (see for example Averill et al.78). Effects of injury on neuronal phenotype are most directly evident in our data by the emergence after axotomy of a large population of neurons that possess moderate CVs indicative of myelinated fibers but show long AP duration characteristic of C-type neurons. This subset of cells exhibits extremes of other features affected by axotomy, including a particularly depolarized RMP, slow upstroke of the AP, and markedly voltage sag, which indicate a substantial and complex alteration of membrane channel function. Because inflammation selectively increases CV of Aδ- and C-type nociceptors,79 it is possible that this novel group of neurons arises through the effect of inflammation on cells that were originally C-type neurons. However, it is evident from figure 11 that the approximately 50% increase in CV attributable to this effect79 is not adequate alone to convert previous C-type cells into the fast conducting neurons represented by the novel group. The histologic counterpart of these cells may have recently been identified by Hammond et al.,80 who described the emergence exclusively in the L5 ganglion after SNL of a novel group of very small neurons that label with N52 antibody, which identifies myelinated fibers. These cells might thus exhibit the AP features of small neurons but have accelerated CV due to myelination. Congruently, Lekan et al.77 have noted the development of an increased population of finely myelinated fiber profiles in the dorsal root of L5 after SNL.

Contribution of Injury-induced Electrophysiologic Changes to Neuropathic Pain

Our strategy was to discern the roles of different components of neuronal injury in the generation of pain by evaluating changes in neurons subjected to different traumatic conditions, including axotomy (SNL L5 group), exposure of surviving axons to fascicular inflammatory mediators (SNL L4 group), and surgical trauma of tissues adjacent to the nerve (sham group). We found that substantial electrophysiologic dysfunction arises principally after axotomy, especially in the Aδ population, in which increased excitability may lead to an increased total nociceptiveafferent traffic. The importance of changes in nonmyelinated neurons may be underestimated in this study because of the small numbers of recorded cells. However, the diminished afterhyperpolarization dimensions noted in this neuronal type...
were also limited to the injury group with axotomy. Our data provide little support for a role of biophysical modifications in neurons with intact axons.

The SNL model used in this study completely disconnects L5 neurons from the periphery, so there is no opportunity for enhancement of excitation initiated by natural stimulation of sensory terminals. However, excitation may arise from other processes directly affecting sensory neuron somata in the DRG, such as mechanical stimulation during movement, ischemia, hypoxia, and sensitivity to circulating and locally released agonists. Clinical conditions rarely result in complete isolation of axotomized neurons from intact ones, and in this setting, injured DRG neurons are also exposed to cross-excitation from activity in adjacent conducting neurons. Irritative mediators released in response to adjacent inflammation or neuronal injury may be a further source of stimulation of hyperexcitable axotomized cells. Our findings suggest that the biophysical consequences of axotomy, through amplification of signals initiated by these mechanisms, may contribute to hyperalgesia after peripheral nerve injury, whereas electrical instability of intact neurons does not play a role.

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