Similar Electrophysiological Changes in Axotomized and Neighboring Intact Dorsal Root Ganglion Neurons

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Ma, Chao, Yousheng Shu, Zheng Zheng, Yong Chen, Hang Yao, Kenneth W. Greenquist, Fletcher A. White, and Robert H. La Motte. Similar electrophysiological changes in axotomized and neighboring intact dorsal root ganglion (DRG) neurons in rats after either a peripheral axotomy consisting of an L5 spinal nerve ligation (SNL) or a central axotomy produced by an L5 partial rhizotomy (PR). SNL produced lasting hyperalgesia to punctate indentation and tactile allodynia to innocuous stroking of the foot ipsilateral to the injury. PR produced ipsilateral hyperalgesia without allodynia with recovery by day 10. Intracelluar recordings were obtained in vivo from the cell bodies (somata) of axotomized and intact DRG neurons, some with functionally identified peripheral receptive fields. PR produced only minor electrophysiological changes in both axotomized and intact somata in L5 DRG. In contrast, extensive changes were observed after SNL in large- and medium-sized, but not small-sized, somata of intact (L4) as well as axotomized (L5) DRG neurons. These changes included (in relation to sham values) higher input resistance, lower current and voltage thresholds, and action potentials with longer durations and slower rising and falling rates. The incidence of spontaneous activity, recorded extracellularly from dorsal root fibers in vitro, was significantly higher (in relation to sham) after SNL but not after PR, and occurred in myelinated but not unmyelinated fibers from both L4 (9.1%) and L5 (16.7%) DRGs. We hypothesize that the changes in the electrophysiological properties of axotomized and intact DRG neurons after SNL are produced by a mechanism associated with Wallerian degeneration and that the hyperexcitability of intact neurons may contribute to SNL-induced hyperalgesia and allodynia.

INTRODUCTION

In animal models of neuropathic pain involving peripheral nerve injury, the measure of pain is typically a reflex withdrawal to cutaneous stimulation. Despite the fact that it is the intact neurons that transmit cutaneous information to the CNS, electrophysiological studies of primary sensory neurons have focused more on the neurons that are axotomized. Among these axotomized neurons are those with hyperexcitable cell bodies (somata) that can become a source of ectopic discharges (Devor and Seltzer 1999; Kajander et al. 1992; Kirk 1974; Wall and Devor 1983).

The spinal-nerve ligation (SNL) model provides a means of separating the somata of neurons with axotomized and intact axons (Kim and Chung 1992). Of the axotomized somata, only those with thickly myelinated axons, and not those with unmyelinated axons, became hyperexcitable (Liu et al. 2000, 2002). In addition, there is evidence that the electrophysiological properties of neighboring, intact L4 DRG neurons can also change after an L5 SNL. For example, abnormal spontaneous activity has been observed in L4 DRG neurons with myelinated axons (Boucher et al. 2000) as well as those with unmyelinated axons (Ali et al. 1999; Wu et al. 2001). Possible changes in the membrane properties of the somata of intact neurons that are in proximity to axotomized neurons have received relatively little attention and are the subject of the present investigation.

Injury to dorsal roots, when compared with peripheral nerve injury, appears to have less of an effect on the properties of DRG neurons (Black et al. 1999; Sleeper et al. 2000). Subsequently, hyperalgesia after a complete transection of the dorsal root is more likely due to central changes triggered by a loss of afferent input rather than to enhanced excitability of the transected DRG neurons that have lost their central connection while retaining their peripheral trophic support (Eschenfelder et al. 2000; Li et al. 2000; Wu et al. 2001). Partial rhizotomy (PR) may be an exception, as injured and intact axons are adjacent to each other, analogous to the situation for degenerating and intact sciatic axons after an L5 spinal nerve transection. Thus it would be useful to compare the membrane properties of the somata of axotomized and intact neurons in the same DRG after PR.

None of the above studies have investigated the receptive field properties of the somata of neighboring intact neurons after a nerve injury, nor has there been a comparison of the effects of peripheral versus central axotomy on the electrophysiological properties of intact DRG somata. With these objectives in mind, the present experiments used two models of neuropathic pain. For one model, SNL, the somata of axotomized and intact neurons were in different ganglia (L5 and L4, respectively), whereas in the other model, PR, the somata of axotomized and intact neurons were in the same ganglion. Extracellular recordings of dorsal root fibers and intracellular recordings of DRG somata were made from axotomized and intact neurons. A novel preparation was developed that...
lowed the visualization of DRG somata in vivo, thereby providing the opportunity of determining the receptive field properties of neurons while maintaining unusually stable conditions for intracellular recording. Some preliminary results of the present study have been published in abstract form (Ma et al. 2001; Zheng et al. 2000).

**Methods**

**Surgical procedures**

Seventy-one adult female Sprague-Dawley rats weighing 150–250 g were used. Groups of three or four animals were housed together in a climate-controlled room under a 12-h light/dark cycle. The use and handling of animals were in accordance with guidelines provided by the National Institutes of Health and the International Association for the Study of Pain and received approval from the Institutional Animal Care and Use Committee of the Yale University School of Medicine.

**Peripheral axotomy: L5 spinal nerve ligation and transection (SNL)** (Fig. 1A). Nine rats received a unilateral, tight ligation and transection of the right L5 spinal nerve using a modification of the surgical procedure described by Kim and Chung (1992). Briefly, under pentobarbital sodium (Nembutal) anesthesia (50 mg/kg ip) and using aseptic precautions, the L5 transverse process was removed and the L5 and L4 spinal nerves identified. The L5 spinal nerve was separated and tightly ligated with 5-0 silk sutures and transected just distal to the ligature. The ligature was located approximately 3–4 mm proximal to the junction with L4 nerve and 5–6 mm distal to the L5 DRG. The incision was closed in layers, and antibiotics were administered prophylactically. Another group of eight rats underwent sham surgery (SNL-sham) that involved the identical surgical exposure without a ligation or transection of the spinal nerve.

**Central axotomy: L5 dorsal root PR** (Fig. 1B). Fifteen rats received a transection of the caudal half of L5 dorsal root on the right side. Anesthesia and aseptic procedures were as described. The spinous process was exposed at the level of L1 to L3, and a laminectomy was made at L2. The dura mater was opened, and the L5 dorsal root was identified at the point where its 4–5 rootlets entered the spinal cord. The caudal 2–3 rootlets were then separated and transected approximately 3 mm distal to the point of entry into the spinal cord. The distal cut ends of the rootlets were immersed for 10 min in a fluorescent dye, Oregon-Green dextran (Molecular Probes, 2 µl, 10% in double-distilled water with 20% DMSO). The fluorescent dye was selectively absorbed by the cut dorsal rootlets and could be retrogradely transported to the somata within 5 days. The rest of the dye was washed out by artificial cerebrospinal fluid (ACSF), and a small piece of gelfoam (sterile sponge, Upjohn) was put on the surface of dorsal roots to prevent compression and adhesion from the surface tissues. The incision was closed in layers. Eleven rats received a sham surgery (PR-sham) consisting of the same surgical exposure but without either the transection of the dorsal root or the loading of dye.

**Behavioral tests**

Behavioral tests were made on each of 3 consecutive days before and 1, 4, and 7 days after one of the following operations: SNL, SNL-sham, PR, or PR-sham surgery. Six PR and six PR-sham rats were additionally tested 10 and 14 days after operation. Tests were carried out without knowledge of the type of surgery and electrophysiological results. Before testing, the rat was placed in a clear plastic cage with a metal mesh floor. After about 15 min of accommodation, the following tests were performed.

**Measurement of the incidence of withdrawal to innocuous stroking.** A wisp of cotton, pulled up but still attached to a cotton swab, was stroked mediolaterally across the middle of the plantar surface of the hindpaw at a velocity of approximately 10 mm/s (Fig. 4C). A total of six strokes were given to each foot, alternating between feet for each stroke with an interstimulus interval of 10–15 s. Because this type of stimulus never elicited a withdrawal in normal, control rats, it was considered to be a valid test for the presence of tactile allodynia. The number of the six strokes that elicited a withdrawal was expressed as a percentage that was taken as an index of tactile allodynia for each foot.

**Measurement of the threshold force eliciting withdrawal to punctate indentation.** A von Frey-type monofilaments each delivering a different bending force in ascending order (5, 10, 20, 40, 60, 80, 100, and 120 mN) but having the same tip diameter of 0.1 mm were delivered to designated loci on the skin (LaMotte et al. 1998; Zhang et al. 1999). Each filament was applied for 1 s, alternately to each foot at intervals of 10–15 s, to each of 10 sites distributed across the plantar surface of the rat hindpaw (Fig. 4A). A Hill equation was fitted (Origin Version 6.0, Microcal Software) to the function relating the percentage of indentations eliciting a withdrawal to the force of indentation. From this equation, the threshold force was obtained, defined as the force corresponding to a 50% withdrawal. Cutaneous hyperalgesia was defined as a postoperative decrease in threshold of 20 mN from the mean of the three preoperative thresholds.

**Intracellular electrophysiological recording in vivo**

Three to 7 days after an L5 SNL (n = 9 rats), SNL-sham (n = 8), PR (n = 9), or PR-sham (n = 7) operation, animals were anesthetized with pentobarbital (Nembutal, initial dose of 50 mg/kg ip followed by 20 mg/kg per hour as needed). The L4 and L5 spinal nerves and the sciatic nerve were exposed and separated from adjacent tissues down to the level of the knee joint. All the other branches of sciatic nerve above the knee joint were cut. A laminectomy was performed at the levels of L2–L6. The L4 and L5 dorsal root ganglia (DRGs) and their corresponding dorsal roots were identified and exposed. The L4–L5 dorsal roots were transected just prior to their entry to the spinal cord. Oxygenated ACSF was dripped periodically onto the surface of the ganglia during the surgical procedure. The ACSF contained (in mM) 130 NaCl, 24 NaHCO₃, 3.5 KCl, 1.25 NaH₂PO₄, 1.2 MgCl₂, 1.2 CaCl₂, and 180 dextrose, bubbled with 95% O₂-5% CO₂ and having a pH of 7.4 and an osmolarity of 290–310 mosM. The L4–L5 dorsal roots, ganglia, and spinal nerves were isolated from the surrounding tissues and transferred to a chamber filled with oxygenated ACSF. The L5 ganglion alone was used for the partial rhizotomy experiments. The sciatic nerve, which still connected to the lower leg, was brought out of the chamber through a petroleum jelly wall and was
kept moist by periodic applications of ACSF through a strip of gauze. The skin incisions on the back and thigh were closed.

Under the dissecting microscope, the sheath covering the surface of the DRG (perineurium and epineurium) was carefully removed using fine forceps and scissors. A metal frame with nylon mesh was used to gently hold the ganglia in the center of the recording chamber. The chamber was then transferred and mounted to a fixed platform under a light microscope (Olympus, model BX50WI; Fig. 2). The rat lay on a second platform, mechanically isolated from the first, to which a clamp pinning the knee was attached. This platform and clamp allowed the lower limb to be manipulated during the search for receptive fields without transmitting unwanted mechanical stimuli to the electrodes or recording chamber (Fig. 2). The ganglia were continuously perfused at a rate of 3–4 ml/min with oxygenated ACSF. The temperature of the ACSF in the chamber was maintained at 36 ± 1°C by a heater and controller (Warner Instrument, TC-344A).

Intracellular recordings were obtained only from the somata of neurons on the surface of DRG, which were visualized under differential interference contrast (DIC) mode (Fig. 3). The recording sharp electrode was filled with 1.0 M KCl (impedance: 50–80 MΩ) and positioned by a Narashige (MC-35A) microdrive. Prior to electrode insertion, the size of a soma to be studied was visually classified as small (≤30 μm), medium (31–45 μm), or large (>45 μm) (Zhang et al. 1999). Electrophysiological recordings were collected with single-electrode continuous current clamp (AxoClamp-2B, Axon Instruments), stored digitally via a Digidata 2200 interface, and analyzed off-line with pClamp 8 software (Axon Instruments). A neuron was accepted for study only when it exhibited a resting membrane potential (RMP) more negative than −45 mV.

CRITERIA FOR CLASSIFYING A NEURON AS SPONTANEOUS ACTIVITY. For each neuron isolated for study, a continuous recording was obtained for 3 min without the delivery of any external stimulus. If during this period, some pattern of spontaneous discharge (regular, bursting, or irregular) was observed, the neuron was classified as spontaneously active (SA) only if the activity could not be attributable to normal, ongoing activation of peripheral receptors. For example, the tonic activity of a muscle spindle afferent was easily manipulated by changing the length of the muscle. Any “injury discharge” that appeared on occasion immediately after electrode insertion and lasted for <30 s but never came back within 3 min was ignored.

DETERMINATION OF CONDUCTION VELOCITY. Action potentials (APs) were evoked either by delivering current pulses (0.1–5 nA, 0.05–0.5 ms duration) through the suction electrodes attached to the cut end of dorsal roots or by injecting current through the amplifier bridge to the somata. The latency of APs electrically evoked from the suction electrode was divided into the distance between the cut end of the dorsal root and the center of the DRG to obtain the conduction velocity (CVdr, m/s). For animals having received a PR, a neuron was identified as having a cut dorsal root axon by the presence of Oregon Green fluorescence (Fig. 3, B and E) and/or APs elicited by electrical stimulation of the formerly axotomized dorsal rootlets.

DETERMINATION OF THE CURRENT-VOLTAGE FUNCTION. A current-voltage (I-V) function was obtained from the voltage responses to

FIG. 2. Method of obtaining intracellular electrophysiological recordings and receptive field properties from visualized DRG somata in the anesthetized rat. The dorsal roots, ganglia, spinal nerve, and the upper portion of the sciatic nerve were dissected free and placed in a recording chamber that was perfused with temperature controlled artificial cerebrospinal fluid (ASCF). The chamber and electrode-positioning micro-drives were mounted on 1 platform and the rat lay on a 2nd one, mechanically isolated from the 1st. The 2nd platform and a clamp that pinned the knee allowed the lower limb to be manipulated during the search for receptive fields without transmitting unwanted mechanical stimuli to the electrodes or recording chamber.

FIG. 3. Visualization of DRG somata during intracellular recording. DRG somata were viewed with a CCD camera combined with infrared DIC (A, C, and E) or fluorescence (B, D, and F) microscopy. A: view of entire DRG under low magnification (40×). B: same view under fluorescence reveals many somata filled with Oregon green that was retrogradely transported from cut dorsal rootlets. C: medium-sized soma (arrow) viewed during intracellular recording under 400× magnification. D: same view under fluorescence reveals that the cell was filled with Oregon green and therefore had a transected dorsal-root axon. E: electrode inserted into soma of a high-threshold, cutaneous mechanoreceptive neuron innervating the dorsum of the foot. Note the functioning blood vessels as indicated by the presence of red blood corpuscles moving through capillaries below and to the right. F: same cell viewed under fluorescence after the 1st electrode had been withdrawn and a 2nd one now inserted and used with iontophoresis to fill the cell with tetramethyl rhodamine. Scale bar in A and B: 20 μm (40×), C–F: 200 μm (400×).
hyperpolarizing and depolarizing current pulses of $-1.0$–$4.0$ nA (100 ms duration) delivered in increments of 0.05 nA until an AP was evoked (or reached 4 nA). The following measurements (1–3) were obtained from the I–V function, and 4–9 were obtained from the AP evoked by stimulating the dorsal root or the receptive fields: 1) the input resistance ($R_{in}$, MΩ), calculated from the slope of a steady-state I–V curve between $-1.0$ and 0.2 nA; 2) the threshold current (nA) of the AP, defined as the minimal current injected that elicited an AP; 3) the threshold voltage of the AP (mV) defined as the first point on the rising phase of the spike at which the change in voltage exceeded 50 mV/ms; 4) the duration of the AP (APD50, ms) measured at one-half of the peak amplitude; 5) the maximal rising rate of the AP (dV/dr, mV/ms) or the maximal slope of the rising limb of the AP, measured by analog differentiation; 6) the maximal falling rate of the AP (dV/dr f., mV/ms) or maximal slope of the falling limb of the AP; 7) the peak amplitude of the afterhyperpolarization (AHP amp, mV); 8) the duration of the afterhyperpolarization (AHP50, ms), measured at one-half of the peak amplitude; and 9) the presence or absence of an inflection on the falling phase of the AP as determined by analog differentiation.

**Classification of the receptive field properties of DRG neurons**

The receptive field properties of DRG neurons were classified using hand-held stimulators according to standard criteria (Burgess and Perl 1967; Lawson et al. 1997). Muscle spindle afferents were classified by their characteristic responses to changes in muscle length, probing of the muscle belly, and slight taps to the tendon. Cutaneous afferents were identified as low or high threshold (LT or HT) by their responses to soft brush, hair movement (hair follicle fibers), or gentle pressure versus mild pinching, mild (37°C versus noxious (51°C) heat stimulation (5s), or noxious cold (ice-water, 0°C, 20 s). Because mechanical stimuli were used as the primary search stimuli for most of the cases in our study, the search procedure was biased against finding mechanical insensitive units.

**Extracellular electrophysiological recording in vitro**

Microfilament recordings were made from dissected dorsal root fiber strands in 12 SNL rats and 4 SNL-sham rats 3–7 days after surgery. The L4 and L5 DRG with the attached dorsal roots, spinal nerves, and sciatic nerve were removed from the rat. The sciatic nerve was cut at the mid-thigh level, and for SNL rat, the L5 spinal nerve was cut approximately 1 mm proximal to the injury (ligated) site. The ganglia and nerves were placed in a recording chamber and perfused via an Axon interface (Digidata 2100, Axon Instruments) and pCLAMP software (Version 6.0, Axon Instruments) for off-line analyses. Usually 20 bundles could be dissected from each dorsal root, and an average of 5 fibers (range, 0–20) were recruited from each bundle.

**Extracellular electrophysiological recording in vivo**

Three to 7 days after an SNL ($n = 9$) or SNL-sham ($n = 3$) operation, animals were anesthetized with pentobarbital (Nembutal) with an initial dose of 50 mg/kg ip followed by 20 mg/kg per hour as needed. The L4 spinal nerve was exposed and gently separated from adjacent tissues. A small pledget of cotton soaked in saline was placed around the L4 spinal nerve at approximately 8 mm distal to the L4 DRG. A laminectomy was performed at the levels of L2–L6. Both L4 and L5 dorsal roots were exposed and covered with a pool of warmed paraffin oil (35 ± 2°C) formed by the edges of the skin sewn to a ring. Extracellular recordings were made from teased L4 and L5 dorsal root microfilaments. The procedure was similar to the in vitro extracellular recording described above. The experiment began with the dorsal root intact, after which microfilaments were cut close to entry into the spinal cord and divided to achieve bundles of about 50 μm in diameter. Recordings were obtained within 6 h from neurons with unanesthetized, intact peripheral axons, or from dorsal roots with an anesthetized spinal nerve (10 min after replacing the cotton pledge around the L4 spinal nerve with one soaked with saline containing 1% chloroprocaine), or from dorsal roots with acutely axotomized spinal nerve fibers (10 min after transection of the anesthetized L4 spinal nerve). The criteria for classifying a neuron as SA are the same as those described for intracellular recording.

**Immunohistochemistry for c-Jun**

Animals were terminally anesthetized at 5 days after SNL ($n = 2$) or SNL-sham operation ($n = 2$) and perfused transcardially with 4% paraformaldehyde. L4/L5 DRGs ipsilateral and contralateral to the injury were dissected and frozen immediately for immunohistochemistry. Cryosections (20–30 μm thick) of the DRG were mounted on Silane-coated slides (Sigma, St. Louis, MO), air-dried, and fixed for 30 min in 4% paraformaldehyde at 4°C before preincubating in a dilution buffer (0.1 M PBS, 0.8% bovine serum albumin, 0.25% Triton X-100, and 5% normal goat serum) for 1 h. After three rinses in PBS, sections were incubated in rabbit anti-c-jun (1:100; Oncogene Research Products, Cambridge, MA) antibody. Immunoreactive cells were visualized with Cy3-conjugated and fluorescein isothiocyanate (FITC)-conjugated anti-rabbit secondary antibodies. Entire ganglia sweeps through every section were made using a 40× objective, and every c-Jun-labeled neuron was counted. Sections from transected L5 DRG were used as positive control.

**Statistical analyses**

SigmaStat software (Version 2.03, SPSS) was used to determine the statistical significance of differences in the mean threshold forces for foot withdrawal to punctate indentation as a function of time and between experimental groups by means of repeated measures analyses of variance (RMANOVA) followed by post hoc pairwise comparisons (Student-Newman-Keuls Method). Student’s $t$-test or one-way ANOVAs with post hoc pairwise comparisons were used to test the significance of differences between experimental conditions in mean values obtained in the electrophysiological experiments. $χ^2$ tests were used to assess differences between experimental groups in the incidence of SA or incidence of foot withdrawal to the cotton wisp. A probability of 0.05 was chosen as the criterion for significance.
RESULTS

Effects of SNL on withdrawal responses to mechanical stimulation of the foot

The mean force thresholds for foot withdrawal to punctate indentation were analyzed separately for each foot for SNL and SNL-sham rats with a two-way ANOVA (group × days) with repeated measures on days. Post hoc comparisons were made between means obtained on each postoperative day and the mean of the 3 consecutive preoperative days of tests. Before surgery, there was no significant difference for the mean foot withdrawal threshold either between SNL and sham or between the ipsilateral and contralateral feet within each group. The mean withdrawal thresholds obtained ipsilateral to the SNL decreased significantly from the preoperative means on the first postoperative day and remained significantly lower up to the seventh postoperative day (P < 0.01; Fig. 4B). In contrast, there were no significant changes in withdrawal thresholds contralateral to the SNL or for either foot in the sham-operated group.

None of the SNL or sham-operated rats exhibited reflex withdrawal to stroking with the cotton wisp prior to surgery (Fig. 4D). Occasionally a rat would move away from stimulus, but this response could easily be discriminated from a reflexive lifting of the foot. In contrast, after surgery, most of the SNL rats, but none of the shams, exhibited significant tactile allodynia. Most SNL rats exhibited a reflex withdrawal to at least some of the strokes on the foot ipsilateral to the injury on postoperative days 1–7 (Fig. 4D), indicative of significant tactile allodynia. A few SNL rats exhibited withdrawal on occasion in response to the strokes delivered to the contralateral foot. The percentage of withdrawals on postoperative days 1, 4, and 7 on the foot ipsilateral to the SNL was significantly greater than it was on any preoperative test and was also significantly greater than on the foot contralateral to the SNL (χ² tests, P < 0.01). The percentage of withdrawals on postoperative days 4, but not 1 and 7, was significantly greater on the foot contralateral to the SNL than it was on any preoperative test.

Effects of PR on withdrawal responses to mechanical stimulation of the foot

The mean force thresholds for foot withdrawal to punctate indentation were analyzed separately for each foot for PR and PR-sham rats with a two-way ANOVA (group × days) with repeated measures on days. Post hoc comparisons were made between means obtained on each postoperative day and the mean of the 3 consecutive preoperative days of tests. Before surgery, there was no significant difference for the mean foot withdrawal threshold either between PR and PR-sham groups or between the ipsilateral and contralateral feet within groups. The mean force thresholds on postoperative days 1, 4, and 7 were each significantly lower than the mean preoperative threshold, but remained unchanged on the contralateral foot and on either foot in the sham-operated group (Fig. 5A). However, by the 10th and 14th postoperative day, thresholds ipsilateral to the PR had returned to preoperative values. Thus, unlike the SNL that reportedly maintains a lower than normal threshold on the ipsilateral foot for upward of 10 wk (Kim and Chung 1992; Liu et al. 2000), the behavioral effects of PR lasted little more than 1 week.

None of the rats exhibited reflex withdrawal to stroking with the cotton wisp before surgery. Postoperatively, there was no significant tactile allodynia after either the PR or the PR-sham surgeries (Fig. 5B). Although a reflex withdrawal was observed ipsilateral (4–14%) or contralateral (0–4%) to the PR in 6 of 15 rats, the mean percentage withdrawal did not reach statis-
tical significance and was not significantly higher than the postoperative values obtained on the controlateral foot, or for the sham-operated, on either foot (χ² tests).

Effects of SNL on the membrane properties of axotomized and intact DRG soma recorded in vivo

Three to 7 days after an L5 SNL (9 rats) or a sham operation (8 rats), intracellular electrophysiological recordings were obtained in vivo from 490 DRG somata. At total of 182 neurons were recorded from the spinally transected L5 DRG and 152 from the adjacent L4 DRG with an intact spinal nerve. For sham-operated rats, 156 neurons were recorded from the L4 or L5 DRG. There were no significant differences in mean conduction velocities of dorsal root axons (CVdr) between SNL and sham groups for each of the three somal size categories (Student’s t-test). For SNL-sham rats, these means ± SE (m/s) were 14.35 ± 1.02 (n = 33) for large-sized, 5.91 ± 0.65 (n = 24) for medium-sized, and 0.41 ± 0.02 (n = 44) for small-sized somata (see Table 3 for combined data of CVdr). These conduction velocities are in accordance, respectively, with conduction in Aβ-, Aδ-, and C-fibers.

EFFECTS OF ACUTE PERIPHERAL AXOTOMY ON THE MEMBRANE PROPERTIES OF DRG SOMATA FROM SHAM RATS. We determined whether the electrophysiological properties of DRG somata from sham rats were similar for neurons with, as opposed to without, peripheral receptive fields. Those without receptive fields were assumed to have received an acute peripheral axotomy during the surgery required to place the DRG in the recording chamber. Student’s t-test revealed no significant differences for any of the properties listed in Table 1 for any of the three categories of cell sizes. Thus acute axotomy had no demonstrable effect on the membrane properties recorded from DRG neurons in sham-operated rats.

EFFECTS OF THE SNL-SHAM SURGERY ON THE MEMBRANE PROPERTIES OF DRG SOMATA. We next examined whether the properties of DRG somata from sham rats were similar to published values obtained from unoperated control rats. The published values were obtained from small-, medium-, and

TABLE 1. Electrophysiological properties of DRG neurons from axotomized and intact ganglia after SNL

<table>
<thead>
<tr>
<th>Size Category</th>
<th>Large</th>
<th>Medium</th>
<th>Small</th>
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<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>DRG L4</td>
<td>DRG L5</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell size, μm</td>
<td>58.7 ± 1.7</td>
<td>58.9 ± 1.3</td>
<td>57.9 ± 0.9</td>
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<td>RMP, mV</td>
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<td>-60.3 ± 1.1</td>
<td>-57.1 ± 0.6</td>
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<tr>
<td>Rin, Mohm</td>
<td>20.2 ± 1.7</td>
<td>27.4 ± 3.0</td>
<td>31.7 ± 2.1</td>
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<td>Curr. thre., nA</td>
<td>1.55 ± 0.22</td>
<td>1.16 ± 0.16</td>
<td>0.72 ± 0.05</td>
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<tr>
<td>Vol. thre., mV</td>
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<td>-45.9 ± 1.6</td>
<td>-45.9 ± 0.9</td>
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<td>APD50, ms</td>
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<td>AHP50, ms</td>
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<td>3.82 ± 0.36</td>
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<td>AHP amp., mV</td>
<td>-9.9 ± 0.6</td>
<td>-9.7 ± 0.5</td>
<td>-7.0 ± 0.4</td>
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</table>

Values are means ± SE, and n = sample size; SNL: L5 spinal nerve ligation and transection; DRG L4 and L5: intact (L4) and axotomized (L5) ganglia from SNL rats; Sham: ganglion from Sham-operated rats; Cell size: mean diameter of neuron; RMP: resting membrane potential; Curr. thre.: current threshold; Vol. thre.: voltage threshold; APD50: action potential (AP) duration at half width; dV/dt r.: maximum rising rate of AP; dV/dt f.: maximum falling rate of AP; AHP50: afterhyperpolarization duration at half width; AHP amp.: amplitude of afterhyperpolarization. * P < 0.05, ** P < 0.01, DRG L4 or DRG L5 vs. Sham; † P < 0.05, †† P < 0.01, DRG L5 vs. DRG L4.

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large-sized somata in intact DRGs using intracellular electrophysiological recording in vivo or in vitro. For each property, the mean values listed in Table 1 for each size category for sham operates were within range of published values (Abdulla and Smith 2001a; Liu et al. 2000; Stebbing et al. 1999; Villiere and McLachlan 1996; Zhang et al. 1999).

Effects of SNL on the membrane properties of DRG somata of axotomized and intact neurons (Fig. 6). The next question was whether the effects of SNL were confined to axotomized neurons in L5 or whether the lesion also affected the membrane properties of intact neurons in L4. For each size category for sham operates, the data in Table 1 represent averages of values obtained from neurons in L5 and L4 DRGs. For each parameter, for a given size category, the means for sham operates were compared with the corresponding means obtained for L5 (DRG L5) and for L4 neurons (DRG L4) from SNL rats using one-way ANOVAs followed by post hoc comparisons. It was found that the effects of SNL on membrane properties were similar for L4 and L5 cells, and furthermore, were confined largely to the large- and medium-sized somata. In comparison with corresponding measurements obtained from sham-operated rats, large-sized somata in both L4 and L5 of SNL rats had significantly higher input resistances, lower current thresholds, lower voltage thresholds (i.e., closer to resting potential), and APs of wider durations and slower maximal falling rates. To investigate the contribution of rising and falling phases to the AP duration, we further compared the rise and fall time of L5/L4 neurons between SNL and sham-operates. The L5 large-sized somata exhibited APs with both a significantly longer mean rise time (0.74 ± 0.06 ms, n = 58, P < 0.05) and longer mean fall time (1.49 ± 0.11 ms, n = 58, P < 0.01) than those of the shams (rise 0.50 ± 0.06 ms, fall 0.70 ± 0.10 ms, n = 23). Although the large-sized somata of L4 DRGs in SNL rats exhibited a similar trend, their rise and fall times (0.69 ± 0.12 and 0.82 ± 0.10 ms, respectively, n = 23), were not significantly different from those of shams. Seven (16%) of the L4 and 13 (14%) of the L5 large-sized somata from SNL rats exhibited an inflection on the falling phase of the AP (Fig. 6) in contrast to only 1 (2%) large-sized somata from sham-operative rats. However, the difference was not statistically significant ($\chi^2$ test).

Some effects of SNL were greater for L5 than for L4 large-sized somata. L5 cells had a significantly lower resting potentials, lower current thresholds, and APs with wider durations, lower AHP amplitudes, and slower falling rates.

For medium-sized somata, the effects of SNL were similar to those of large-sized cells for some parameters but negligible for others. In comparison with values for sham rats, both L4 and L5 medium-sized somata from SNL rats exhibited APs with significantly wider durations and slower maximal rising and falling rates. The rise and fall times of medium-sized somata from either L5 (rise 1.78 ± 0.21 ms, fall 1.97 ± 0.15 ms, n = 27) or L4 (rise 2.47 ± 0.62 ms, fall 1.95 ± 0.29 ms, n = 22) DRGs of SNL rats were significantly longer than those from sham rats (rise 1.17 ± 0.10 ms, fall 0.98 ± 0.13 ms, n = 26). The effects of SNL were slightly greater for L5 than for L4 medium-sized somata. The L5 medium-sized somata exhibited significantly lower current and voltage thresholds than somata of either L4 or sham cells and significantly higher input resistances than those of L4 (but not sham).

SNL had a lesser effect on the membrane properties of small-sized neurons than on those of medium- or large-sized cells. Only two effects were observed. First, the maximum falling rates of the AP in small-sized somata of L5 and L4 SNL were significantly slower than those of sham rats. Second, small-sized somata in L5 had significantly lower voltage thresholds than those of L4 SNL or sham (with no difference between values obtained from L4 SNL and from sham rats).

Effects of PR on the membrane properties of axotomized and intact DRG somata recorded in vivo

Three to 7 days after PR, intracellular electrophysiological recordings were obtained in vivo from 86 DRG neurons obtained from nine rats. The central axons had been transected by the PR for 38 of these neurons but remained intact for the remaining 48. Thirteen cells were without the fluorescent dye but responded to electrical stimulation of the cut root and were therefore classified as “axotomized” neurons. Fifty-five neurons were recorded from seven rats 3–7 days after the PR-sham operation.

Effects of PR-sham surgery on the membrane properties of DRG somata. Because the mean values of each parameter for SNL-shams (averaged for L4 and L5) were within range of published values, they were assumed to be normal and therefore used to assess the effects of the PR-sham operation. The mean values of each parameter for PR-sham somata in each category of cell size were compared with corresponding values averaged from L4 and L5 somata of the SNL-sham-operated rats (Student t-test). The means for most parameters in Table 1 were not significantly different for PR-shams and SNL-shams and were within range of published values obtained using intracellular recording methods from the intact DRGs of unoperated control rats (Liu et al. 2000; Villiere and McLachlan 1996; Zhang et al. 1999). There were two exceptions. First, the mean input resistance for medium- and large-sized somata was significantly lower for PR- than SNL-shams (in Mohm, 8.98 ± 1.49, n = 16 vs. 20.24 ± 1.69, n = 36, for large somata and 15.77 ± 2.04, n = 24 vs. 29.94 ± 2.70, n = 33, for medium somata). Second, the mean current threshold of medium-sized cells for PR-shams (1.76 ± 0.27 nA, n = 23) was significantly higher than that of SNL-shams (0.88 ± 0.12 nA, n = 31).
PR: L5 dorsal root partial rhizotomy; unc: intact dorsal root fibers, cut: neurons with cut dorsal root fibers in PR rats, Sham: DRG neurons from Sham-operated rats; Cell size: mean diameter of neuron; * P < 0.05, ** P < 0.01, cut or unc vs. Sham; † P < 0.05, cut vs. unc. Other parameters are same as Table 1.

EFFECTS OF PR ON THE MEMBRANE PROPERTIES OF DRG SOMATA. Medium- and large-sized DRG somata were more affected by central axotomy than were small-sized somata just as they were after peripheral axotomy. However, in contrast to the effects of SNL, those of PR were relatively minor (Table 2). Both axotomized and intact PR somata of medium or large size had significantly lower voltage thresholds than somata of corresponding size from PR-shams. In addition, the axotomized medium- and large-sized somata (but not the intact) had a significantly higher input resistance than corresponding somata from PR-shams. The only significant difference between intact and axotomized neurons was a higher input resistance for axotomized medium-sized somata. PR had no significant effects, in relation to PR-sham, on the membrane properties of small-sized somata.

Functional properties of intracellulary recorded neurons with peripheral receptive fields

Among the 449 neurons recorded intracellularly, peripheral receptive fields (RFs) were found in 129 neurons, including 55 with large-sized somata, 28 with medium-sized somata, and 46 with small-sized somata. The RFs were mainly distributed in the lower leg and foot, because most of the sciatic nerve branches above the knee joint were transected during the surgical preparation for in vivo recording. Because there were no significant differences in RF properties due to the nerve lesion (SNL or PR), the RF data were pooled in Table 3 for SNL (L4 DRG only), SNL-sham (L4 and L5 DRG), PR, and PR-sham (L5 DRG).

Large-sized somata had muscle spindles (n = 27, 49%),

### Table 2. Electrophysiological properties of DRG neurons with axotomized or intact central axons after PR

<table>
<thead>
<tr>
<th>Size Category</th>
<th>Large</th>
<th>Medium</th>
<th>Small</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
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<td></td>
<td></td>
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<tr>
<td>Cell size, um</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>18</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>RMP, mV</td>
<td>-56.4 ± 2.0</td>
<td>-57.2 ± 2.0</td>
<td>-58.6 ± 1.6</td>
</tr>
<tr>
<td>n</td>
<td>18</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Km, Mohm</td>
<td>9.0 ± 1.5</td>
<td>13.1 ± 2.0</td>
<td>20.2 ± 4.0</td>
</tr>
<tr>
<td>n</td>
<td>16</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Curr. thre., nA</td>
<td>1.85 ± 0.33</td>
<td>2.18 ± 0.34</td>
<td>1.70 ± 0.38</td>
</tr>
<tr>
<td>n</td>
<td>16</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>Vol. thre., mV</td>
<td>-44.4 ± 4.6</td>
<td>-54.9 ± 2.1</td>
<td>-56.2 ± 2.0</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>12*</td>
<td>14</td>
</tr>
<tr>
<td>APD50, ms</td>
<td>0.60 ± 0.04</td>
<td>0.62 ± 0.06</td>
<td>0.66 ± 0.11</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>dV/dr, mV/ms</td>
<td>198 ± 20.6</td>
<td>225 ± 19.9</td>
<td>229 ± 30.7</td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>12</td>
<td>10</td>
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<tr>
<td>dV/dr, mV</td>
<td>-133 ± 11.9</td>
<td>-131 ± 9.1</td>
<td>-144 ± 22.3</td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>AHP50, ms</td>
<td>3.35 ± 1.18</td>
<td>4.71 ± 0.79</td>
<td>4.81 ± 1.44</td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>AMP amp., mV</td>
<td>-13.1 ± 2.3</td>
<td>-11.1 ± 0.8</td>
<td>-10.2 ± 1.1</td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>12</td>
<td>10</td>
</tr>
</tbody>
</table>

Values are given in means ± SE, and n = sample size. Neurons were classified by peripheral receptor type, i.e., muscle spindle (MS), cutaneous low-threshold mechanoreceptor (LTM), cutaneous high-threshold mechanosensitive nociceptor (HTM), and cutaneous mecha-no-heat nociceptor (MH) (3 of which also responded to cold). One mecha-no-heat sensitive nociceptor medium sized soma and one heat-only nociceptive small sized soma are not included. CVdr: dorsal root conduction velocity, AP amp.: amplitude of action potential. Other parameters are same as Table 1.

### Table 3. Electrophysiological properties of DRG neurons with functionally identified peripheral receptive fields

<table>
<thead>
<tr>
<th>Size Category</th>
<th>Large</th>
<th>Medium</th>
<th>Small</th>
</tr>
</thead>
<tbody>
<tr>
<td>Submodality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>27</td>
<td>27</td>
<td>10.6</td>
</tr>
<tr>
<td>CVdr, m/s</td>
<td>16.6 ± 1.4</td>
<td>14.7 ± 1.4</td>
<td>11.0 ± 1.5</td>
</tr>
<tr>
<td>n</td>
<td>17</td>
<td>25</td>
<td>2</td>
</tr>
<tr>
<td>Curr. Thre., nA</td>
<td>1.19 ± 0.26</td>
<td>1.41 ± 0.16</td>
<td>0.58 ± 0.14</td>
</tr>
<tr>
<td>n</td>
<td>14</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>APD50, ms</td>
<td>0.48 ± 0.08</td>
<td>0.53 ± 0.07</td>
<td>0.55 ± 0.10</td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>AP amp., mV</td>
<td>54.4 ± 3.1</td>
<td>57.1 ± 2.7</td>
<td>50.5 ± 1.5</td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>AHP50, ms</td>
<td>2.35 ± 0.28</td>
<td>3.69 ± 0.40</td>
<td>2.01 ± 0.23</td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>18</td>
<td>6</td>
</tr>
</tbody>
</table>

Values are given in means ± SE, and n = sample size. Neurons were classified by peripheral receptor type, i.e., muscle spindle (MS), cutaneous low-threshold mechanoreceptor (LTM), cutaneous high-threshold mechanosensitive nociceptor (HTM), and cutaneous mecha-no-heat nociceptor (MH) (3 of which also responded to cold). One mecha-no-heat sensitive nociceptor medium sized soma and one heat-only nociceptive small sized soma are not included. CVdr: dorsal root conduction velocity, AP amp.: amplitude of action potential. Other parameters are same as Table 1.
cutaneous low-threshold mechanoreceptors (LTM, 27, 49%), and a cutaneous high-threshold mechanoreceptor with no response to heat or cold (HTM, 2%).

Medium-sized somata had cutaneous LTM (n = 11, 39%), HTMs (9, 32%), muscle spindles (7, 25%), and a cutaneous high-threshold mecanooheat receptor (MH, 4%) that was unresponsive to cold.

All but 1 of the 46 small neurons with RFs had nociceptors, including 27 (59%) HTMs with no response to heat or cold, 14 (30%) MHS with no response to cold, 3 (7%) MHCs (MHs that responded to cold), and 1 (approximately 2%) responsive only to heat (H). An additional C-neuron had a high-threshold mechanoreceptor (LTM). The search procedure used mainly mechanical stimuli and was therefore biased against finding mechanically insensitive neurons.

There were certain features of the shapes of the APs that were more characteristic of nociceptive than the nonnociceptive neurons. All the C-nociceptors (including the HTMs, MHS, MHCs, and H) exhibited APs with an inflection on the falling phase, as did most of the A-nociceptors (82%, with the exception of 1 large- and 1 medium-sized HTM). In contrast, only one medium-sized LTM (approximately 2%) and none of the muscle spindles had APs with inflections. In addition, the AP amplitude and AHP duration (AHP50) of A- and C-nociceptive neurons were significantly larger than those with muscle spindles or LTM. Further comparisons of the membrane properties of A-neurons (large- and medium-sized somata) with RFs revealed that the current thresholds, AP durations, AP amplitudes, and AHP durations increased in the order of those with muscle spindles, LTM, and HTMs (Table 3). These findings are in general agreement with those obtained with intracellular recording from DRG somata of cat (Koerber et al. 1988), rat (Ritter and Mendell 1992), and guinea pig (Djourhi et al. 1998).

SNL, but not PR, increased the incidence of abnormal SA

Abnormal SA was defined as discharges that persisted for more than 3 min, typically having a bursting, tonic, or irregular pattern. The incidence of SA was electrophysiologically recorded three different ways: 1) in vivo, intracellularly from DRG somata; 2) in vitro, extracellularly from dorsal-root fibers; and 3) in vivo, extracellularly from dorsal root fibers. The intracellular recordings were confined to somata on the surface of the DRG, whereas the extracellular recordings presumably sampled the SA of neurons with somata distributed throughout the DRG. Consequently the extracellular recordings were thought to yield an incidence of SA that was more representative of SA in the DRG population. The patterns of SA (regular, bursting, or irregular) were similar for intracellular and extracellular recordings and some examples are shown in Fig. 7, E–G and H–J, respectively.

In vitro extracellular recording of SA after SNL. None of the 84 large- and medium-sized somata of “A-neurons” from sham-operated rats exhibited SA (Fig. 7A)—neither was the SA recorded in any of the 72 small-sized somata of “C-neurons” from sham rats (Fig. 7B). In contrast, after SNL, 11 of 143 A-neurons and 1 of 39 C-neurons from axotomized L5 DRG exhibited SA. Most of the cells with SA were recorded from axotomized L5 somata with only 1 of 78 A-neurons and 0 of 74 C-neurons sampled from intact somata in the L4 DRG (Fig. 7, A and B). The incidence of SA of L5 A-neurons was significantly higher after SNL than after the SNL-sham operation (P < 0.05, χ² test). However, we never found a SA neuron that had a RF. For neurons with RFs, any ongoing activity, for example, the tonic activity generated by muscle spindles, appeared normal and could always be manipulated by applying adequate stimuli to the RF. Because we could only access the RFs on the foot and lower leg, it is conceivable that some of the “SA” neurons may have had RFs on the upper leg, hip, or the back.

In vivo intracellular recording of SA after PR. Only 2 of the 86 cells recorded intracellularly after PR exhibited SA. These two cells were medium-sized with transected central axons and were without peripheral receptive fields. None of the 55 cells from sham rats exhibited SA.

In vitro extracellular recording of SA after PR. Recordings were obtained from 618 fibers from the injured portion of the L5 dorsal root and 607 fibers from the intact portion of L5 dorsal root for which electrical stimulation of the sciatic nerve evoked APs. Only seven A-fibers (1.13%), recorded from the injured portion of the dorsal root, exhibited SA. Of the 315 fibers activated from sciatic nerves from two PR-sham rats, none exhibited SA.

Thus the incidence of SA was not increased after central axotomy (PR) in contrast to the significant increase found in both axotomized and intact DRG neurons after chronic peripheral axotomy (SNL).

Acute peripheral axotomy may enhance the incidence of SA in intact A-neurons after SNL

During intracellular recordings from L4 somata, only one cell without peripheral RFs exhibited SA. The neurons without peripheral receptive fields were presumably acutely axotomized during the surgical procedure required to place the DRG into the recording chamber. There was also a tendency for the alterations in various membrane properties in cells to be more prominent in cells without receptive fields. Among the 44 large-sized somata recorded from the intact L4 DRG after SNL, peripheral RFs were located for 13 neurons. The remaining 31 neurons were presumably acutely axotomized during the surgical procedure required to place the DRG into the record-
ing chamber. Of the 44 large-sized somata from the L4 or L5 DRG after SNL-sham operation, RFs were identified for 27, with the remaining 17 considered to be acutely axotomized (for L4 DRG alone, there were 15 of 26 with and 11 without RFs). No significant differences between means for any of the electrophysiological parameters were found between the neurons with as opposed to without RFs. A possible exception was the higher incidence for large-sized somata without a RF to exhibit APs with an inflection on the falling phase. After SNL, 7 of 31 neurons (22.6%) without RF had an inflection on the falling phase of their APs, whereas 0 of the 13 neurons with RFs had the inflection. After the sham operation, only 1 of the 17 neurons (5.9%) without RFs and 0 of the 27 neurons with RFs had an inflection. After SNL, but not after SNL-sham, large-sized somata without RFs exhibited a tendency toward lower current thresholds (L4 without RF vs. with RF: 1.14 ± 0.19 nA, n = 17 vs. 1.25 ± 0.19 nA, n = 4), more negative voltage threshold (−47.16 ± 1.90 mV, n = 17 vs. −42.96 ± 2.80 mV, n = 7), and longer APD50 (0.71 ± 0.13 ms, n = 16 vs. 0.58 ± 0.14 ms, n = 7) than those with RFs. However, these numerical differences did not quite reach statistically significance. Similar tendencies were not observed for medium- or small-sized neurons with versus without RFs.

An experiment was undertaken to determine whether SA could be observed in A-neurons (including Aβ and Aδ) with peripheral RFs and whether the incidence of SA in intact DRG neurons after SNL was enhanced by acute peripheral axotomy. The criteria for “SA” would be an ongoing pattern of discharge (e.g., bursting, irregular) not characteristic for activity generated by the activation of peripheral receptors. Extracellular recordings from teased L4-dorsal root fibers were obtained in vivo 3–5 days after an ipsilateral L5 SNL. The spinal nerve of L4 was gently separated from adjacent tissues so that a small pledget of cotton soaked either in saline or in saline with 1% chloroprocaine could be placed around the distal portion approximately 8 mm from the DRG. The incidence of SA was recorded under each of three experimental conditions: the distal portion of the spinal nerve was exposed either to saline only, to the local anesthetic, or locally anesthetized and then transected. Attempts to electri-
cally stimulate the proximal, unanesthetized portion of the nerve were not successful, owing in part to difficulties in access to the nerve and stimulus spread to the recording electrode. Consequently, the incidence of SA was defined in terms of the number of fibers with SA per filament bundle of approximately 50 μm diameter. Individual fibers were distinguished by the shapes of their APs each of which also had to have a minimal signal to noise ratio of 3:1.

The incidence of SA obtained in the in vitro fiber recording experiments was expressed in Fig. 8A as the mean number of SA fibers per bundle. The incidence of SA was significantly higher for L5 than for L4 dorsal roots after SNL and lowest after the sham operation.

The incidence of SA recorded in vivo from L4 dorsal root fibers of three sham-operated rats was 0.061 (5 SA fibers in 82 intact L4 or L5 dorsal root bundles; Fig. 8B). This value was not significantly different from that recorded in vitro from sham dorsal roots (Fig. 8A; χ² test). The incidence of SA recorded in vivo from dorsal roots 3–5 days after SNL (3 rats; Fig. 8B) was 0.89 for axotomized L5 neurons (58 SA fibers from 65 bundles) and 0.125 for intact L4 neurons (7 SA fibers from 56 bundles). The latter was not significantly different from that recorded from sham L4, but significantly lower than that obtained from L4 with in vitro recordings (Fig. 8A; χ² test, P < 0.01).

The anesthetic was effective in eliminating activity originating from peripheral tissues innervated distal to the nerve block. After applying the anesthetic for about 10 min, the incidence of SA in the L4 dorsal root was 0.23 (24 SA fibers from 88 bundles, 3 rats)—significantly higher than that obtained from sham L4 (0.061), but not significantly different from that recorded in the absence of anesthetic (0.125; χ² test; Fig. 8B). However, the incidence of SA was significantly higher after the spinal nerve was anesthetized and then transected. After transecting the nerve the incidence was 0.55 (43 fibers of 78 bundles, 3 rats)—a value significantly greater than that obtained when the nerve was intact with or without the anesthetic (χ² test, P < 0.01; Fig. 8B).

A test for the possible injury of L4 neurons after ipsilateral L5 SNL

Five days after SNL (2 rats) or sham operations (2 rats), cell counts were obtained from L4 and L5 DRGs ipsilateral and contralateral to lesion. For sham-operated rats, there was no significant difference in the number of c-Jun immunopositive cells (<1%) between ipsilateral L4 and L5 DRG. The effect of SNL on the number of c-Jun immunopositive cells in L5 was dramatic. Most ipsilateral L5 DRG neurons were immunopositive for c-Jun 5 days after L5 spinal nerve transection (Fig. 9A). However, only about 1% of neurons were c-Jun positive in L4 DRG ipsilateral to L5 spinal nerve transection (Fig. 9B). In ipsilateral L4 DRG, there was a strong likelihood that the few c-Jun-positive cells presented had peripheral targets in the dorsal skin of the back that were severed during surgery. There was no significant difference in the number of positive cells between the ipsilateral L4 and contralateral L4/L5 DRG of SNL rats.
DISCUSSION

Central axotomy produced only transient hyperalgesia and minor changes in electrophysiological properties of DRG neurons

The transection of central axons by a PR produced transient mechanical hyperalgesia on the ipsilateral foot, without allodynia to stroking. After surgery, the withdrawal threshold of ipsilateral foot to Von Frey hair stimulation was significantly lowered through the seventh day of testing, but returned to the preoperative level by day 10. A short lasting hyperalgesia was also obtained after a complete transection of the L5 dorsal root (Li et al. 2000; Sheth et al. 2002). In these studies, mechanically evoked withdrawal threshold returned to normal within 7 days.

The electrophysiological effects of the partial rhizotomy were rather small. There was no increase in the incidence of SA in either axotomized or intact neurons, and the minor changes in membrane properties were limited to medium- or large-sized somata. For these, the input resistance was lowered by the sham operation itself but increased by the effects of axotomy, suggesting a predominantly nonspecific effect of the surgical procedure. Possibly the opening of the dura, carried out for both sham and experimental rats, might have produced a local inflammation on the surface of exposed dorsal roots that, in turn, might have altered the membrane properties of some primary afferent neurons (DeLeo and Yezierski 2001).

Our findings of transient hyperalgesia and few significant electrophysiological changes in axotomized and intact DRG neurons after PR are at odds with the results obtained by Zhou and Xie (2000) and Xie et al. (1999). These investigators ligated one-half of the dorsal root of L5 and left the suture in place. Ipsilateral mechanical hyperalgesia persisted through 30 days of testing. In addition, abnormal SA was recorded from both axotomized and intact dorsal root fibers. Possibly their implanted suture may have had a chronic irritating effect on both axotomized and intact roots. However, there is no direct evidence for this at the present time. In any event, we conclude that the transient mechanical hyperalgesia we observed in the present experiment was not due to a change in the excitability of primary sensory neurons but rather to changes in central processing caused by a loss of afferent input (Eschenfelder et al. 2000; Li et al. 2000).

Peripheral axotomy produced lasting hyperalgesia and allodynia and major changes in the excitability of DRG neurons with myelinated axons

SNL produced significant mechanical hyperalgesia to punctate indentation of the ipsilateral foot and allodynia to innocuous tactile stroking each lasting through the seventh postoperative day of testing. A lowered withdrawal threshold to Von Frey stimuli ipsilateral to SNL has been amply demonstrated in previous studies and found to persist for at least 10 wk (e.g., Kim and Chung 1995; Liu et al. 2000).

After peripheral axotomy by SNL, somata of large and medium size, in both chronically axotomized and the neighboring intact DRG, exhibited a higher input resistance, lower current and voltage threshold, longer AP duration, and slower AP rise and falling rates. There were no major changes in the membrane properties of C-neurons with small-sized somata. Correspondingly, SA was recorded in vitro from the dorsal root fibers of A- but not C-neurons of L4 as well as L5.

Our finding of the absence of c-jun labeling in L4 (in contrast to L5) was taken as evidence that the similarity in electrophysiological changes between the two ganglia was not due to the possibility that the L4 axons were stressed or injured (Decosterd et al. 2002; Jenkins and Hunt 1991; Kenney and Kocsis 1997a,b).

Possible mechanisms of enhanced excitability of axotomized and intact DRG neurons after SNL

The changes in excitability and the shapes of APs of A-neurons after peripheral axotomy are well described in the literature and similar to present findings (Abdulla et al. 2001a; Kim et al. 1998; Liu et al. 2000; Stebbing et al. 1999). A novelty of our findings is that qualitatively similar changes occurred in intact A-neurons with somata in the L4 DRG adjacent to the peripherally axotomized L5. Thus it seems reasonable to postulate that similar ionic mechanisms were responsible for the changes in membrane properties observed in both axotomized and intact neurons. In addition, the signaling mechanisms that trigger these changes may be similar as well. Our working hypothesis is that there is a de novo synthesis of cytokines from either neurons or nonneuronal cells in response to axonal injury and that these cytokines induce changes in the cell bodies of A-type neurons.

Voltage-clamp studies of isolated currents in dissociated DRG cells from nerve-injured animals have focused on the altered properties of axotomized but not intact neurons. For example, after peripheral axotomy, the axotomized somata exhibited an up-regulation of tetrodotoxin-sensitive (TTX-S) Na⁺ current and down-regulation of tetrodotoxin-resistant (TTX-R) Na⁺ currents (see Black et al. 1999; Rizzo et al. 1995; Sleeper et al. 2000; Waxman et al. 1999 for review), together with a reduction of K⁺ and Ca²⁺ currents (Abdulla and Smith 2001b; Baccini and Kocsis 2000; Everill et al. 1998; Hogan et al. 2000). Although some of these findings might be argued as contributing in various ways to the changes we observed in the shape of the AP or the enhanced excitability of the axotomized L5 neurons, there is scant evidence in the literature that the same changes occur for intact L4 neurons. In addition, explanations for the cause of the changes in current expression in an axotomized neurons based on the loss of trophic support (e.g., NGF) from the periphery (Dib-Hajj et al. 1998; Oyelese et al. 1997; for review, Waxman et al. 2000) cannot be used to explain similar changes in intact neurons in a neighboring ganglion that still has this support.

Because L4 afferent fibers comingle with degenerating L5 axons in the peripheral nerve, Wallerian degeneration may be the cause of the events leading to the enhanced excitability of intact neurons with somata in L4 DRG adjacent to the axotomized L5 DRG. For example, nerve growth factor (NGF) or glial cell line-derived neurotrophic factor (GDNF), cytokines [e.g., tumor necrosis factor (TNF)α, interleukin (IL)-1β], or other inflammatory mediators released by immune cells and Schwann cells activated by L5 axonal degeneration could diffuse to neighboring intact axons of L4 and be either retrogradely transported or locally induce signal transduction cascades in L4 DRG, thereby influencing gene expression in these
neurons (Cui et al. 2000; Li et al. 2000; Ramer et al. 1997; Shamash et al. 2002; Sommer and Schafer 1998; Wagner and Myers 1996; Wu et al. 2001). There is increasing evidence for the altered expression of receptors, neuropeptides, and ion channels in the intact L4 DRG adjacent to L5-SNL. Increases in the expression of vanilloid receptor (VR)-1 (Hudson et al. 1996; Wu et al. 2001). There is increasing evidence for altered excitability of unmyelinated peripheral nerve fibers from the L4 DRG after an L5 SNL. NaV1.8 protein and the TTX-R C-wave of the compound AP were up-regulated in the intact C-fibers in the sciatic nerve (Gold et al. 2001). In addition, Wu et al. (2001) found an early onset of SA at a low rate (approximately 7 pulses/5 min) in L4 dorsal root C-fibers after L5 SNL. This SA originated distal to the L4 DRG and was present in nociceptive fibers with cutaneous receptive fields. We did not observe any SA in intact, small-sized somata with C-fibers and peripheral cutaneous nociceptors. However, the observation period we allowed for SA, prior to continued study of a neuron, was relatively short (3 min), leaving open the possibility that a very low rate of SA may have been overlooked. It may also be that the C-neurons of L4, while altered in certain ways by the SNL injury, do not express SA unless they are repetitively activated, for example, by the electrical search stimuli used in the study by Wu et al. (2001).

The cellular events set in motion by peripheral axotomy may increase the excitability of second order nociceptive neurons in the dorsal horn receiving input both from nociceptive and low threshold mechanoreceptive DRG neurons. As discussed in the literature, such a state of central sensitization might be initiated and maintained by abnormal processing in central as well as peripheral neurons (for review, see Devor 1999; Ji and Woolf 2001).

**Effect of acute axotomy on the excitability of intact L4 neurons**

In rats with no previous nerve injury, a transection of the L5 spinal nerve does not result in abnormal SA until 16–20 h after the axotomy (Liu et al. 2000). We found that after SNL, intact L4 neurons still connected with their peripheral receptors exhibited no SA. However, starting approximately 30 min after acute peripheral axotomy, SA could be recorded in approximately 9.1% of the A-neurons. Boucher (2000) obtained a similar incidence of SA in 14 ± 2% of the myelinated dorsal root fibers from L4 DRG in vivo (with spinal nerve acutely transected) after an L5 SNL.

We consider three possibilities for the rapid appearance of SA in certain A-neurons after acute axotomy. The simplest explanation is that, after nerve section, it may be easier to distinguish abnormal SA from ongoing activity produced by certain peripheral receptors such as muscle spindles. Against this idea was the finding that the incidence of SA was not significantly different with versus without anesthetization of the nerve (which eliminated, for example, the competing, normal, ongoing activity from muscle spindles). Alternatively, the ectopic generator in the soma may be suppressed (or continu-

ally reset) by APs generated from the periphery. A third possibility, and one that we favor, is that the nerve section either interrupts a signal transmitted along the axon from the periphery to the DRG that normally acts to suppress the excitability of the soma or a positive signal is generated at the proximal stump of the nerve that is also transported anterogradely and acts to further increase the excitability of the soma. After SNL, the intact L4 somata and/or peripheral axons may develop an enhanced sensitivity to neuroactive agents (e.g., TNFα, IL-1, ATP, bradykinin, epinephrine, and protons) released from the axotomized axons, Schwann cells, blood vessels, and other tissues in the acute transected proximal nerve stump (see review by Stoll and Muller 1999).

**Evaluation of the in vivo recording preparation**

Our in vivo preparation provided a relatively stable environment for the somata of primary sensory neurons by isolating the DRG from its original site and perfusing it with oxygenated ACSF, while maintaining at least a portion of its blood supply. The preparation also allows the determination of the receptive field properties of a portion of the DRG neurons, but only those with somata on the surface. The extent to which the properties of these surface somata are the same of somata located deeper in the ganglion remains to be determined. The location of the cell bodies within the DRG was not well specified in previous intracellular recordings from intact DRG after L5 SNL. For most of the membrane properties recorded intracellularly with our preparation, the mean values for each size category for axotomized and sham-operated rats were within range of published values (Abdulla and Smith 2001a; Liu et al. 2000; Stebbing et al. 1999; Villiere and McLachlan 1996; Zhang et al. 1999), except for some discrepancies in AP shape (duration, rise/fall rates and percentage of inflected A-neurons), input resistance, and AP threshold. As noted in previous studies, for example, Amir and Devor (1996), Stebbing et al. (1999), and Liu et al. (2000), such discrepancies might arise from differences in the type of injury, the type of electrophysiological recording (patch- or sharp-electrode), the temperature of the preparation, and whether recordings are carried out in vitro or in vivo.

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