Nerve injury induces a new profile of tactile and mechanical nociceptor input from undamaged peripheral afferents

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Boada MD, Gutierrez S, Aschenbrenner CA, Houle TT, Hayashida K, Ririe DG, Eisenach JC. Nerve injury induces a new profile of tactile and mechanical nociceptor input from undamaged peripheral afferents. J Neurophysiol 113: 100–109, 2015. First published October 1, 2014; doi:10.1152/jn.00506.2014.—Chronic pain after nerve injury is often accompanied by hypersensitivity to mechanical stimuli, yet whether this reflects altered input, altered processing, or both remains unclear. Spinal nerve ligation or transection results in hypersensitivity to mechanical stimuli in skin innervated by adjacent dorsal root ganglia, but no previous study has quantified the changes in receptive field properties of these neurons in vivo. To address this, we recorded intracellularly from L₄ dorsal root ganglion neurons of anesthetized young adult rats, 1 wk after L₄ partial spinal nerve ligation (pSNL) or sham surgery. One week after pSNL, hindpaw mechanical withdrawal threshold in awake, freely behaving animals was decreased in the L₄ distribution on the nerve-injured side compared with sham controls. Electrophysiology revealed that high-threshold mechanoreceptive cells of A-fiber conduction velocity in L₄ were sensitized, with a seven-fold reduction in mechanical threshold, a seven-fold increase in receptive field area, and doubling of maximum instantaneous frequency in response to peripheral stimuli, accompanied by reductions in after-hyperpolarization amplitude and duration. Only a reduction in mechanical threshold (minimum von Frey hair producing neuronal activity) was observed in C-fiber conduction velocity high-threshold mechanoreceptive cells. In contrast, low-threshold mechanoreceptive cells were desensitized, with a 13-fold increase in mechanical threshold, a 60% reduction in receptive field area, and a 40% reduction in instantaneous frequency to stimulation. No spontaneous activity was observed in L₄ ganglia, and the likelihood of recording from neurons without a mechanical receptive field was increased after pSNL. These data suggest massively altered input from undamaged sensory afferents innervating areas of hypersensitivity after nerve injury, with reduced tactile and increased nociceptive afferent response. These findings differ importantly from previous preclinical studies, but are consistent with clinical findings in most patients with chronic neuropathic pain.

chronic pain model; in vivo electrophysiology; sensory neurons; spinal nerve ligation

Animal models of neuropathic pain typically involve surgical damage to peripheral nerves, and hypersensitivity to mechanical stimuli is commonly used to assess the development and magnitude of the neuropathological condition. Withdrawal to a stimulus in the behaving animal relies on mechanical activation of intact cells from neighboring ganglia innervating the dermatome of testing. Paradoxically, most studies have focused on the axotomized rather than the undamaged sensory afferents. Ectopic discharges from these axotomized cells are speculated to drive spontaneous pain and long-lasting central sensitization (Kajander et al. 1992; Kirk 1974; Wall and Devor 1983), but whether input from intact afferents is altered has been less well studied.

In addition to central sensitization, hypersensitivity could reflect altered input from intact sensory afferents, as supported by numerous changes in the protein expression in these cells (Gold et al. 2003; Obata et al. 2003), as well as responses recorded in teased fibers preparations (Ali et al. 1999; Shim et al. 2005). Although the focus has been on sensitization of slow- and fast-conducting nociceptors, some studies have suggested sensitization of low-threshold tactile afferents, and alterations in their neuropeptide content and sprouting into the superficial laminae of the spinal cord dorsal horn (Kohama et al. 2000). This finding is curious, given the presence of negative findings, including hypoesthesia, numbness, and reduction in detection threshold to mechanical vibration frequently encountered in patients with neuropathic pain (von Henn et al. 2012). In addition, loss rather than enhancement of large-fiber input would be expected to enhance transmission of pain at the spinal level (Melzack and Wall 1965). Indeed, indirect data suggest that early decreased activity of tactile afferents, not increased activity, drives hypersensitivity (Komagata et al. 2011).

Intracellular recording provides a definitive method to investigate the effects of nerve injury on intact afferents, since it does not rely on imprecise immunohistochemical or cell size definition of afferent subtype. Few investigations previously recorded from intact dorsal ganglia neurons with receptive fields (RFs) in the area of neuropathic hypersensitivity (Djouhri et al. 2012; Ma et al. 2003; Zhu et al. 2012; Zhu and Henry 2012). Their findings were highly discrepant, likely reflecting differences in surgical injury, the temperature of the neuronal soma at the time of study, focus only on cells with mechanism-ly sensitive RFs, and failure to measure the RF areas.

The purpose of the present study is to define the passive and active membrane properties and RF characteristics of neurons in the uninjured dorsal root ganglion (DRG) adjacent to the injured one at the time when the animal shows maximal

IN HUMANS, NEUROPATHIC PAIN includes both spontaneous and evoked pain, often accompanied by hypersensitivity to normal nociceptive stimuli (hyperalgesia) and normally nonnociceptive stimuli (allodynia). This pathological condition is also sometimes accompanied by dysesthesias and paresthesias (von Henn et al. 2012), suggesting that both tactile and nociceptive information channels and/or processing are disrupted.

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mechanical hypersensitivity. Based on previous preclinical (Boada et al. 2012) and clinical (von Hehn et al. 2012) observations, we hypothesized that nociceptive and nonnociceptive L₄ afferents would be affected in an opposite manner by damage to the neighboring L₅ ganglion. This hypothesis was confirmed across several domains of neurophysiological sensitivity, and, after adjacent injury, a large increase was observed in the proportion of cells with electrical signatures similar to tactile afferents but with no peripheral RF at all.

METHODS

Animals

Twenty-three juvenile female Sprague-Dawley rats (postnatal day 28) were used. Animals were housed together in pairs, in a climate-controlled room under a 12:12-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 Wake Forest School of Medicine.

L₅ Partial Spinal Nerve Ligation

The animals were deeply anesthetized with isoflurane, and, under aseptic conditions, the skin was incised at the midline over the lumbar spine. The right L₅ spinal nerve was ligated and identified approximately 1/3 to 1/2 thickness of the L₅ spinal nerve was ligated with 9-0 nylon suture under a dissecting microscope, as previously described (Guan et al. 2010). Care was taken not to pull the nerve or contact the intact L₄ spinal nerve. After hemostasis was achieved, the muscle layer was approximated with 4-0 synthetic absorbable suture (Look, Reading, PA), and the skin closed with absorbable suture. In a sham control group, the surgical procedure was identical to that described above, except that the left L₅ spinal nerve was not injured. After the surgery, the rats were returned to their cages, kept warm under a heat lamp, and monitored during recovery.

Behavioral Testing

Animals were placed on a mesh surface in a plastic cage and were acclimated for 20 min before testing. Withdrawal threshold in awake rats was assessed on the hindpaws resting on the mesh surface using calibrated von Frey filaments to determine withdrawal thresholds to application of the filament on the footpad until the filaments bent. This was done by a person blinded to the surgical treatment. The von Frey filaments used were 3.84, 4.08, 4.31, 4.56, 4.74, 4.93, 5.18, 5.46, and 5.88, corresponding to 0.5, 0.9, 1.7, 3.7, 5.5, 8.0, 12.4, 21.5, and 53.0 g, respectively. This was done three times, with a positive response determined by brisk withdrawal of the foot from the filament. The force resulting in withdrawal with a 50% probability of withdrawal (termed withdrawal threshold by convention) was determined using the up-down method, as previously described (Chaplan et al. 1994). Withdrawal thresholds were determined before partial spinal nerve ligation (pSNL) and 1 wk after pSNL. All animals were included in the data analysis, and no animal in the study had a wound dehiscence or infection during the study.

Electrophysiology

A week after pSNL or sham surgery and behavioral assessment, rats were deeply anesthetized with isoflurane 3%. The trachea was intubated, and animals ventilated using pressure-controlled ventilation (Inspira PCV, Harvard Apparatus, Holliston, MA) with humidified oxygen. Heart rate was monitored throughout as a guide to depth of anesthesia. Anesthetized animals were immobilized with pancuronium bromide (2 mg/kg), and inspired isoflurane maintained at 2% throughout the study (Tevan Pharmaceuticals). As illustrated in Fig. 1, a dorsal midline incision was made in trunk skin, and the L₄ DRG and adjacent spinal cord were exposed by laminectomy, as previously described (Boada et al. 2010). The tissue was continuously superfused with oxygenated artificial cerebrospinal fluid (in mM: 127.0 NaCl, 1.9 KCl, 1.2 KH₂PO₄, 1.3 MgSO₄, 2.4 CaCl₂, 26.0 NaHCO₃, and 10.0 d-glucose). The spinal column was secured using custom clamps, and the preparation was transferred to a preheated (32–34°C) recording chamber where the superfusate was slowly raised to 37°C (MPRE8, Cell MicroControls, Norfolk, VA). Pool temperature adjacent to the DRG was monitored with a thermocouple (IT-23, Physitemp, Clifton, NJ). Rectal temperature (RET-3, Physitemp) was maintained at 34 ± 1°C with radiant heat.

The electrophysiological recordings from L₄ DRG neurons were limited to a maximum duration of 4,250 s (70.8 min) to diminish the likelihood that experimental manipulation would result in sensitization and to allow equal time to search for afferents in sham and pSNL animals. DRG soma were impaled with borosilicate microelectrodes (80–250 MΩ) containing 1 M potassium acetate. Intracellular penetrations with a resting membrane potential (Eᵣ) of −40 mV or less were characterized further. DC output from an Axoclamp 2B amplifier (Axon Instruments/Molecular Devices, Sunnyvale, CA) was digitized and analyzed off-line using Spike2 (CED, Cambridge, UK). Sampling rate for intracellular recordings was 21 kHz throughout (MicroPower1401, CED).

In this study, both cells with positive mechanically sensitive RF (P-RF) and no mechanically sensitive RF (N-RF) were included. Only cells capable of generating a somatic action potential (AP) (by current somatic injection, 25- and 500-ms pulses) and with impalements stable long enough to adequately explore the full extent of the skin at the L₄ dermatome (>2 min) were included. Unexcitable cells (stable impalements of more than 5 min with steady Eᵣ of −40 mV or greater but unexcitable to peripheral mechanical stimuli and intrasomal injection of current) were noted for general statistical purposes only.

Cellular Classification Protocol

To identify the RF, the skin was searched, applying gentle pressure with a fine-tipped brush. For nonresponding afferents, subsequent
searches used increasingly stiffer probes and finally sharp-tipped watchmaker forceps. Afferents with cutaneous RFs were distinguished from those with deep RFs by displacing skin to ensure that RFs tracked rather than remained stationary. Mechanical thresholds were characterized with calibrated von Frey filaments (Stoelting, Wood Dale, IL) with the mechanical threshold (MT) being the minimum von Frey hair producing neuronal activity. The cellular classification process was performed in a sequential manner and by the combination of multiple parameters to narrow down a given afferent identity. For example: response to brushing = high-sensitive mechano → rapidly adapting (RA) response + MT < 1.6 mN = low-threshold mechanoreceptor (LTMR) → response to single hair displacement + conduction velocity (CV) > 5 m/s = LTMR = fast-conducting hair (Hair). The same step-by-step procedures were used for every recorded cell, and, when in conflict, additional test and evaluations were performed (e.g., presence of poststimuli discharge and presence of spontaneous activity). The result of these procedures was combined with specific cellular properties (AP shape and somatic passive characteristics) to assign every cell into one of three simplified categories: LTMR, A-fiber high-threshold mechanoreceptor (AHTMR), C-fiber high-threshold mechanoreceptor (CHTMR), based on the strongest defining characteristics (Boada et al. 2011) and to compare afferents between sham and pSNL groups, innervating both types of skin (glabrous and hairy) (Boada et al. 2010). In particular, their adaptation rate [RA or slow adapting (SA) units] was evaluated in all cells since “on-off” responses to steady suprathreshold mechanical stimulation are characteristic of a large percentage of tactile afferents (LTMRs) and never observed in nociceptors (AHTMRs and CHTMRs) (2 s per trial, 3 trials per cell, 2 × MT). This procedure used a micromanipulator-based probe to stretch the skin in and around the cellular RF. In addition, the application of vibratory stimuli was used to assess cellular response characteristics within the RF (tuning fork of 256 and 512 Hz; SKLAR Instruments, West Chester, PA) because tracking of vibration has been shown to be 100% predictive of the LTMR population (Boada et al. 2010, 2011). In all cases, RFs were characterized and measured with the aid of a zoom stereomicroscope (for details see RF Analysis section).

**P-RF and N-RF Neurons: Somatic Electrical Properties**

Active membrane properties of all excitable neurons were analyzed, including the amplitude and duration of the AP and afterhyperpolarization (AHP) of the AP, along with the maximum rates of spike depolarization and repolarization; AP and AHP durations were measured at half-amplitude (D50 and AHP50, respectively) to minimize hyperpolarization-related artifacts. Passive properties were analyzed including $E_{\text{Na}}$, input resistance (Ri), time constant ($\tau$), inward rectification, and, where possible, rheobase; all but the latter were determined by injecting incremental hyperpolarizing current pulses ($\pm 0.1 \, \text{nA}, 500 \, \text{ms}$) through balanced electrodes.

The N-RF cells were separated in two different populations based on the shape of the AP (Cabanes et al. 2002; Gallego and Eyzaguirre 1978; Yoshida and Matsuda 1979): neurons with inflection in the repolarizing phase (S-type neurons) and neurons without this inflection (F-type neurons). To more clearly determine the presence of this inflection, the differentially recorded data of the AP were used (presence or absence of a second additional negative component in the time course of the AP derivative). Since RF properties, especially response characteristics, were used to define differences in the fast-conducting afferents (those without inflected APs), the ability to accurately define and categorize these two populations further was not possible.

All included cells satisfied the following requirements: $E_{\text{Na}}$ more negative than $-40 \, \text{mV}$, AP amplitude $\geq 30 \, \text{mV}$ and the presence of AHP. Passive membrane properties indicative of poor (extremely low Ri and/or $\tau$) impairment were also reasons for exclusion.

**P-RF Neurons Only: CV**

Because intact lumbar DRGs serve multiple nerves, spike latency was obtained by stimulating the RF at the skin surface using a bipolar electrode (0.5 Hz); this was performed following all natural stimulation to prevent potential alterations in RF properties by electrical stimulation. All measurements were obtained using the absolute minimum intensity required to excite neurons consistently without jitter; this variability (jitter) in the AP generation latency (particularly at significantly shorter latencies), seen at traditional (i.e., two- to threefold threshold) intensity has been presumed to reflect spread to more proximal sites along axons. Any neuron with jitter was rejected (3 cells). Stimuli ranged in duration from 50 to 100 μs; utilization time was not taken into account. Conduction distances were measured for each afferent on termination of the experiment by inserting a pin through the RF (marked with ink at the time of recording) and carefully measuring the distance to the DRG along the closest nerve.

**Maximal Instantaneous Frequency Response**

Since it is extremely difficult to deliver a controlled mechanical stimuli (both force and duration) we used Maximal Instantaneous Frequency Response (IF max) rather than firing rate as another parameter to detect a change in the response characteristics of these neurons. IF max was defined as the reciprocal of the minimum interval between impulses in response to suprathreshold mechanical stimulation of the cellular RF.

**RF Analysis**

After establishing the afferent identity, the RF was carefully searched with suprathreshold mechanical stimuli. During the skin RF mapping, two parameters were recorded: number and location of “spots” with the highest sensitivity (lowest threshold responses) and absolute RF area ($\text{mm}^2$) at threshold intensity. Values where obtained by tracing the cellular RF on the skin to later establish absolute area. The area measurements were performed using StereoInvestigator 7.0 (MicroBrightField, Williston, VT) that was supported by an Olympus BX51 microscope and a digital camera (Microfire A/R, Optronics, Goleta, CA). The perimeter of the traced RF was captured at ×4 magnification. StereoInvestigator automatically calculated the area of the traced RF.

**Data Analysis**

Data are presented as appropriate to their underlying distributions with mean and SE for normally distributed data, and median and range for nonparametric data. For behavioral withdrawal analysis, a two-way ANOVA was performed with experimental group as a between-subjects factor and pre- and postsurgery as a repeated-measures factor. Correction for multiple comparisons was made when appropriate. Prior to analysis of electrophysiological variables, we examined the assumption that measurements taken on neurons from the same animal are independent of one another (i.e., that neurons selected randomly across animals are as similar as those from within animals) using the intraclass correlation. For all measurements, the intraclass correlations were <0.22, indicating support for the independence assumption; thus all analyses were conducted assuming each neuron represented an independent measurement of others taken from the same animal. An interim power analysis was conducted at $N = 68$ cells to examine effect sizes and cell representation. It was determined that the total sample size of $N = 136$ cells was needed to provide 80% power, assuming a two-sided $\alpha = 0.05$ and to detect an effect size of $d = 0.43 \, \text{SD}$.

Differences between cell population proportions in the sham and pSNL groups were examined using $\chi^2$. Differences in the distributions...
between sham and pSNL groups for scaled measurements were examined using Mann-Whitney U.

To accomplish the exploratory aims of this study, we designed statistical analyses that interpret hypotheses in light of the number of comparisons needed to examine the pool of hypotheses. To accomplish this, we report hypothesis tests that are adjusted using Holm’s step-down procedure ($P \leq 0.05$/the number of remaining inferences to be tested in that family). We applied the adjustments within a family of comparisons (i.e., all electrical measurements in one cell type is one family) and report this with each analyses.

RESULTS

Effect of pSNL on Withdrawal Threshold to Hindpaw Stimulation

Withdrawal threshold prior to surgery did not differ between groups (population means $= 21.8 \pm 2.1$ g). One week after surgery, withdrawal threshold ipsilateral to pSNL (4.4 $\pm 0.5$ g) was significantly lower compared with sham surgery animals (22.2 $\pm 2.0$ g; $P < 0.01$). In contrast, withdrawal threshold contralateral to surgery did not differ between groups and did not differ from presurgery baseline (data not shown).

Effects of L5 pSNL on the Properties of Intact L4 DRG Sensory Neurons

The distribution of cells recorded and analyzed by group in the study is diagrammed in Fig. 2 and detailed in the text below. Of note, the likelihood of impaling cells which met passive membrane property definitions but which were not excitable was considerably increased in recordings from animals after pSNL group, as was the likelihood of impaling cells that were excitable, but for which a mechanical RF could not be identified. Additionally, a larger proportion of LTMR cells in the pSNL did not meet criteria for hair, D-hair, or mechano-cold definitions, as in the sham group.

As showed in the diagram (Fig. 2), the present study includes a total of 277 cells recorded in a stable fashion at lumbar level (L4) with an average of 9 cells (sham) and 10 cells (pSNL) per experiment. This number comprised both sham (116 cells/13 animals) and pSNL (161 cells/15 animals) recordings in all three categories (P-RF, N-RF and unexcitable) [sham: P-RF (101/116, 87%), N-RF (15/116, 13%), unexcitable (0/116, 0%); pSNL: P-RF (54/161, 33%), N-RF (82/161, 51%), unexcitable (25/161, 16%)]. The P-RF cells in both groups were classified further using defined criteria (see METHODS), as tactile (LTMR: sham: 42/101; pSNL: 23/54), fast nociceptors (AHTMR $> 1.2$ m/s; sham: 21/101; pSNL: 17/54) and slow nociceptors (CHTMR $< 1.2$ m/s; sham: 22/101; pSNL: 11/54), innervating both glabrous and hairy skin. N-RF afferents were classified by the absence or presence of inflection into F-types neurons (sham: 0/15; pSNL 51/82) and S-type neurons (sham: 15/15; pSNL: 31/82), respectively (not shown in Fig. 2).

Muscle Spindles

This population of afferents innervating skeletal muscle stretch receptors was found in both sham (16/101) and pSNL (3/54) animals. Most were readily identifiable as spindle afferents, with discharge that was tightly correlated with muscular stretch, or stimulus-evoked discharge that could be reproducibly modulated by alternating flexion/extension of the extremity. In either case, RFs failed to track with skin and remained fixed with deeper tissues. All of these cells (19/19) showed intense ongoing activity and subthreshold membrane potential oscillations. After being identified as muscle spindles afferents, they were excluded from further analysis.

Characteristics of P-RF Cells

As with electrical properties of the soma (see below), after pSNL there was a greater effect on RF properties of LTMRs and AHTMRs than on the RF properties of CHTMRs. Representative recordings of these cellular subtypes are provided in Fig. 3. LTMRs from pSNL animals were desensitized com-

Fig. 2. Classification of neurons included in the study. pSNL, sham; AP, action potential; RF, receptive field; MS, muscle spindle; CHTMR, C-fiber low-threshold mechanoreceptor; AHTMR, A-fiber high-threshold mechanoreceptor; LTMR, low-threshold mechanoreceptor; RA, rapid adapting; SA, slow adapting; Unclass, unclassified; M-Cold, mechano-cold. Statistically significant differences in distributions are shown by shaded bars.
pared with sham, with large increases in MT and reductions in RF area (Fig. 4, A and B). AHTMRs exhibited an opposite effect, with reductions in MT and increases in RF area. Additionally, the maximum IF max of response to a peripheral mechanical stimulus was increased in AHTMR after pSNL (Fig. 4C), consistent with the reduction in their AHP50 described above, whereas this measure of responsiveness was reduced in LTMRs.

Tactile LTMR-sham. These neurons had fibers conducting in the A range (<1.2 m/s) (mean: 16.02 ± 2.5 m/s), with low MT (median: 0.3 mN; range: 0.07–5.8 mN), and were capable of following vibratory stimulation (1:1 response to a 256-Hz tuning fork application), large multispot RF (2–3 points/RF) (median: 11.5 mm²; range: 2–28 mm²) and exhibited a relatively high IF max (median: 250 Hz; range: 112–556 Hz). Despite their physiological identity (unclassified RA units: 16/42; Hair: 19/42; D-Hair: 3/42; mechano-cold: 2/42 and SA units: 2/42), in the present study both RA (RA of different types) and SA units were considered LTMRs.

LTMR-pSNL. Despite their physiological identity (unclassified RA units: 19/23; Hair: 2/23; and SA units: 2/23), all were considered LTMRs. Compared with sham, these neurons 1 wk after pSNL demonstrated a reduced ability to follow 256-Hz vibratory stimulation (cells fail to achieve a steady 1:1 response, yet still effectively responded to the vibration stimulus; 13/23), a significantly reduced mechanical sensitivity (median: 3.9 mN; range: 0.4–39 mN) (P < 0.001) (Fig. 4A), a significantly decreased RF area (median: 5.1 mm²; range: 2–15 mm²) (P < 0.05) (Fig. 4B) and a significant decrease in their IF max (median: 150 Hz; range: 30–505) (P < 0.05) (Fig. 4C). No significant change in CV of this afferents was observed (mean: 17.6 ± 1.3 m/s).

Nociceptive AHTMR-sham. These neurons had fibers conducting in the A range (mean: 9.7 ± 0.9 m/s), with high MT (median: 99 mN; range 9.8–147 mN), were incapable of following vibratory stimulation (256 Hz) with fidelity, and exhibited a small spot-like RF (≤1 mm²) and relatively low IF max (median: 77.5 Hz; range: 22–175 Hz).

Fig. 3. A: examples of typical APs from different types of functionally identified L4 dorsal root ganglion sensory neurons in both sham (black trace) and pSNL (gray trace) animals. B: impulse responses to identical suprathreshold mechanical stimulation (gray above trace) in sham (I) and pSNL (II) are shown. Note the poststimuli discharge in the AHTMR-pSNL trace (arrow). C: impulse response to vibration (512 and 256 Hz) applied to the cellular RF (black above trace) presented with it maximal instantaneous frequency response (IF max; dotted upper trace). Calibration bars: 4 ms (A), 0.2 s (B), 0.1 s (C).
Compared with the sham group, these neurons 1 wk after pSNL exhibited no change in CV (mean: 9.7 ± 1.3 m/s). However, they showed a significant increase in sensitivity represented by a reduction in their MT (median: 13.7 mN; range: 0.6–58 mN) (P < 0.001) (Fig. 4A), a significantly increased RF area (median: 6.8 mm²; range: 1–20.3 mm²) (P < 0.01) (Fig. 4B), frequently with (9/17) multiple spots (≥2), poststimulus discharges (14/17) and a significantly increased IF max (median: 133 Hz; range: 47–303 Hz) (P < 0.01) (Fig. 4C). These cells remained unresponsive to vibratory stimulation (256 Hz), except for a few asynchronous APs (out of phase with the stimulus frequency) related temporally only to the initial contact of the tuning fork with the skin.

CHTMR-sham. These neurons had fibers conducting in the C range (median: 0.8 ± 0.1 m/s), with high MT (median: 99 mN; range: 3.9–147 mN), were incapable of following vibratory stimulation (256 Hz), and exhibited a small spot-like RF (≤1 mm²), and relatively low IF max (median: 47 Hz; range: 2–131 Hz).

CHTMR-pSNL. Compared with the sham group, these neurons 1 wk after pSNL showed a significantly increased mechanical sensitivity (median: 14 mN; range: 1.5–99 mN) (P < 0.05) (Fig. 4A). However, neither RF size (≤1 mm²) nor IF max (median: 32 Hz; range: 10–71 Hz) was significantly affected after injury (Fig. 4, B and C, respectively). No significant change in CV of these afferents was observed (mean: 0.4 ± 0.06 m/s).

**Effect on the Cellular Membrane Electrical Properties**

Passive electrical properties of LTMRs, AHTMRs, and CHTMRs in sham-treated animals were within ranges previously described by us and others (Boada 2013; Boada et al. 2010, 2011; Boada and Woodbury 2007, 2008; Ma et al. 2003) and were not affected by pSNL (Table 1). In contrast, there were several changes in active electrical properties between the groups, some according to class. Note that we applied a Holm’s step-down correction for multiple comparisons, a more conservative statistical approach than is often applied to electrophysiological studies and which reduces error inflation and the risk of type 1 error. Using this approach, there was an effect on LTMR AP slope, with maximum and minimum values being reduced in afferents recorded from pSNL animals (Table 1). In addition, there was an effect on AHTMR AHP50, which was decreased in afferents from pSNL animals (Table 1).

As previously described (Boada 2013; Boada et al. 2010, 2011; Boada and Woodbury 2007, 2008), all LTMR neurons exhibited an F-type AP profile, and all CHTMR neurons exhibited an S-type AP profile. This was true in both the sham and pSNL conditions. Most AHTMR neurons exhibited an S-type AP profile, and the proportion of S-type to F-type AP profiles of AHTMR neurons did not differ between sham (15 S-type, 6 F-type) and pSNL (11 S-type, 3 F-type).

**AP Time Course and Duration**

In sham animals, 100% (42/42) of the LTMRs and 29% (6/21) of the AHTMRs had an F-type neuron profile, whereas 71% (15/21) of the AHTMRs and 100% (22/22) of the CHTMRs exhibited an S-type profile based on the presence of an inflection in their repolarizing phase. These percentages were unchanged after injury [F-type neurons: LTMR: 100% (23/23);
AHTMR: 18% (3/17); CHTMR: 0% (0/11); S-type neurons: LTMR: 0% (0/23); AHTMR: 72% (14/17); CHTMR: 100% (11/11), despite a general slowing in the AP time course of all cells (Table 1).

**AHP Duration**

Only the fast nociceptive subtype (AHTMR) displayed a significant decrease in both magnitude and duration of its AHP (\(P < 0.05\)) (Table 1) (Fig. 4D).

**Characteristics of N-RF Cells**

All 15 of the neurons in sham animals without mechanical RF exhibited an S-type AP profile. Their electrical signature was most similar to the CHTMR population (Tables 1 and 2).

In contrast, there was a nearly even mix of AP profiles in neurons without RFs in pSNL animals, with 30 S-type and 51 F-type. As with sham, the S-type neurons without RF most closely resembled CHTMRs, although they had a broader, more slurred AP than sham (Tables 1 and 2). Those with an F-type AP did not display an inflection in their repolarization phase, and their electrical signature was intermediate between LTMR and AHTMR (Tables 1 and 2).

**DISCUSSION**

In this report, we describe the effects of L5 pSNL on both the RF characteristics and electrical properties of primary sensory neurons in the adjacent L4 ganglion. The behavioral study shows that pSNL produces significant mechanical sensitization of the ipsilateral paw, which is consistent with previous studies using this model (Guan et al. 2010). The principal observations and conclusions are as follows: 1) the injury of L5 (pSNL) afferents deeply compromises the normal physiological response of primary sensory afferents in the neighboring ganglion (L4); 2) this effect differs by afferent class, with hypersensitivity in the nociceptive population and hyposensitivity in the tactile population; 3) both tactile and nociceptive afferents show significant changes in their normal electrical signature (electrical properties) and in their skin RFs; and 4) there is an apparent increase in the number of cells without mechanical sensitivity (F-type) that may represent changes to the electrical properties of one of the population subtypes from injury.

**Technical Considerations**

The present study was performed in young adolescent female rats and includes L4 afferents innervating both glabrous and hairy skin. Although there is enough evidence to suggest that primary sensory neurons in these rats are similar to adult animals (Boada et al. 2010, 2011, 2012), whether these observations would parallel those in older or aging animals is uncertain. As regards sex, there is no available information or evidence of any fundamental difference in the response or electrical properties of primary sensory neurons between sexes in rodents. Therefore, while possible, it is highly improbable that the estrous cycle plays a role in the sensory effects
described in this study after injury. Further studies are required to clarify this matter. Finally, we combined L₄ afferents innervating both types of skin (glabrous and hairy). This is perhaps the most important limitation of our study (Boada et al. 2010), since the density, composition, and properties of these afferents can vary widely, depending upon their target. We classified these afferents into three groups (LTMR, AHTMR, CHTMR) based on their most fundamental properties. In our opinion, the classification methodology is useful and helps to clearly identify cellular subtypes based on multiple properties and characteristics (Boada et al. 2011) and allows us to compare populations from two conditions (sham and pSNL) with some confidence of correct classification. In particular, the preservation of response characteristics to vibration remains useful to further classify cells. Since injury resulted in a narrowing, if the differences in cellular responses to mechanical activation (increase in threshold in the LTMR and the decrease in threshold in the AHTMR), the unique ability of LTMRs to respond to vibration remained, adding confidence to correct classification. Without this test, it is likely that misclassification of AHTMR as LTMR neurons after injury could easily occur. Furthermore, the lack of RF in the mechanically insensitive subset of cells makes definitive classification of cells unreliable, thus the reduced ability to further classify mechanically insensitive cells further from the F- and S-type based on repolarization properties. This simplification of classification into three does, however, neglect subclasses of afferents within these groups. Therefore, a more detailed study focused on the effect of nerve injury on individual afferent subtypes innervating particular types of skin would further our understanding of nerve injury-induced changes in peripheral sensory activation. This would be particularly valuable if a method of staining specific skin terminals becomes available.

L₃ pSNL Induces Major Changes in the Excitability of L₄ Afferents

These data show that both nociceptive and nonnociceptive afferents are affected by injury to adjacent afferents. Unexpectedly, the most profound effects appear to be confined to myelinated fibers rather than unmyelinated fibers.

L₄ RF Changes: Nociceptors

Our study shows that after L₅ pSNL, both fast- and slow-conducting nociceptors (AHTMR and CHTMR) are sensitized in the neighboring uninjured DRG (L₄). The reduction in MTs of both neuronal subsets and the increased size of the RF of the AHTMR neurons is consistent with the normal sensitization process after skin injury (Boada et al. 2012) and the sensitization process previously described for both of these mechanonociceptors (Djouhri et al. 2012; Ma et al. 2003). Although our data largely support the observations by Djouhri et al. (2012), our findings of a largely increased AHTMR RF size after injury suggest that these neurons may be important in both alldynia and hyperalgesia resulting from nerve injury, a finding that is surprising in putative undamaged cells (Decosterd et al. 2002; Jenkins and Hunt 1991; Kenney and Kocsis 1997a, 1997b). Additionally, our data show that CHTMR afferents are less affected than AHTMRs with respect to effects of nerve injury on the RF size and on the maximum IF responses. This suggests that the AHTMR have the potential to play a major role in the responses to nerve injury at this early time point after injury.

Tactiles

Previous studies using similar methods to the current one observed sensitization rather than desensitization of LTMRs (Djouhri et al. 2012; Ma et al. 2003), and others have suggested that, after injury, the increased activity in these cells contributes to the development of paresthesias/dysesthesias and even contribute to mechanical allodynia after injury (Baron 2009; Na et al. 1993). In contrast, patients with neuropathic pain from a variety of etiologies, including peripheral nerve injury, typically exhibit a reduction in sensitivity to vibration, leading the authors of that study to conclude that large-fiber neuropathy is present (von Hehn et al. 2012). The desensitization in LTMRs we observed 1 wk after injury is similar to that we previously observed in this class of afferents within minutes after nociceptive stimuli (Boada et al. 2012; Boada and Woodbury 2007). Even though these afferents invade dorsal horn superficial laminae, a role for them in the inhibition of nociceptive transmission has been suggested (Boada and Woodbury 2007, 2008; Lu and Perl 2003; Melzack and Wall 1965; Narikawa et al. 2000). As we know, substantia gelatinosa neurons integrate both light tactile and nociceptive information (Bennett et al. 1980; Light et al. 1993) (for review, see Light 1992); therefore, we speculate that, after injury, reduced or silenced tactile inputs should provide uninhibited access of greatly sensitized nociceptors to substantia gelatinosa circuits, and this may contribute to the development of a chronic pain state. It is also tempting to speculate that the large population of silent afferents with an F-type signature, typical of LTMRs found after injury, may reflect a terminal desensitization state of some cells of this class, although further studies (anatomical and immunohistochemical) are required to definitively elucidate their identity and contribution, if any, to pain and hypersensitivity after injury.

L₅ pSNL-induced Changes in the Electrical Properties of L₄ Afferents

Others have previously shown that L₅ axotomized neurons exhibit several electrical and functional changes after injury (Abdulla and Smith 2001; Stebbing et al. 1999). Furthermore, the elegant in vivo work of Ma and colleagues (2003) demonstrated that L₅ ganglia cells could also be affected by injury at L₅. However, very little is known about this process. For instance, we know that undamaged L₅ afferents overlap L₅ disrupted innervations to the same dermatome (Wu et al. 2012). This makes it conceivable that different inflammatory mediators or neuroactive agents could be released by the damaged afferents (e.g., TNF-α, IL-1, ATP, bradykinin, epinephrine, and protons) at the point of injury and directly into the innervated skin, modifying both the excitability of undamaged afferents in the affected ganglia (Liang et al. 2010; Ma et al. 2003; Pogatzki et al. 2002; Stoll and Muller 1999), as well neighboring L₅ afferents innervating the same area in the skin. This concept is consistent with the development of primary hyperalgesia by direct effect on the nociceptive terminals themselves, but it does not explain the desensitizing process observed in the undamaged L₅ LTMR afferents.

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To some extent, our findings are consistent with previous descriptions of the effect of L5 injury in the membrane electrical properties of the L4 intact afferents (Djouhri et al. 2012; Ma et al. 2003). In general, these studies show greater effects on fast- than slow-conducting intact afferents after injury. Interestingly, our results show that this process appears to affect tactile and nociceptive afferents in an opposite manner (tactile desensitization, nociceptive sensitization), along all CV ranges (A and C). We have considered the possibility that the cause of these changes is as simple as the development of an inflammatory process that increases the membrane cellular surface area reducing its Ri, therefore affecting the time course of the AP generation. However, we found no evidence of such Ri change suggesting the potential variable regulation of voltage-gated channels (Na\(^+\) and K\(^+\)) as the foremost explanation for the observed changes. For example, after injury axotomized somata exhibit an upregulation of tetrodotoxin-sensitive Na\(^+\) current and downregulation of tetrodotoxin-resistant Na\(^+\) currents (Black et al. 1999; Rizzo et al. 1995; Sleeper et al. 2000; Waxman et al. 1999). Alternatively, or perhaps even concomitantly, voltage-gated K\(^+\) channels can also be disrupted after injury (see below). In this particular cellular subtype (large-diameter cells), the prominence of I\(_K\) currents has been a focus of intensive but so far inconclusive studies (Biel et al. 2009; Gao et al. 2012; Hogan and Poroli 2008; Scroggs et al. 1994) about their role in cellular electrical changes detected in axotomized myelinated afferents (ABDδ).

On the other hand, in our study, L4 AHTMR show two marked signs of enhanced somatic electrical excitability after L5 injury: 1) a greatly diminished AHP (AHP50), which is likely to increase firing frequency of these cells; and 2) an extended AP duration (D50). It has been suggested that cutaneous afferent DRG neurons can express different combinations of three K\(^+\) currents (I\(_A\), I\(_D\), I\(_K\)) (Everill et al. 1998). Also, after injury, the maximal K\(^+\) current is reduced by 52% by a significant decrease in 2/3 types of their K\(^+\) currents (Everill et al. 1998, cited by Ishikawa et al. 1999). This has been partially corroborated by more recent work showing that, after injury (transsection of the sciatic nerve), voltage-gated K\(^+\) current is downregulated (Abdulla and Smith 2001; Takeda et al. 2006). Unfortunately, all of these studies have focused on injured afferents and few on intact afferents. Despite the logic of these electrical changes in the membrane of hyperexcitable nociceptors, the limited evidence in the literature and the differences in the type of injury and procedures may explain our discrepancies with similar studies (Djouhri et al. 2012; Ma et al. 2003).

Conclusions

In summary, peripheral nerve injury results in sensitization of high-threshold mechano-sensitive nociceptors, but desensitization of low-threshold units in adjacent intact afferents innervating the area of hypersensitivity observed in the behaving animal. This is accompanied by expansion and contraction of RF areas in high- and low-threshold units, respectively. Injury also produces the appearance of a large proportion of intact afferents which cannot be excited by mechanical stimulation, yet with somatic electrical profiles resembling LTMRs. These data suggest that peripheral input 1 wk after neuropathic injury is grossly abnormal and not merely due to sensitization of afferents across all fiber types. Rather, it suggests that the LTMR desensitization may play a role in peripheral pain type sensations after nerve injury. Furthermore, it may be possible that a strategy to target the desensitization processes of LTMRs could be effective in altering the hypersensitivity or dysesthesias associated with neuropathic pain.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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