Hyperexcitability of Axotomized and Neighboring Unaxotomized Sensory Neurons Is Reduced Days After Perineural Clonidine at the Site of Injury

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Liu, Baogang and James C. Eisenach. Hyperexcitability of axotomized and neighboring unaxotomized sensory neurons is reduced days after perineural clonidine at the site of injury. J Neurophysiol 94: 3159–3167, 2005; doi:10.1152/jn.00623.2005. Hyperexcitability after peripheral nerve injury occurs in axotomized and neighboring unaxotomized dorsal root ganglion (DRG) neurons and contributes to hypersensitivity. Previous studies have focused on proximal nerve injury and have not examined unaxotomized neurons innervating the site of sensory testing. The current study used a distal nerve injury (partial sciatic nerve ligation [PSNL]), and identified, using fluorescent tracers, axotomized and unaxotomized neurons innervating the site of hypersensitivity. We hypothesized that reduced hypersensitivity after perineural clonidine was associated with reduced hyperexcitability of DRG neurons. Rats underwent sham or PSNL surgery, followed 2 wk later by a single injection at the injury site of clonidine or saline. PSNL, but not sham surgery, reduced hindpaw mechanical withdrawal threshold, and clonidine, but not saline, partially reversed this effect 3 days after injection. Intracellular recording of neurons in whole DRG demonstrated similar changes in membrane properties and excitability in unaxotomized and axotomized neurons after PSNL compared with sham surgery, primarily depolarized resting membrane potential, reduced rheobase, presence of oscillations, and capability to fire repetitively. Most of these changes were present in small-, medium-, and large-diameter neurons. Perineural clonidine 3 days later significantly reversed many of these effects, whereas saline was without effect. We speculate that perineural clonidine reduces signals, likely proinflammatory cytokines and prostaglandins produced during Wallerian degeneration in the nerve result in excitability in their uninjured neighbors (Ma et al. 2003). That study, however, did not determine whether the unaxotomized neurons projected to the site of sensory testing. Additionally, they injured the nerve at a very proximal site (spinal nerve ligation). The presence and extent of many of changes in afferent excitability depend heavily on the site of nerve injury relative to the cell body (Liu et al. 2000; Ma et al. 2003). For example, distal nerve axotomy results in a larger proportion of A fibers with capability to repetitively fire, but a smaller proportion with spontaneous activity than proximal axotomy (Liu et al. 2000). The effect of distal nerve injury on unaxotomized neighboring fibers has not previously been examined. One purpose of the current study was to test whether similar changes occur in axotomized neurons and unaxotomized neurons projecting to the hindpaw (the site of behavioral testing) after nerve injury in a more peripheral location (Seltzer et al. 1990).

The α2-adrenoceptor agonist clonidine has been approved for epidural administration in the treatment of neuropathic pain for many years, and the assumed site of action of this drug by this route is on α2-adrenoceptors in the spinal cord (Eisenach et al. 1996). Other studies have suggested a peripheral site of action. For example, transdermal clonidine reduces pain in patients with diabetic neuropathy (Zeigler et al. 1992) and postherpetic neuralgia (Kirkpatrick et al. 1992). Additionally, peripheral nerve injury results in a buildup of α2-adrenoceptors in the nerve just proximal to the site of lesion (Birder and Perl 1999; Gold et al. 1997) as well as in immune cells, primarily macrophages and T lymphocytes, surrounding the injury site and within the nerve (Lavand’homme et al. 2002). Perineural injection of clonidine at the injury site reduces hypersensitivity in the paw after partial sciatic nerve ligation (PSNL) with a delayed onset of days and a prolonged duration of weeks, and

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this inhibition is prevented when clonidine is coadministered with an α2-adrenoceptor antagonist (Lavand’homme et al. 2002). Clonidine-treated animals also demonstrate reduced concentrations of proinflammatory cytokines in the injured nerve and DRG at the time of clonidine’s antihypersensitivity effect (Lavand’homme and Eisenach 2003). A key proinflammatory cytokine, tumor necrosis factor (TNF)α, sensitizes and induces spontaneous activity in primary afferents (Sorkin et al. 1997) and in DRG somata (Liu et al. 2002a), and it is conceivable that the association between reduced cytokine expression and reduced hypersensitivity after clonidine administration is linked by reduced afferent excitability. The second purpose of the current study was therefore to determine whether perineural clonidine diminished excitability after PSNL, and whether it did so similarly in unaxotomized or injured neurons.

METHODS

Animals

All experiments were approved by the Animal Care and Use Committee of Wake Forest University School of Medicine. Forty-three adult male Wistar rats (Harlan, Indianapolis, IN) were used, weighing 200–250 g on the day of the experiments. Animals were housed at 22°C and under a 12 h:12 h light:dark cycle, with free access to food and water.

Surgical preparation

PSNL was performed as previously described (Seltzer et al. 1990). Briefly, the left sciatic nerve was carefully exposed at mid thigh level. The dorsal half of the sciatic nerve was carefully separated and sectioned, and the distal part of the sectioned nerve tightly ligated using a 6/0 silk suture. The proximal part of sectioned nerve was carefully separated from the unaxotomized nerve along a 4- to 6-mm distance and the cut end was inserted into a small glass capsule filled with 10% fluororuby (Molecular Probes, Paisley, UK), dissolved in 0.9% sterile saline, wt/vol, for 10 min. The proximal end of the sectioned nerve was then tightly ligated using a 6/0 silk suture. The incision was closed in layers.

A different retrograde tracer was used to identify unaxotomized neurons in the DRG innervating the site of sensory testing. As previously described (Hudson et al. 2001), after closure of the PSNL incision, 10 μl 1% of fast blue (FB, Sigma-Aldrich, Manchester, UK), wt/vol dissolved in sterile water, were injected intradermally into the sterilized glabrous hairy border and plantar surface of the lateral aspect of the left hind paw, using a 32-g needle. Anesthesia was then discontinued.

Behavioral testing

Withdrawal threshold to punctate mechanical stimuli was determined before and on days 1, 3, 5, 7, 9, 11, 13, and 14 after peripheral nerve injury by the application of calibrated von Frey filaments (Stoelting, Wood Dale, IL). Animals were placed on a plastic mesh floor in individual clear plastic boxes and allowed to accommodate to their environment for ≥30 min. The von Frey filaments were applied vertically to the plantar surface of the hindpaw ipsilateral to the partial nerve ligation, and gently pushed to the bending point. Filaments were applied three to four times. If no response was elicited, a larger-diameter filament was applied in the same manner. The filaments were applied in increasing order (0.53, 0.93, 1.14, 1.58, 2.81, 4.25, 6.58, 9.08, 11.69, 22.28, and 58.6 g) until a brisk withdrawal or paw flinching was elicited, which was considered as a positive response. The withdrawal threshold was determined using an up–down statistical method (Chaplan et al. 1994) twice, with testing separated by 10 min, and the mean withdrawal threshold was used for data analysis.

Drug administration

To determine the influence of perineural clonidine on behavior and neuronal excitability, rats with reduced withdrawal thresholds 2 wk after PSNL were brieﬂy anesthetized and clonidine (0.3 ml, 30 μg) or saline (0.3 ml) was injected perineurally by percutaneous injection, using a fanning motion, as previously described (Thalhammer et al. 1995). In control experiments, the same dose of clonidine was injected intramuscularly to determine whether clonidine acted in a systemic manner. All injections were performed by an investigator who did not perform behavioral or electrophysiological studies, guaranteeing that these observations were obtained in a blinded manner. Withdrawal threshold was then determined 1, 2, and 3 days after perineural injection.

Microelectrode intracellular recording

Intracellular recording of DRG neurons in an isolated, whole preparation was performed as previously described (Liu et al. 2002a). Briefly, after surgical dissection, the left L4 or L5 DRGs were placed in the recording chamber and mounted on the stage of an upright microscope (BX51-WI, Olympus, Tokyo, Japan). A U-shaped stainless steel wire on which three to four ﬁne nylon ﬁbers spanned the two sides was used to gently hold the ganglion immersed at the bottom of the chamber. The DRG was continuously perfused with oxygenated artiﬁcial cerebrospinal ﬂuid containing (in mM): 130 NaCl, 3.5 KCl, 1.25 NaH₂PO₄, 2.4 NaHCO₃, 10 dextrose, 1.2 MgCl₂, and 1.2 CaCl₂ (pH = 7.3) at a rate of 3– 4 ml/min, and the temperature was maintained at 37 ± 1°C.

Intracellular electrophysiological recordings were made with microelectrodes ﬁlled with 2.5 M potassium acetate (pH = 7.2). Satisfactory recordings were obtained with electrodes of 50–80 MΩ. Before electrode penetration, the DRG soma was viewed with a CCD camera combined with infrared DIC or ﬂuorescence microscopy. Only neurons identiﬁed as axotomized, which contained ﬂuororuby, or unaxotomized and innervating the hindpaw, which contained FB, were studied. Soma of DRG were visually classiﬁed according to diameter as small (≤30 μm), medium (30–49 μm), or large (≥50 μm) as previously described in this preparation (Liu et al. 2002a). Electrophysiological data were collected with the use of single-electrode continuous current clamp (AxoClamp-2B, Axon Instruments, Foster City, CA) and analyzed with Clampex 8 software (Axon Instruments).

Membrane properties and AP parameters were measured as previously reported (Czeh et al. 1977; Liu et al. 2002a; Zhang et al. 1999), including Vₑ, voltage and current thresholds, whole cell input resistance (Rₑ), and afterhyperpolarization (AHP). Depolarizing currents of 0.05–4.0 nA (100-ms pulse duration) were delivered in increments of 0.05 nA until an AP was evoked. The threshold current (rheobase) was deﬁned as the minimum current required to evoke an AP. The AP voltage threshold was deﬁned as the ﬁrst point on the rising phase of the spike at which the change in voltage exceeded 50 mV/ms. The duration of the AP (APD) was measured at the AP threshold level. The AP amplitude was measured between the peak and the AP threshold. The Rₑ for each cell was obtained from the slope of a steady-state I–V plot in response to a series of hyperpolarizing currents of 100–ms duration, delivered in decreasing steps of 0.05 nA from 0.2 to –2 nA. The AHP amplitude was measured from the valley peak to the baseline and the AHP duration was measured at amplitude halfway between.

For each neuron, a continuous recording was obtained for ≥3 min after electrode penetration. Only neurons with stable resting membrane potential were studied. Cells were considered to show oscillations if, before action potential recording, they displayed increasing
and decreasing potential >1.5 times resting threshold, and were 
considered to show spontaneous activity if they exhibited any action 
potentials in 3 min of continuous recording in the absence of a 
stimulus. Capability for repetitive firing and the number of volleys 
were determined by injection of a depolarizing current with amplitude 
1.5 times the rheobase and of 100-ms duration.

Statistical analyses

Data are presented as means ± SE. Student’s t-test or two-way 
ANOVA were used to compare groups or times with withdrawal 
threshold and membrane and AP properties. χ² or Fisher’s exact 
test were used to compare groups for the incidence of cells expressing 
membrane oscillation, accommodation, and spontaneous activity. P < 
0.05 was considered significant.

RESULTS

Behavioral effects of surgery and perineural injections

The mean paw withdrawal threshold to von Frey filament 
testing did not differ between sham and PSNL groups before 
surgery. The overall mean paw withdrawal threshold at this 
time was 22 ± 1.7 g. After surgery, the withdrawal threshold 
did not change in the sham group. In contrast, paw withdrawal 
threshold 14 days after PSNL was dramatically reduced to 
1.8 ± 0.2 g. A single perineural injection of saline 0.3 ml did 
not change the withdrawal threshold 3 days after injection (n = 
12). In contrast, a single perineural injection of clonidine, 30 
µg, significantly increased withdrawal threshold 3 days later to 
8.5 ± 0.4 g, although this value was still significantly lower 
than the pre-PSNL baseline (n = 11, Fig. 1). A single intra-
muscular injection of the same dose of clonidine failed to alter 
withdrawal threshold, which was 1.0 ± 0.05 before and 1.4 ± 
0.1 g 3 days after clonidine injection.

Electrophysiologic properties of neurons and their response 
to clonidine

Intracellular recordings were obtained from a total of 387 
neurons. Of these, 89 were from the sham group, 134 were 
from the PSNL plus perineural saline group, 129 were from the 
PSNL plus perineural clonidine group, and 35 were from the 
PSNL plus systemic clonidine group.

Perineural injections

LARGE-DIAMETER DRG NEURONS. Axotomized and unaxoto-
mized large-diameter neurons in the PSNL plus perineural 
saline group differed in membrane properties compared with 
the sham group in a largely similar, but not identical manner. 
As such, both axotomized and unaxotomized large-diameter 
neurons after PSNL had more depolarized Vm, lower voltage 
and current thresholds, larger APD, increased Rin, and slower 
rising and falling phases of the AP than those of large-diameter 
neurons in the sham group. There were, however, some sig-
ificant differences after PSNL in large-diameter unaxotomized 
versus injured neurons. Thus unaxotomized large-diameter 
neurons demonstrated a shorter APD (1.5 ± 0.09 ms) and 
slower falling phase of AP (−101 ± 8.6 mV/ms) than axoto-
mized large-diameter neurons (2.2 ± 0.1 ms and −69.6 ± 4.5 
mV/ms, respectively).

Perineural clonidine reversed or partially reversed many of 
the membrane and AP properties in large-diameter DRG neu-
rons. Thus clonidine reversed the effect of PSNL on Vm in both 
unaxotomized and axotomized neurons, reversed the effect on 
under threshold voltage, and partially reversed the effect on rheobase 
(Table 1). In contrast, neurons from clonidine-treated animals 
did not differ from perineural saline control neurons in APD, 
Rin, and the rising and falling rates of the AP in large-diameter 
nurons (Table 1).

MEDIUM-DIAMETER DRG NEURONS. The effects of PSNL and 
perineural clonidine on unaxotomized and axotomized medi-
um-diameter neurons were similar to those of large-diameter 
nurons, with the exception of Vm, which was not altered in 
unaxotomized neurons after PSNL (Table 2). Thus medium-
diameter neurons after PSNL had more depolarized Vm (axo-
tomized only), lower voltage and current thresholds, larger 
APD, increased Rin, and slower rising and falling phases of the 
AP than those of medium-diameter neurons in the sham group 
(Table 2). As with large-diameter neurons, there were some 
significant differences between medium-diameter unaxoto-
mized versus injured neurons after PSNL. Unaxotomized me-
dium-diameter neurons demonstrated a shorter APD (2.0 ± 
0.25 ms) and slower falling phase of AP (−86.2 ± 8.13 
mV/ms) than that of axotomized medium-diameter neurons 
(2.8 ± 0.01 ms, −51.8 ± 3.25 mV/ms, respectively).

Perineural clonidine also reversed or partially reversed many 
of the membrane and AP properties in medium-diameter DRG 
nurons. Thus clonidine reversed the effect of PSNL on Vm in 
axotomized neurons, and reversed the effect on voltage thresh-
old, and partially reversed the effect on rheobase in both axoto-
mized and unaxotomized medium-diameter neurons (Table 2).

SMALL-DIAMETER DRG NEURONS. The effects of PSNL on un-
axotomized and axotomized small-diameter neurons were less 
pronounced than those of large-diameter neurons, but clear 
effects were observed. Thus although small-diameter neurons 
from PSNL plus perineural saline did not differ from sham in 
Vm, both unaxotomized and axotomized small-diameter neu-
nons had significantly decreased voltage and current thresholds 
(Table 3). There were some significant differences after PSNL 
in small-diameter unaxotomized versus injured neurons. Un-

![Fig. 1](http://jn.physiology.org/)

**Fig. 1.** Withdrawal threshold to von Frey filament testing of the hindpaw before (Pre) and 14 days after (Post) partial sciatic nerve ligation (PSNL), then for 3 days after injection of saline (filled bars) or clonidine (open bars) perineurally at the injury site. *P < 0.05 compared with pre-PSNL. #P < 0.05 compared with PSNL saline group value. §P < 0.05 compared with post-
PSNL clonidine group value.
neurons (Table 3).

Thus clonidine reversed the effect of PSNL on voltage thresh-

TABLE 2. Electrophysiological properties of medium-diameter DRG neurons

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sham</th>
<th>Saline Axot</th>
<th>Saline Unaxot</th>
<th>Clonidine Axot</th>
<th>Clonidine Unaxot</th>
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<tr>
<td>$V_m$, mV</td>
<td>$-64.2$</td>
<td>$-60.7$</td>
<td>$-61.8$</td>
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<td>36</td>
<td>27</td>
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<tr>
<td>Voltage threshold, mV</td>
<td>$-34.7$</td>
<td>$-40.9$</td>
<td>$-40.9$</td>
<td>$-36.7$</td>
<td>$-37.9$</td>
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<td>$n$</td>
<td>36</td>
<td>27</td>
<td>18</td>
<td>27</td>
<td>22</td>
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<tr>
<td>AP height, mV</td>
<td>63.3</td>
<td>65.2</td>
<td>66.1</td>
<td>66.5</td>
<td>62.7</td>
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<tr>
<td>$n$</td>
<td>36</td>
<td>27</td>
<td>18</td>
<td>27</td>
<td>22</td>
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<tr>
<td>Rheobase, nA</td>
<td>1.6</td>
<td>0.8</td>
<td>0.9</td>
<td>1.2</td>
<td>1.2</td>
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<tr>
<td>$n$</td>
<td>36</td>
<td>27</td>
<td>18</td>
<td>27</td>
<td>22</td>
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<tr>
<td>AP-D, ms</td>
<td>0.9</td>
<td>2.2</td>
<td>1.5</td>
<td>2.0</td>
<td>1.8</td>
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<td>36</td>
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<td>18</td>
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<td>22</td>
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<tr>
<td>AHP, mV</td>
<td>11.6</td>
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<td>9.3</td>
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<tr>
<td>AHP-D, ms</td>
<td>2.8</td>
<td>3.5</td>
<td>3.6</td>
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<tr>
<td>$R_{m}$, MΩ</td>
<td>15.2</td>
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<td>$\alpha V/\alpha T$, mV/ms</td>
<td>326.7</td>
<td>205.4</td>
<td>220.8</td>
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<td>$\alpha V/\alpha T_{f}$, mV/ms</td>
<td>154.2</td>
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</table>

Values, expressed as means ± SE, were obtained from both L4 and L5 dorsal root ganglion large neurons; $n =$ recorded number of neurons. Cell size is the diameter. *$P < 0.05$, PSNL group versus Sham group; §$P < 0.05$, unaxotomized (unaxot) group versus corresponding axotomized (axot) group; # $P < 0.05$, clonidine-treated group versus corresponding saline control.

Three unaxotomized small-diameter neurons demonstrated a slower falling phase of AP ($-45.2 ± 3.05$ mV/ms) than that of axotomized small-diameter neurons ($-35.2 ± 1.58$ mV/ms).

Perineural clonidine also reversed the effects of PSNL on the membrane and AP properties in small-diameter DRG neurons. Thus clonidine reversed the effect of PSNL on voltage threshold and rheobase in both unaxotomized and axotomized DRG neurons (Table 3).

MEMBRANE POTENTIAL OSCILLATIONS. Membrane potential oscillations were assessed in a total of 335 neurons. Of the 56 neurons from sham group, only two (3%) exhibited oscillations. In contrast, 28% of axotomized and 18% of unaxotomized neurons after PSNL plus perineural saline demonstrated oscillations ($P < 0.05$ compared with sham; Fig. 2). The proportion of unaxotomized and axotomized neurons with oscillations after perineural clonidine treatment remained greater than that of sham, but was reduced in axotomized neurons compared with PSNL plus saline control (Fig. 2). Thus clonidine treatment resulted in a partial reduction in the proportion of neurons with membrane oscillations after PSNL.
SPONTANEOUS ACTIVITY. Spontaneous activity was determined by injecting a current of magnitude 1.5 times the rhobase, and this was performed in a total of 247 neurons. An example of repetitive firing induced by this protocol is shown in Fig. 3A, top. The proportion of neurons with repetitive firing and the number of spikes generated by a single current injection were increased in axotomized neurons after PSNL compared with that of sham (Fig. 3B). This effect was completely reversed by clonidine, and neurons from animals treated with perineural clonidine did not differ from sham in either the proportion of axotomized neurons with repetitive firing or the number of spikes induced by current injection (Fig. 3B). PSNL did not alter repetitive firing in unaxotomized neurons compared with sham control.

SPONTANEOUS ACTIVITY. Spontaneous activity was determined in all neurons. No neuron in the sham group exhibited spontaneous activity. In contrast spontaneous activity was observed in a small proportion of neurons after PSNL, always in neurons with membrane oscillations and typically resulting in an irregular firing pattern (Fig. 4, top). The proportion of neurons with spontaneous activity after PSNL and perineural saline differed from sham for axotomized (three small, one medium, and one large) neurons (Fig. 4, bottom). In contrast to clonidine’s significant effect to reduce the proportion of axotomized neurons with membrane oscillations, neurons from clonidine-treated animals did not differ from saline-treated animals after PSNL in the proportion with spontaneous activity (two small axotomized neurons and one medium unaxotomized neuron). Given the low proportion of neurons with spontaneous activity in the saline-treated group, however, this study was not powered to observe even a 70% reduction in this proportion by clonidine, should it have existed.

**Systemic clonidine injections**

To determine whether the reduced excitability by clonidine arose from a local or systemic action, some PSNL animals received a systemic injection of clonidine and DRGs were studied 3 days later. In contrast to perineural administration, discussed in the preceding paragraphs, intramuscular clonidine failed to alter membrane properties of axotomized large-, medium-, or small-diameter neurons after PSNL (Table 4).

**Discussion**

There are two key findings in the current study. First, these results significantly add to extend our understanding that axotomized and unaxotomized sensory neurons increase their excitability after proximal nerve injury in a similar manner (Ma et al. 2003), by demonstrating extensive changes in small-diameter neurons and by specifically examining unaxotomized neurons that innervate the area of hypersensitivity.
Second, these results confirm previous observations of a curiously delayed onset of reversal of hypersensitivity after PSNL by perineural but not systemic clonidine injection (Lavand’homme et al. 2002) and extend them by demonstrating an associated reduction in excitability of sensory neurons. This reduced excitability after clonidine treatment was interestingly similar in all cell sizes and in unaxotomized neurons innervating the testing site as well as axotomized neurons. A discussion of the essential limitations of these observations is introduced next, followed by interpretation of these two novel findings.

Several factors limit interpretation of the current study. The dose of perineural clonidine and timing of DRG removal were based on our previous observations of dose and time responses of perineural clonidine to coincide with clonidine’s maximal dose and peak effect (Lavand’homme et al. 2002). We recognize that examining a range of doses and times would further strengthen the confidence in the association between clonidine’s effect on behavior and neurophysiologic properties. We also recognize that the percutaneous method of perineural injection does not guarantee administration of the drug close to the nerve. This method, however, results in flaccid paralysis of the hindlimb in >95% of injections of local anesthetic (Lavand’homme et al. 2002), suggesting it is reasonably reliable. Removal of the entire DRG is not as destructive as acute dissociation, in that it maintains the anatomy of cell–cell contacts between neurons and support cells and does not require enzymatic digestion of tissue, but does nonetheless produce an axotomy in all DRG neurons. We believe the results obtained in the current study are likely to reflect cell properties in vivo because DRG neurons were studied within a few hours of this axotomy, before electrophysiologic changes are commonplace (Liu et al. 2000) and because even acutely dissociated sensory neurons exhibit many of the nerve injury–induced changes as those recorded in vivo (Ma et al. 2003). We recognize that we cannot determine the conduction velocity or fiber type of DRG neurons by measuring cell diameter. Nonetheless, the categorization we used in the current study of large-, medium-, and small-diameter neurons correlates remarkably well with Aβ, Aδ, and C neurons, respectively, as determined by measuring conduction velocity in vivo (Ma et al. 2003). Finally, we recognize that membrane depolarization itself can induce oscillations and spontaneous firing in normal sensory neurons (Amir et al. 1999), although oscillations occur at more depolarized potentials than seen in our neurons from PSNL animals, and spontaneous firing is very rare in normal sensory neurons.

**Distal peripheral injury–induced changes in excitability** extend to small-diameter sensory afferents

Nerve injury–induced changes in membrane properties, primarily depolarized *V*<sub>m</sub>, decreased rheobase, and altered AP properties (increased APD and capability for repetitive firing), that were observed in the current study are typical of numerous previous reports after axotomy (Kajander and Bennett 1992; Kim et al. 1993; Liu et al. 2000; Wall and Devor 1983) and in neighboring unaxotomized neurons (Ali et al. 1999; Boucher et al. 2000; Ma et al. 2003; Wu et al. 2001). The current study extends these previous observations by indicating that unaxotomized and axotomized neurons change in a similar manner with respect to membrane properties and excitability after this more distal nerve injury. In contrast to proximal injury (Ma et al. 2003), however, membrane properties were more severely affected in small- and medium-diameter unaxotomized and axotomized neurons after more distal peripheral nerve injury in the current study. As such, AP threshold and rheobase were reduced in unaxotomized medium- and small-diameter sensory neurons in the current study with peripheral injury, but these parameters were unaffected after proximal injury (Ma et al. 2003). Whether these differences in unaxotomized neurons between the two studies reflect proximal versus distal injury or different inclusion criteria (selectively those innervating the...
paw in the current study) cannot be determined from these experiments.

The role of small-diameter DRG neurons, presumably most of which are C fibers, to ongoing pain and hypersensitivity after peripheral nerve injury is controversial, and may vary with the type of nerve injury. For example, chronic constriction injury of the mid sciatic nerve results in a maintenance of C fibers and pronounced thermal hypersensitivity (Laird and 2002) suggest the possibility of a temporary reversion of the altered phenotype of sensory afferents. Nerve injury is known to produce phenotypic shifts in primary afferents neurons including ion channels, neuropeptides and their receptors, and synaptic vesicle proteins (Xiao et al. 2002), which are thought to result in enhanced excitability. Perineural, but not intramuscular clonidine treatment partially or completely reversed each of the membrane properties that differed between PSNL and sham treatment, and this reversal extended across neurons of all sizes and in axotomized as well as unaxotomized neurons. Similar reductions in the proportion of neurons with abnormal membrane oscillation by clonidine after PSNL suggests that spontaneous activity could be reduced by this treatment, although the current study lacked adequate power to directly test this. We recognize that changes in physiologic properties at the cell soma do not necessarily reflect changes that may occur at peripheral nerve terminals (Shim et al. 2005). Because periph-

### Table 4. Membrane properties of axotomized large-, medium-, or small-diameter neurons after PSNL

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Large</th>
<th>Medium</th>
<th>Small</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>Saline</td>
<td>Clonidine</td>
</tr>
<tr>
<td>Cell size, μm</td>
<td>54.6 ± 0.9</td>
<td>53.2 ± 1.1</td>
<td>55.1 ± 1.2</td>
</tr>
<tr>
<td>n</td>
<td>36</td>
<td>27</td>
<td>13</td>
</tr>
<tr>
<td>V&lt;sub&gt;ma&lt;/sub&gt;, mV</td>
<td>-64.2 ± 0.6</td>
<td>-60.7 ± 1.2*</td>
<td>-59.9 ± 1.6*</td>
</tr>
<tr>
<td>mV</td>
<td>36</td>
<td>27</td>
<td>13</td>
</tr>
<tr>
<td>AP height, mV</td>
<td>34.7 ± 1.4</td>
<td>40.9 ± 1.4*</td>
<td>42.0 ± 2.5*</td>
</tr>
<tr>
<td>n</td>
<td>36</td>
<td>27</td>
<td>13</td>
</tr>
<tr>
<td>mV</td>
<td>36</td>
<td>27</td>
<td>13</td>
</tr>
<tr>
<td>AP-D, ms</td>
<td>0.9 ± 0.1</td>
<td>2.2 ± 0.1*</td>
<td>2.0 ± 0.1*</td>
</tr>
<tr>
<td>n</td>
<td>36</td>
<td>27</td>
<td>13</td>
</tr>
<tr>
<td>AHP, mV</td>
<td>11.6 ± 0.6</td>
<td>8.2 ± 0.6*</td>
<td>8.8 ± 1.0*</td>
</tr>
<tr>
<td>n</td>
<td>35</td>
<td>22</td>
<td>13</td>
</tr>
<tr>
<td>R&lt;sub&gt;m&lt;/sub&gt;, Mohm</td>
<td>15.2 ± 1.2</td>
<td>21.5 ± 2.1*</td>
<td>17.0 ± 1.9</td>
</tr>
<tr>
<td>n</td>
<td>35</td>
<td>22</td>
<td>13</td>
</tr>
<tr>
<td>aV/A f&lt;sub&gt;r&lt;/sub&gt;, mv/ms</td>
<td>326.7 ± 14.6</td>
<td>205.4 ± 13.1*</td>
<td>218.0 ± 27.0*</td>
</tr>
<tr>
<td>n</td>
<td>36</td>
<td>27</td>
<td>13</td>
</tr>
<tr>
<td>aV/A f&lt;sub&gt;r&lt;/sub&gt;, mV/m</td>
<td>-154.2 ± 14.1</td>
<td>-69.6 ± 4.5*</td>
<td>-95.9 ± 11.8</td>
</tr>
<tr>
<td>n</td>
<td>36</td>
<td>27</td>
<td>13</td>
</tr>
</tbody>
</table>

Values, expressed as means ± SE, were obtained from both L4 and L5 dorsal root ganglion axotomized neurons; n = recorded number of neurons. *P < 0.05, PSNL group versus Sham group.
eral nerve blockade reduces ongoing pain as well as allodynia in patients with neuropathic pain (Gracely et al. 1992), reduction in spontaneous activity by perineural clonidine would be expected to reduce ongoing pain. This could not be tested in the current study, given the lack of a validated measure for ongoing pain in this rodent nerve injury preparation.

The mechanisms by which clonidine reversed hyperexcitability after PSNL were not tested in the current study. A direct effect of clonidine on neural activity is unlikely because clonidine affects axonal conduction only at much higher concentrations than those of the current study, is not reversed by α2-adrenoceptor antagonists, and lasts for only a few hours (Gaumann et al. 1994; Leem et al. 2000), rather than the slow onset and prolonged duration observed in the current study. We speculate that perineural clonidine reduces primary afferent excitability across neuron size and injury state by reducing a global signal for alterations in primary afferent phenotype leading to increased excitability, and that proinflammatory cytokines represent this signal. Peripheral nerve injury results in acute release of TNFα from injured Schwann cells, followed by recruitment of macrophages that respond with a second wave of TNFα release (Shubayev and Myers 2000). TNFα is known to enhance excitability in primary afferent fibers (Sorkin et al. 1997) and their cell bodies (Liu et al. 2002a) and contributes to the development of pain and hypersensitivity in animal models of local inflammation or peripheral neuropathy (Cunha et al. 1992; Sommer et al. 1998; Woolf et al. 1997). In addition to its acute effects on ion channel properties and excitability (Liu et al. 2002a; Sorkin et al. 1997; Zhang et al. 2002), TNFα also, with a 24-h delay, alters calcium currents in cultured sympathetic (Soliven and Albert 1992) and hippocampal neurons (Furukawa and Mattson 1998). Other proinflammatory products may be important as well. For example, perineural injection of a cyclooxygenase inhibitor also reduces hypersensitivity after PSNL, suggesting a role for prostaglandins (Ma and Eisenach 2002). We therefore speculate that reduced excitability after perineural clonidine after PSNL in the current study likely reflects clonidine’s action to reduce proinflammatory cytokine and prostaglandin production by immune and other resident cells.

In summary, PSNL increases excitability of all size neurons that have been axotomized as well as their unaxotomized neighbors, which innervate the distal testing site of mechanical hypersensitivity. Increased excitability of large-diameter unaxotomized neurons is consistent with behavioral hypersensitivity, and increased spontaneous activity in both axotomized and unaxotomized neurons could play a role in ongoing pain. Perineural clonidine, administered 3 days earlier, reduces both behavioral hypersensitivity and measures of primary afferent excitability globally across neuron size and injury state. We speculate that clonidine’s effect on excitability likely reflects a reduction in a signal, perhaps TNFα or prostaglandins, that drives changes in primary afferent phenotype after peripheral nerve injury. These studies provide a rationale for controlled clinical trials of perineural clonidine injection at sites of nerve injury in patients with neuropathic pain and for further investigation into the mechanisms by which clonidine reduces primary afferent excitability.

### Grants

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### References


