Spinal Nerve Injury Enhances Subthreshold Membrane Potential Oscillations in DRG Neurons: Relation to Neuropathic Pain

CHANG-NING LIU, MARTIN MICHAELIS, RON AMIR, and MARSHALL DEVOR

INTRODUCTION

Ectopic afferent discharge is widely believed to be a major contributor to chronic pain following peripheral nerve injury [neuropathic pain; reviewed in Devor and Seltzer 1999]. This motivates a deeper understanding of the neural processes responsible for the discharge, and the specific cellular role played by nerve injury. The most prominent sites of origin of ectopic discharge in axotomized primary afferent neurons are the site of injury itself (e.g., the nerve end neuroma) and the dorsal root ganglion (DRG) (Burchiel 1984; Govrin-Lippmann and Devor 1978; Kirk 1974; Study and Kral 1996; Wall and Devor 1983; Wall and Gutnick 1974). Interestingly, the relative contribution of these two sources depends on the location of the nerve injury. Following sciatic nerve injury, at a distance from the DRG, the neuroma is the most prominent impulse generator. However, following spinal nerve injury, in which afferent neurons are axotomized close to their soma, most of the ectopic barrage originates in the DRG (Liu et al. 1999, 2000).

Ectopic afferent discharge originating in neuromas and DRG neurons contributes to neuropathic pain in several ways (Devor and Seltzer 1999). The spontaneous component of the pain is presumed to result from spontaneous discharge of injured afferents. Pain on movement and on deep tissue palpation, on the other hand, is probably due to the mechanosensitivity of sensory endings of neurons that survived the injury. According to this hypothesis, the spinal amplification process (“central sensitization”) is triggered and maintained by the ectopic afferent barrage (Gracey et al. 1992; Liu et al. 2000; Rowbotham and Fields 1996; Sheen and Chung 1993; Woolf and Thompson 1991; Yoon et al. 1996).

All three of these pain components are probably present in the Chung model of neuropathic pain, an experimental preparation in which the L4 or L5 spinal nerves are tightly ligated and then cut (Kim and Chung 1992). There is direct evidence in the Chung model of a role for ectopic afferent firing. For example, Chung and collaborators report that abnormal sensory symptoms are eliminated when the ectopic activity is prevented from entering the spinal cord by selective...
dorsal rhizotomy (Sheen and Chung 1993; Yoon et al. 1996). Likewise, we have recently shown that there is a sudden increase in the intensity of ectopic afferent discharge at precisely the time of onset of tactile allodynia, 16–24 h after transection of the spinal nerve (Liu et al. 2000).

The repetitive discharge of DRG neurons depends on intrinsic resonance properties of the cell membrane (Amir et al. 1999). Most neuronal somata in the DRG are incapable of generating repetitive spike trains on sustained depolarization. Step depolarization usually evokes only a single spike, or a brief burst, and slow ramp depolarization rarely evokes any discharge at all. Only a relatively small subpopulation of cells, those with subthreshold membrane potential oscillations, are able to fire repetitively. In the present study we show that the time of onset of tactile allodynia in the Chung model is marked by a sudden increase in the proportion of DRG neurons with subthreshold oscillations. The increased prevalence of oscillatory behavior, in turn, leads to an augmentation in repetitive discharge in DRG neurons, both at resting membrane potential and during sustained depolarization. These observations strengthen the link between oscillatory behavior in DRG neurons, ectopic firing, and positive sensory symptoms in neuropathy.

**METHODS**

**Animals and surgery**

Experiments were carried out using 68 adult (230–520 g) male rats of the Wistar-derived Sabra strain (Lutzky et al. 1984). All procedures followed national and University regulations for the care and use of laboratory animals, and the ethical guidelines of the International Association for the Study of Pain (Zimmermann 1983). Many of the animals underwent tight ligation and transection of the L₄ spinal nerve as described by Kim and Chung (1992). Briefly, under Nembutal anesthesia (50 mg/kg ip) and using aseptic precautions, the left paraspinal muscles were separated from the spinous processes at the site of incision. The site of incision was then marked with a Lempert nipper and the point of convergence of the L₄ and L₅ spinal nerves was identified. The L₄ spinal nerve was then tightly ligated with 5-0 silk and transected with iridectomy scissors just distal to the ligature. The ligature was placed 3–5 mm proximal to the junction with L₅ nerve, 5–10 mm distal to the L₅ DRG. The incision was then closed in layers, and antibiotics were administered prophylactically (tropical bacteriostatic powder and penicillin 50 kilounits/kg im). Six rats underwent sham surgery that involved the identical surgical incision but without ligation or transection of the spinal nerve.

Following surgery rats were returned to the animal colony and maintained postoperatively in standard transparent plastic shoebox cages bedded with wood shavings, with a 12:12 light:dark cycle, and with food and water available ad libitum. Some of the rats were tested behaviorally to confirm the development of tactile and thermal allodynia (Liu et al. 2000).

**Electrophysiological recording**

Between 9 h and 9 days postoperatively (hpo, dpo) animals were overdosed with pentobarbital sodium (Nembutal) and killed by carotid exsanguination. DRGs L₄ and L₅ were rapidly excised with their dorsal roots (DRs) and spinal nerve attached, and in controls also with their corresponding DRGs. The tissue was immersed in ice-chilled modified Krebs solution (Liu et al. 2000).

**TABLE 1. Prevalence of subthreshold oscillations and repetitive firing, at Vₑ and on depolarization, in the three types of control ganglia (data from A₀ and Aᵣᵣ neurons combined)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Vₑ Cells Without Oscillations, mV</th>
<th>Vₑ Cells With Oscillations, mV</th>
<th>Action Potential Duration, *ms</th>
<th>Oscillations at Vₑ, n/N, %</th>
<th>Firing at Vₑ, n/N, %</th>
<th>Oscillations at Vₑ or on Depolarization, n/N, %</th>
<th>Firing at Vₑ or on Depolarization, n/N, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact L₄</td>
<td>−63.7 ± 6.8 (119)</td>
<td>−58.8 ± 4.6 (16)</td>
<td>0.64 ± 0.15 (38)</td>
<td>7/140, 5.0</td>
<td>6/140, 4.3</td>
<td>16/140, 11.4</td>
<td>16/140, 10.7</td>
</tr>
<tr>
<td>Sham L₄</td>
<td>−64.1 ± 6.0 (31)</td>
<td>−63.0 ± 1.4 (2)</td>
<td>0.72 ± 0.12 (18)</td>
<td>1/33, 3.0</td>
<td>1/33, 3.0</td>
<td>2/33, 6.1</td>
<td>2/33, 6.1</td>
</tr>
<tr>
<td>Intact L₄ (iraxotimized)</td>
<td>−61.1 ± 6.9 (52)</td>
<td>−52.8 ± 5.4 (43)</td>
<td>0.59 ± 0.16 (43)</td>
<td>4/56, 7.1</td>
<td>4/56, 7.1</td>
<td>4/56, 7.1</td>
<td>4/56, 7.1</td>
</tr>
<tr>
<td>Combined control DRGs</td>
<td>−63.1 ± 6.8 (202)</td>
<td>−58.1 ± 5.2 (22)</td>
<td>0.63 ± 0.16 (99)</td>
<td>12/229, 5.2</td>
<td>11/229, 4.8</td>
<td>22/229, 9.6</td>
<td>22/229, 9.2</td>
</tr>
</tbody>
</table>

Values in Vₑ Cells Without Oscillations, Vₑ Cells With Oscillations, and Action Potential Duration are means ± SD with number of cells in parentheses. DRGs, dorsal root ganglia. * A₀ neurons alone.
subthreshold oscillations, spike-free was taken as the asymptotic potential between spikes. To assess membrane potential in actively spiking neurons that sometimes gave rise to action potentials (Amir et al. 1999). Membrane potential in actively spiking neurons was averaged from a sample of 30–40 cycles. The first cycle in the runs of subthreshold oscillations, peak-to-peak oscillation amplitude was usually obvious, but when necessary we used as a formal criterion that amplitude peaks be at least 1.5 times the amplitude of the background noise level present during brief pauses in the oscillations, and/or that there be a distinct peak in the FFT plot at the frequency expected from visual inspection of the voltage trace.

All cells were first examined at $V_r$. They were then depolarized in a slow ramp (about 20 mV/s) by intracellular current injection until oscillations and/or repetitive spiking occurred, or until $-20$ mV. Data are expressed as means ± SD. Differences were compared using $\chi^2$ or two-tailed $t$-tests with a significance criterion of $P = 0.05$ except in the case of multiple comparisons (Table 4) in which a criterion of $P = 0.02$ is recommended (Sigmastat v2.0, Jandel Scientific).

### Results

In this report we focus exclusively on A-neurons because a prior study in vivo showed that at $\leq 9$ dpo these cells alone contribute to the ectopic barrage responsible for hindlimb allodynia in the Chung model of neuropathic pain (Liu et al. 2000).

#### Subthreshold oscillations

**CONTROL GANGLIA.** Neurons from the three types of control preparations ($L_5$ DRG from unoperated, $n = 140$, and sham operated animals, $n = 33$, and $L_5$ DRG from animals with $L_5$ cut, $n = 56$) behaved similarly (Table 1) and will therefore be considered as a group. Most had a stable membrane potential at $V_r$. However, a minority (12/229, 5.2%) exhibited high-frequency, subthreshold sinusoidal oscillations in their membrane potential (Fig. 1). All of these were $A_0$ neurons (Table 2). Oscillations were generally sustained, but some had intermittent brief pauses of $\leq 200$ ms. Oscillation frequency formed a single major peak in FFT histograms, what we call the “dominant oscillation frequency” (Fig. 2). The dominant frequency varied somewhat among neurons, and it was voltage sensitive (see VOLTAGE SENSITIVITY OF OSCILLATION FREQUENCY AND THE EFFECT OF AXOTOMY). At $V_r$ the dominant oscillation frequency was 95.5 ± 27.6 (SD) Hz with a peak-to-peak amplitude of 1.9 ± 1.1 mV (Table 3). These values are very similar to ones reported previously in a separate sample of DRG neurons in intact juvenile rats (Amir et al. 1999).

#### Table 2. Proportion of A-neurons with subthreshold oscillations and with repetitive spike discharge in intact DRGs and at various times following spinal nerve transaction

<table>
<thead>
<tr>
<th>Type of A-Neuron in Each Group</th>
<th>Subthreshold Oscillations</th>
<th>Repetitive Spike Discharge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At $V_r$</td>
<td>Depolarized to $\leq -20$ mV*</td>
</tr>
<tr>
<td>Control (combined)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$A_0$</td>
<td>12/198 (6.1)</td>
<td>22/198 (11.1)</td>
</tr>
<tr>
<td>$A_{inf}$</td>
<td>0/31 (0)</td>
<td>0/31 (0)</td>
</tr>
<tr>
<td>Both</td>
<td>12/229 (5.2)</td>
<td>22/229 (9.6)</td>
</tr>
<tr>
<td>Chung, 9–16 hpo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$A_0$</td>
<td>0/28 (0)</td>
<td>3/28 (10.7)</td>
</tr>
<tr>
<td>$A_{inf}$</td>
<td>0/9 (0)</td>
<td>0/9 (0)</td>
</tr>
<tr>
<td>Both</td>
<td>0/37 (0)</td>
<td>3/37 (8.1)</td>
</tr>
<tr>
<td>Chung, 16–24 hpo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$A_0$</td>
<td>4/30 (13.3)</td>
<td>6/30 (20)</td>
</tr>
<tr>
<td>$A_{inf}$</td>
<td>0/12 (0)</td>
<td>0/12 (0)</td>
</tr>
<tr>
<td>Both</td>
<td>4/42 (9.5)</td>
<td>6/42 (14.3)</td>
</tr>
<tr>
<td>Chung, 1–9 dpo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$A_0$</td>
<td>26/126 (20.6)</td>
<td>38/126 (30.2)</td>
</tr>
<tr>
<td>$A_{inf}$</td>
<td>0/46 (0)</td>
<td>1/46 (2.2)</td>
</tr>
<tr>
<td>Both</td>
<td>26/172 (15.1)</td>
<td>39/172 (22.7)</td>
</tr>
</tbody>
</table>

Numbers in parentheses are percentages. DRGs, dorsal root ganglia; hpo and dpo, hours and days postoperative, respectively. * Includes cells with oscillations at $V_r$.
The resting membrane potential \( V_r \) of the 12 A0 neurons that had oscillations at \( V_r \) was \(-56.8 \pm 5.9 \) mV, a value depolarized by 6–7 mV compared with the remainder of the neurons in which there were no oscillations at \( V_r \) (–63.1 ± 6.8 mV for all A-neurons, –63.4 ± 6.7 mV considering only A0 neurons, both \( P < 0.001 \)). This suggests that the oscillatory behavior may have been due to the depolarized state of the membrane. However, when we injected current intracellularly so as to generate a slow ramp depolarization, subthreshold oscillations appeared in only an additional 10 neurons (all A0) that did not already have them at \( V_r \) (Table 2). Moreover, in these 10 neurons oscillations were not seen until the membrane potential reached \(-36.5 \pm 9.0 \) mV, far positive to the \(-56.8 \pm 5.9 \) value of cells with oscillations at \( V_r \) \( (P < 0.001) \). We conclude that oscillatory behavior is not a general characteristic of DRG neurons. Rather, it can be demonstrated in only a select subpopulation of A0 neurons, even on deep depolarization.

### Table 3. Dominant frequency and peak-to-peak amplitude of subthreshold oscillations in neurons from control DRGs, and DRGs 9 h to 9 days after spinal nerve injury (Chung model)

<table>
<thead>
<tr>
<th>Group</th>
<th>Oscillations Present at ( V_r ) or Only on Depolarization</th>
<th>Potential at Which Oscillation Parameters Measured*</th>
<th>Membrane Potential, mV</th>
<th>Oscillation Frequency, Hz</th>
<th>Oscillation Amplitude, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>( V_r ) or Only on Depolarization</td>
<td>( V_r )</td>
<td>(-56.8 \pm 5.9 ) (12)</td>
<td>95.5 ± 27.6 (11)</td>
<td>1.9 ± 1.1 (12)</td>
</tr>
<tr>
<td></td>
<td>Optimal Depolarization</td>
<td>Threshold</td>
<td>(-36.5 \pm 9.0 ) (10)</td>
<td>105.8 ± 23.3 (10)</td>
<td>1.2 ± 0.4 (10)</td>
</tr>
<tr>
<td>Chung</td>
<td>( V_r ) or Only on Depolarization</td>
<td>Optimal</td>
<td>(-32.2 \pm 7.9 ) (10)</td>
<td>116.0 ± 28.2 (10)</td>
<td>1.7 ± 0.4 (10)</td>
</tr>
<tr>
<td></td>
<td>Optimal Depolarization</td>
<td>Threshold</td>
<td>(-52.5 \pm 5.7 ) (30)</td>
<td>68.9 ± 22.0 (21)</td>
<td>2.9 ± 1.1 (24)</td>
</tr>
<tr>
<td></td>
<td>Optimal Depolarization</td>
<td>Optimal</td>
<td>(-52.5 \pm 4.6 ) (22)</td>
<td>69.5 ± 21.4 (21)</td>
<td>3.1 ± 1.2 (23)</td>
</tr>
<tr>
<td></td>
<td>Optimal Depolarization</td>
<td>Threshold</td>
<td>(-47.0 \pm 7.0 ) (17)</td>
<td>78.1 ± 32.9 (11)</td>
<td>1.9 ± 1.1 (11)</td>
</tr>
<tr>
<td></td>
<td>Optimal Depolarization</td>
<td>Optimal</td>
<td>(-39.1 \pm 7.1 ) (16)</td>
<td>84.6 ± 33.5 (11)</td>
<td>2.6 ± 1.2 (11)</td>
</tr>
</tbody>
</table>

Values are means ± SD with number of cells in parentheses. * “\( V_r \)” is the cell’s resting membrane potential, “threshold” is the most negative potential at which oscillations were discernable, “optimal” is the potential at which oscillation amplitude was maximal.
tions were no longer discernable above the background noise (Figs. 2 and 4 A). In all neurons that oscillated at $V_r$, the oscillations were quenched by hyperpolarization.

Spinal nerve injury had a marked effect on the relation of oscillation amplitude to membrane potential. First, it shifted the function to more negative (hyperpolarized) potentials. Following axotomy, fewer cells required deep depolarization before generating oscillations (Fig. 4, A and B), and the mean threshold and optimal potentials for generating oscillations shifted in the negative direction ($P < 0.01$, Table 3). Second, axotomy caused a net increase in oscillation amplitude (Table 3). For example, oscillation amplitude at the optimal membrane potential averaged $1.9 \pm 1.0 \text{ mV}$ ($n = 21$) in control neurons versus $2.9 \pm 1.2 \text{ mV}$ ($n = 34$) in axotomized neurons ($P = 0.002$).

**VOLTAGE SENSIBILITY OF OSCILLATION FREQUENCY AND THE EFFECT OF AXOTOMY.** Like oscillation amplitude, oscillation frequency increased systematically when the cells were depolarized (Amir et al. 1999). However, unlike amplitude, there was no optimum value. Rather, oscillation frequency continued to increase with depolarization, in a monotonic manner (Fig. 4 C). The dependency of frequency on membrane potential in control neurons was $0.84 \pm 0.66 \text{ Hz/mV}$ (8 cells). This value was not significantly affected by axotomy [$0.72 \pm 0.36 \text{ Hz/mV}$ (10 cells, 1–9 dpo, $P > 0.2$)].

Figure 4 D plots the dominant oscillation frequency at the membrane potential at which oscillation amplitude was maximal. Axotomy caused a clear shift to lower frequencies ($106.0 \pm 29.3 \text{ Hz}$ in 21 control neurons vs. $74.7 \pm 26.7$ in 32 axotomized neurons, $P < 0.001$, Table 3). Part of this change is expected from the relatively hyperpolarized optimal mem-
Ectopic spiking and the effect of axotomy

Subthreshold membrane potential oscillations often gave rise to repetitive spike discharge. In all cases spikes arose from the rising (depolarizing) phase of the oscillatory sinusoid (Table 2). In neurons with oscillations at \( V_r \) (both intact and axotomized) oscillation amplitude was often already large enough to trigger spontaneous spike discharge (Fig. 3B). In neurons that generated oscillations only on depolarization, repetitive spiking usually appeared when the membrane was depolarized by an additional 2–4 mV beyond the threshold for appearance of oscillations (Figs. 5 and 6). In both cases depolarization had its effect by 1) increasing oscillation amplitude and 2) bringing the peaks of the oscillatory sinusoids closer to spike threshold. Overall, 66 of the 70 neurons studied (94%) that had subthreshold oscillations also fired repetitively at rest or on depolarization (Table 2, Fig. 3). For the cells that were not already firing at \( V_r \), the threshold for repetitive firing was \(-34.3 \pm 7.4 (n = 10)\) in controls and \(-43.8 \pm 8.3 \text{ mV} (n = 13)\) in Chung preparations (\(P = 0.01\)). At first, firing rate increased when the cells were depolarized beyond firing threshold. However, still deeper depolarization led to a reduction in oscillation amplitude and eventually to the cessation of spiking (Fig. 5).

Repetitive firing often took the form of irregular discharge of single spikes, where individual oscillation sinusoids triggered spikes at irregular intervals. Alternatively, there were trains of spike bursts (“interrupted autorhythmicity,” Fig. 1). The mean interval between spikes or spike bursts decreased as the cell was depolarized. Likewise, the probability of burst firing increased, as did the duration of bursts. The factors that control discharge pattern will be presented in detail elsewhere.

In contrast to neurons with subthreshold oscillations, neurons that did not either oscillate at \( V_r \) or begin to generate oscillations on depolarization never showed repetitive spiking (410 of 410 cells observed). This includes 313 \( A_0 \) neurons (176 in control DRGs, 137 in axotomized DRGs) and 97 \( A_{inf} \) neurons (31 in control DRGs, 66 in axotomized DRGs). Despite their inability to support repetitive firing, all of the non-oscillating \( A_0 \) and \( A_{inf} \) neurons generated a single action potential at the beginning of a suprathreshold depolarizing step, and in some there was a short burst of action potentials. The association of repetitive spiking with subthreshold oscillations was, of course, highly significant statistically (\(P < 0.001, \chi^2\)). The one axotomized \( A_{inf} \) neuron encountered that had oscillations fired repetitively on depolarization.

Since axotomy increased the proportion of neurons with subthreshold oscillations, it also increased the overall ectopic impulse barrage (Table 2, Fig. 3B). This increase was particularly prominent at \( V_r \) and hence relevant to the intensity of the spontaneous ectopic barrage in vivo (Figs. 4, A and B, and 6). There was nearly a fourfold increase in the prevalence of spontaneous firing of \( A_0 \) neurons at \( V_r \) in axotomized compared with control ganglia (5.6 vs. 20.6% 1–9 dpo). We have reported a similar dramatic increase in ectopic discharge in vivo (Liu et al. 2000). This appears to be the principal factor responsible for positive neuropathic symptoms in the Chung model of neuropathic pain.

Biophysical characteristics of oscillating neurons and effects of axotomy

We made systematic measurements of a number of cellular parameters thought to be associated with neuronal excitability aiming to identify the ones most closely associated with oscillatory behavior. Results for \( A_0 \) neurons are given in Table 4. Differences in \( V_r \) were noted above and will not be repeated here.

Oscillations at \( V_r \). Considering first only cells with oscillatory capability, neurons that oscillated only when depolarized...
from rest had narrower spikes than those with oscillations at $V_r$. This was so in both control and axotomized DRGs and was associated with faster rates of rise and fall of the spike and larger spike amplitude. A small part of these differences is accounted for by the slightly more positive $V_r$ of the neurons that oscillated at $V_r$. In direct measurements we found that spikes became broader on depolarization (by 16 $\mu$mV) and that dV/dt of the rising and falling phases of the spike decreased (by 3.4 and 1.8 V/s/mV, respectively). However, this factor accounts for only about 20% of the observed difference in spike waveform (Table 4). Moreover, as noted above, oscillations emerged in the neurons requiring depolarization at potentials far positive to the $V_r$ of the cells that oscillated at $V_r$, and $R_{in}$ did not differ between the two cell groups. Finally, threshold for evoking action potentials, both single spikes and sustained firing, was higher in the cells that oscillated only on depolarization. These data suggest that the neurons with oscillations at $V_r$ constitute a distinct population, different from those that oscillate only on depolarization (see discussion).

**Table 4. Properties of DRG A0 neurons that had subthreshold oscillations at $V_r$, or on depolarization, and neurons without oscillatory capability, in intact animals and in the Chung model of neuropathic pain (spinal nerve section 9 hpo to 9 dpo)**

<table>
<thead>
<tr>
<th>Oscillation at $V_r$</th>
<th>Amplitude, mV</th>
<th>Duration, ms</th>
<th>dV/dt Rise, V/s</th>
<th>dV/dt Fall, V/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (cells with oscillations)</td>
<td>-54.1 ± 5.9 (40)</td>
<td>67.1 ± 10.2 (34)</td>
<td>0.95 ± 0.55 (34)</td>
<td>160.9 ± 68.6 (32)</td>
</tr>
<tr>
<td>Chung (9 hpo to 9 dpo)</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>No oscillations (control + Chung)</td>
<td>-59.1 ± 5.4 (25)</td>
<td>78.7 ± 12.5 (25)</td>
<td>0.57 ± 0.24 (25)</td>
<td>238.4 ± 60.0 (21)</td>
</tr>
<tr>
<td>$P$ (t-test)</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**Discussion**

Afferent discharge arising ectopically in DRG neuronal somata is believed to contribute significantly to spontaneous dysaesthesias, pain on movement, and tissue tenderness in neuropathic pain (spinal nerve section 9 hpo to 9 dpo). Axotomy had similar effects on all three groups of neurons, those oscillating at $V_r$, those with oscillations only on depolarization, and those without oscillations. These were generally consistent with many prior reports (Kim et al. 1998; Stebbing et al. 1999; Titmus and Faber 1990). Specifically, axotomy caused a broadening of the action potential, due to slowing of both the rising and the falling limbs of the spike. There was no consistent change in spike height or in $R_{in}$. The amplitude of the AHP was significantly reduced, but AHP duration was unchanged. Most importantly, there was a significant reduction in the single spike threshold (Table 4) and the threshold for evoking sustained firing (Fig. 6).
patients with neuropathy (Devor and Seltzer 1999). During the first few days after nerve injury in the Chung model of neuropathic pain virtually all of the ectopic activity that originates in the DRG occurs in neurons with myelinated axons (A-neurons) (Liu et al. 2000). In the present study we recorded intracellularly from A-neurons in excised DRGs to evaluate the process whereby axotomy gives rise to this ectopia. The most striking observation was that repetitive firing in DRG neurons does not result from the classical (Hodgkin-Huxley) repetitive firing process in which a sustained (generator) depolarization repeatedly draws the membrane potential to spike threshold (Jack et al. 1985). Rather, it is due to subthreshold oscillations in the resting membrane potential (Amir et al. 1999). Spiking occurs when individual oscillation sinusoids reach threshold. Nearly all neurons with subthreshold oscillations fired repetitively either at \( V_r \) or when depolarized; none of the cells without subthreshold oscillations fired repetitively at any membrane potential. Spinal nerve injury enhances the subthreshold oscillations in DRG neurons triggering intensified ectopic discharge and hence neuropathic paresthesias and pain.

**Categories of A0 neurons**

The relation between repetitive firing in DRG neurons and subthreshold membrane potential oscillations was first reported by Amir et al. (1999), although related resonance behavior has been noted previously in primary afferent neurons in the trigeminal ganglion and in the mesencephalic nucleus of the trigeminal brain stem (Pedroarena et al. 1999; Pelkey and Marshall 1998; Puil and Spigelman 1988). Consistent with the observations of Amir et al. (1999), we found that in DRG A-neurons, subthreshold oscillations occur almost exclusively in (noninflected) A0 neurons. Moreover, these appear to be a specific A0 subpopulation, statistically distinguishable from A0 neurons without oscillatory capability on the basis of definable biophysical characteristics. Specifically, A0 neurons incapable of generating oscillations have a smaller and more prolonged AHP than those with oscillations. In A0inf neurons, which virtually never show subthreshold oscillations, the AHP is much longer than in A0 neurons (Amir and Devor 1997; Liu et al. 2000). We conclude that at least one requirement for oscillations is rapid recovery from the postspike hyperpolarization.

A priori, the somata of DRG neurons, particularly A0 neurons, are poorly adapted for sustained firing (Eng et al. 1988; Everill et al. 1998; Kocsis et al. 1982; Stansfeld et al. 1986). They are relatively large cells, with a large membrane area (and capacitance) and have a very prominent delayed (outward) rectification. The outwardly rectifying conductance opposes inward (generator) currents and decreases \( R_{in} \). Both effects cause the trajectory of depolarization after the first spike to be sufficiently shallow that membrane accommodation tends to prevent a second and third spike from being triggered. For this reason step depolarization rarely triggers more than one or two action potentials (at the beginning of the pulse) unless the delayed rectifier is blocked pharmacologically.

Oscillatory behavior can be viewed as a means of enabling repetitive firing despite the strong outward rectification. Specifically, the rapid rise time of the depolarizing limb on the oscillatory sinusoid, which scales with oscillation frequency, has the effect of overcoming membrane accommodation. In this way oscillations simulate rapid-fire (tetanic) step depolarizations. Depolarization brings oscillation peaks closer to threshold, hence favoring repetitive discharge. However, an even more important effect of depolarization is to increase oscillation amplitude and the slope (dV/dt) of the depolarizing phase of the oscillatory sinusoids. When cells become too deeply depolarized, however, oscillation amplitude declines, and the Na+ channels responsible for the rising limb of the action potential become progressively inactivated. As a result, spike generation begins to fail, despite the continued presence of oscillations. Possible reasons why evolution provided some DRG neuronal somata with this special repetitive firing capability are discussed elsewhere (Devor 1999).

Among the A0 neurons with oscillatory capability in intact DRGs, about one-half showed subthreshold oscillations at \( V_r \), while in the remainders it was necessary to apply tonic depolarization. Since the resting membrane potential of neurons with oscillations at \( V_r \) was itself relatively depolarized (Table 4), we initially presumed that these two groups form a continuum of oscillatory A0 neurons. However, we now believe that they constitute distinct functional subtypes on the basis of two observations. First, in neurons requiring depolarization, subthreshold oscillations became detectable at a mean of \(-36.5 \pm 9.0 \) mV, a value almost non-overlapping with the resting potential of cells with oscillations at \( V_r \) (\(-56.8 \pm 5.9 \) mV, \( P < 0.001 \)). Second, neurons requiring depolarization had much narrower action potentials than those with oscillations at \( V_r \), although AHP characteristics were no different (Table 4). These differences cannot be explained on the basis of resting membrane potential alone.

Axotomy greatly increased the population of neurons with oscillations, shifting cells from the nonoscillating to the two oscillating categories. Interestingly, early in this process (1–2 dpo), essentially all of the newly oscillating neurons oscillated at \( V_r \) (80% of all oscillating neurons in this time window oscillated at \( V_r \)). Only later (2–9 dpo) did the ratio between the two categories return to its control value of about 50% (Fig. 3A). Moreover, the cells newly oscillating at \( V_r \) had spike waveform characteristics very similar to the overall value for cells with oscillations at \( V_r \) \([0.91 \pm 0.43 \) ms \((n = 17 \) cells, 1–2 dpo) \] vs. \(0.95 \pm 0.35 \) ms \((n = 34 \), \( P > 0.2 \), Table 4\]), while the cells that oscillated only on depolarization 1–2 dpo were very similar to the overall population of cells with oscillations only on depolarization \([0.52 \pm 0.06 \) ms \((n = 6 \) cells, 1–2 dpo) \] vs. \(0.57 \pm 0.24 \) ms \((n = 25 \), \( P > 0.2 \), Table 4\]). This means that the first change in cellular membrane characteristics induced by axotomy causes an increase in spike duration. This observation strengthens the link between spike width and oscillatory phenotype. By the same token, it suggests that individual A0 neurons can shift between the three categories (i.e., nonoscillating and 2 categories of oscillating neurons).

The existence of distinct A0 neuronal subtypes is of considerable practical significance since electrical properties of the DRG cell soma tend to correlate with those of the peripheral sensory ending (Harper 1991; Reeh and Wadell 1990). Therefore resonance properties of the soma may reflect the type of afferent signal carried by the neuron in question. For example, A0 neurons with oscillations at \( V_r \) may correspond to slowly or nonadapting afferent types such as muscle spindle afferents, while A0 neurons that oscillate only on depolarization may correspond to low-threshold mechanoreceptors, which have more rapid adaptation. The quality of abnormal sensations
resulting from ectopic firing, in health and disease, depends on the functional class of the active neurons. It is not known yet whether oscillatory behavior also underlies ectopic repetitive firing in neuroma end bulbs or normal sensory endings (but see Kapoor et al. 1997). However, if so, this difference could account for the observation that spontaneous activity in DRG neurons and neuroma endings originates largely in muscle afferents, while most injured cutaneous afferents require a depolarizing stimulus to evoke ectopic firing (Johnson and Munson 1991; Michaelis et al. 2000; Proske et al. 1995; Tal et al. 1999).

Effects of axotomy

The prevalence of subthreshold oscillations observed here in intact (control) DRGs was similar to that reported by Amir et al. (1999), although the present study includes a much larger sample of A-neurons. Combining data from the two studies, the proportion of A0 neurons with oscillations and spiking at Vr was 4–5%. This corresponds well with the prevalence of spontaneous discharge originating in the DRG of intact rats in vivo (Wall and Devor 1983). Likewise, the proportion of A0 neurons with oscillations and spiking on depolarization was nearly identical to values reported by Amir et al. (1999). Interestingly, the effects of axotomy were also similar despite the fact that Amir et al. (1999) cut the sciatic nerve (rather than the spinal nerve), severing axons much farther from the cell soma. The most prominent differences were the timing of the most intense ectopic barrage (later in the case of sciatic nerve injury), and its intensity (less at the peak in the case of sciatic nerve injury). Detailed comparisons of the effects of distal versus proximal axotomy are given in Liu et al. (2000).

Axotomy increased the incidence of oscillations and firing at Vr. This effect, however, was not caused primarily by shifting Vr in the direction of depolarization. Rather, it was mostly a result of the shift in the voltage dependence of oscillation amplitude toward Vr (Fig. 4). An additional effect of axotomy was to lower the frequency of oscillations at any given membrane potential. Amir et al. (1999) showed that the depolarizing limb of the oscillatory sinusoid is dependent on Na+ conductance. The specific resonance characteristics of different DRG neurons, including the heterogeneity in the voltage dependence of oscillation amplitude and frequency, are presumably related, at least in part, to the heterogeneity in the Na+ channel subtypes expressed in different classes of DRG neurons. Axotomy is known to differentially alter the expression of certain Na+ channel subtypes, although for other subtypes the nature of the change, if any, has not yet been determined (Waxman et al. 1999).

Unfortunately, the currently known changes in Na+ channel subtypes do not account in any obvious way for the observed alterations in spike waveform and oscillatory phenotype. For example, it is known that the kinetically fast TTX-sensitive type III Na+ channels are up-regulated following axotomy (Cummins and Waxman 1997; Rizzo et al. 1995; Waxman et al. 1994), and that the slower TTX-resistant PN3/SNS and NaN/SNS2 Na+ channels are downregulated (Cummins and Waxman 1997; Dib-Hajj et al. 1998; Novakovic et al. 1998; Waxman et al. 1999). This might have lead one to predict that an early effect of axotomy would be to decrease spike width, and increase rates of spike rise and fall (dV/dr). In fact, the opposite was observed. Shortly after axotomy (1–2 dpo), newly oscillating neurons showed an increase in spike width, which only later (2–9 dpo) narrowed. Type III Na+ channels are normally expressed at very low levels in adult DRG neurons (Waxman et al. 1994). Perhaps the early increase in spike width reflects reduced K+ conductance (Everill and Kocsis 1999), with the subsequent narrowing due to up-regulated type III Na+ channels. Alternatively, an early change in some other TTX-sensitive Na+ channel subtype may be involved.

It is likely that the marked reduction in oscillation frequency induced by axotomy is more closely related to changes in K+ conductance than in changes in Na+ conductance (unpublished observations). Axotomy is known to downregulate the expression of at least some K+ channel transcripts, and to reduce whole cell K+ conductance, including both Ik and IA, by about 50% (Everill and Kocsis 1999; Ishikawa et al. 1999). This change also probably contributes to the overall facilitation of resonance behavior and firing in axotomized neurons (Devor et al. 1994; Amir et al., unpublished observations). Finally, in addition to voltage-sensitive Na+ and K+ channels, axotomy alters the expression of a variety of other channel and receptor subtypes in DRG neurons (e.g., Baccei and Kocsis 2000; Hokfelt et al. 1997; Kelly et al. 1986). The observed changes in oscillatory phenotype no doubt result from the integrated effects of the entire spectrum of axotomy-induced changes in membrane electrical properties.

Relation to neuropathic pain

In most cases A0 neurons are the cell bodies of low-threshold mechanoreceptors (e.g., Koerber and Mendell 1992). Ectopic firing in these neurons is therefore expected to be felt as touch, pressure, stretch (including proprioception), or vibration. However, it has been well documented in recent years that in the presence of central sensitization, afferent input along low-threshold afferents can give rise to a sensation of pain (Aβ pain) (Campbell et al. 1988; Cook et al. 1987; Koltzenburg et al. 1997; Kelly et al. 1998). Indeed, Aβ pain is probably the most important component of tenderness (tactile allodynia) in everyday minor injuries (within the zone of primary as well as secondary hyperalgesia). Therefore spontaneous discharge originating in DRG A0 neurons and ectopic activity evoked by depolarization (e.g., secondary to mechanical stimulation of the DRG, crossed- afterdischarge or sympathetic efferent activity) may well contribute to frank pain in addition to neuropathic paresthesias and dysaesthesias (Devor and Seltzer 1999).

The question remains as to the origin of central sensitization in neuropathy. At least two options present themselves. First, in the event of nerve injury, central sensitization may be triggered and maintained by C-nociceptor activity originating within the DRG, the nerve injury site, or residual intact afferents (Devor and Seltzer 1999). Second, there is accumulating evidence that following axotomy, the structural and neurochemical phenotype of A0 neurons may change in such a way that they, too, can trigger and maintain central sensitization (Liu et al. 2000; Ma and Woolf 1996; Noguchi et al. 1995; Woolf 1992). Thus the enhancement of cellular resonance followed by nerve injury, and the augmented ectopic discharge that it triggers, may well be the most fundamental of the cellular changes that underly neuropathic pain.
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