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# *Painful Nerve Injury Shortens the Intracellular $Ca^{2+}$ Signal in Axotomized Sensory Neurons of Rats*

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***Background:*** **Neuropathic pain is inadequately treated and poorly understood at the cellular level. Because intracellular $Ca^{2+}$ signaling critically regulates diverse neuronal functions, the authors examined effects of peripheral nerve injury on the $Ca^{2+}$ transient that follows neuronal activation.**

***Methods:*** **Cytoplasmic $Ca^{2+}$ levels were recorded by digital microfluorometry from dissociated dorsal root ganglion neurons of hyperalgesic animals after ligation of the fifth lumbar spinal nerve and control animals. Neurons were activated by field stimulation or by $K^+$ depolarization.**

***Results:*** **Transients in presumptively nociceptive, small, capsaicin-sensitive neurons were diminished after axotomy, whereas transient amplitude increased in axotomized nonnociceptive neurons. Axotomy diminished the upward shift in resting calcium after transient recovery. In contrast, nociceptive neurons adjacent to axotomy acquired increased duration of the transient and greater baseline shift after $K^+$ activation. Transients of nonnociceptive neurons adjacent to axotomy showed no changes after injury. In nociceptive neurons from injured rats that did not develop hyperalgesia, transient amplitude and baseline offset were large after axotomy, whereas transient duration in the adjacent neurons was shorter compared with neurons excised from hyperalgesic animals, which show normalization of these features.**

***Conclusions:*** **A diminished $Ca^{2+}$ signal in axotomized neurons may be in part due to loss of $Ca^{2+}$ influx through voltage-gated $Ca^{2+}$ channels. The upward shift in resting $Ca^{2+}$ level after activation, which is diminished after axotomy in presumed nociceptive neurons, is a previously unrecognized aspect of neuronal plasticity. These changes in the critical $Ca^{2+}$ signal may mediate various injury-related abnormalities in $Ca^{2+}$-dependent neuronal.**

NEUROPATHIC pain is a maladaptive, persistent pain syndrome that results from damage to the peripheral or central nervous system.[1] It accompanies diverse diseases such as diabetes mellitus, herpes zoster, intervertebral disc herniation, or trauma and is difficult to treat.[2] A hallmark of neuropathic pain is hyperexcitability of peripheral and central nociceptive pathways. Patients report spontaneous pain, allodynia (pain from stimuli at intensities that normally do not produce pain), or hyperalgesia (greater pain from a normally painful stimulus), which result from complex pathologic processes at the site of injury, the dorsal root ganglion (DRG), the spinal cord, and the brain.

Substantial membrane modifications have been identified in primary sensory neurons in different animal models of neuropathic pain. Loss of current through potassium channels,[3] altered expression of various sodium channel isoforms,[4] and diminished influx through plasmalemmal voltage-gated calcium channels[5] produce electrical abnormalities of injured sensory neurons that contribute to hyperexcitability and pain.[6,7]

The level of cytoplasmic calcium ($[Ca^{2+}]_c$) is closely regulated by a complex system of buffers, pumps, and release mechanisms that shape the pattern of rise and fall of $[Ca^{2+}]_c$ (the $Ca^{2+}$ transient) initiated by depolarization-induced calcium influx. The duration of such $[Ca^{2+}]_c$ events are considerably prolonged compared with membrane depolarization, with durations of the transient spanning many seconds compared with the few-millisecond duration of an action potential (AP).[8] In addition, the amplitude and duration of the $Ca^{2+}$ transient expand in response to repetitive neuronal AP generation. Therefore, the $[Ca^{2+}]_c$ signal provides a cellular integration and memory function. Calcium transients critically regulate diverse neuronal functions, including excitability, kinase activity, neurotransmitter release, genetic expression, and apoptotic cell death.[9]

Our previous data have identified reduced $Ca^{2+}$ influx through high- and low-voltage–activated $Ca^{2+}$ channels of primary sensory neurons after painful peripheral nerve injury.[5,10,11] We have further observed that resting $Ca^{2+}$ levels are lower in sensory neurons after injury.[12] The influence of these alterations on depolarization-induced $Ca^{2+}$ transients is unknown. We hypothesized that the documented injury-induced loss of inward $Ca^{2+}$ flux across the plasmalemma will lead to diminished amplitude and duration $Ca^{2+}$ transients in sensory neurons. We therefore used digital microfluorometry to examine $Ca^{2+}$ transients in dissociated DRG neurons from control animals and animals with hyperalgesia after peripheral nerve trauma by spinal nerve ligation (SNL). This model allows separate examination of neurons that are injured by axotomy in the fifth lumbar (L5) ganglion *versus* L4 neurons exposed to inflammatory mediators induced by wallerian degeneration[13,14] of L5 fibers in the distal nerve. Both field stimulation and depolarization by $K^+$ elevation were used to trigger $Ca^{2+}$ transients, and neurons were categorized by size and sensitivity to cap-

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saicin as a means of segregating predominantly nociceptive and nonnociceptive neurons.

## Materials and Methods

All procedures were approved by the Medical College of Wisconsin Animal Care and Use Committee, Milwaukee, Wisconsin.

### *Animal Preparation*

Male adult Sprague-Dawley rats (Charles River Laboratories Inc., Wilmington, MA) weighing 160–180 g were randomly assigned to an SNL group or a control group. SNL was performed similar to the originally reported technique.[15] The right lumbar paravertebral region was exposed during anesthesia with halothane (2–3%) in oxygen. After subperiosteal removal of the sixth lumbar transverse process, both the right fifth and the sixth lumbar spinal nerves were tightly ligated with 6-0 silk suture and transected distal to the ligatures. In contrast to the original description,[15] no muscle was removed, the intertransverse fascia was incised only at the site of the two ligations, and articular processes were not removed. The lumbar fascia was closed by 4-0 resorbable polyglactin suture, and the skin was closed with three staples. In control rats, only lumbar skin incision and closure was performed. After surgery, the rats were returned to the colony, where they were kept in individual cages under normal housing conditions. All animals used for further study had fully healed incisions.

### *Sensory Testing*

Identification of hyperalgesia was performed as previously described.[16] At least 1 day after arrival at the animal care facility, rats were brought to the testing area for 4 h of familiarization with handling and the environment. Hind paws were stimulated in random order with a 22-gauge spinal needle applied with pressure adequate to indent but not penetrate the plantar skin 2 days before surgery and on the 10th, 12th, and 14th postoperative days. We tabulated the frequency of two types of induced behavior. The response typical of control rats was only a brief withdrawal. A hyperalgesia-type response was found in SNL animals, consisting of sustained lifting, licking, chewing, or shaking of the paw. With the exception of four nonhyperalgesic rats evaluated as a separate group, rats that did not develop hyperalgesia responses after SNL were not studied further.

### *Cell Isolation and Plating*

The L4 and L5 DRGs were removed from control and SNL rats after halothane anesthesia and decapitation. The operative field was perfused with cold, oxygenated calcium and magnesium chloride–free Hanks Balanced Salt Solution. Ganglia were cut into three pieces and enzymatically dissociated in a solution containing 0.018% liberase blendzyme 2 (Roche Diagnostics Corp., Indianapolis, IN), 0.05% trypsin (Sigma, St. Louis, MO), and 0.01% deoxyribonuclease 1 (150,000 U; Sigma) in 4.5 ml DMEM F12 (Gibco, Invitrogen Corp., Carlsbad, CA) for 90 min in a shaker bath at 32°C. Cells were harvested by centrifugation and resuspended in a culture medium consisting of 0.5 mM glutamine, 0.02 mg/ml gentamicin, 100 ng/ml nerve growth factor 7S (Alomone Labs, Jerusalem, Israel), 2% (vol/vol) B-27 supplement (Life Technologies, Rockville, MD), and 98% (vol/vol) neurobasal medium A 1X (Life Technologies) for plating onto poly-L-lysine–coated 12-mm glass coverslips (Deutsche Spiegelglas; Carolina Biologic Supply, Burlington, NC), plating from two to four slips per ganglion. Cells were incubated for 2–3 h in humidified incubator at 37°C with 95% air and 5% $CO_2$ before dye loading, and were studied within 5 h of dissociation.

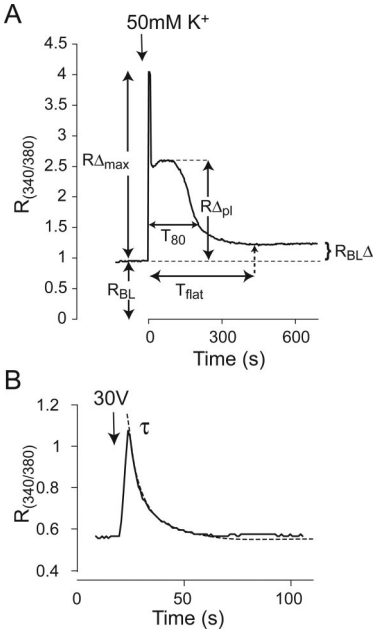


**Fig. 1. Measures of the $Ca^{2+}$ transient indicated by the fluorescence ratio ($R_{340/380}$). Typical trace after $K^+$ stimulation (*A*) shows baseline ratio ($R_{BL}$), transient amplitude above baseline ($R\Delta_{max}$), transient duration to 80% recovery ($T_{80}$), duration until the transient becomes flat ($T_{flat}$), level of the plateau above baseline ($R\Delta_{pl}$), and the offset of the posttransient baseline above the original baseline ($R_{BL}\Delta$). In cells without a plateau, as in this trace after field stimulation (*B*), an exponential function (*dotted line*) was fit from which the time constant ($\tau$) was determined.**

### *Calcium Microfluorometry*

Cells were loaded with the ratiometric $Ca^{2+}$ indicator fura-2 AM (2.5 μm in 0.1% Pluronic F-127; Molecular Probes, Eugene, OR) for 45 min at room temperature and then washed three times with a Tyrode solution consisting of 140 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM glucose, and 10 mM HEPES. Cells were left in a dark environment for 30 min for dye de-esterifica-

tion. Coverslips were mounted in a 500-$\mu$l recording chamber perfused with room temperature (22°C) Tyrode solution at a gravity driven flow rate of 2 ml/min, and imaged at 400× magnification using an inverted microscope (Nikon Diaphot 200; Nikon, Tokyo, Japan) and cooled charge-coupled device camera (Cool Snap; fx-Photometrics, Tucson, AZ). Cell diameter was determined by calibrated video image. On bright-field examination, neurons were excluded from measurement if they showed evidence of lysis or crenulation of their surface, because these cells showed unstable recordings, and also if they had overlying glial satellite cells. Only one field was studied per slip. Autofluorescence of unloaded cells had a signal strength of less than 5% of the fluorescence of loaded cells. Emitted fura-2 fluorescence was recorded at 510 ± 20 nm wavelength during alternating 340- and 380-nm excitation (DG-4; Sutter, Novato, CA), and the ratio of fluorescence at 340 and 380 nm (R) was calculated on a pixel-by-pixel basis. The frame capture period was 200 ms at intervals of 3 s. Each neuron was specified as a region of interest in the digital image (MetaFluor; Universal Imaging Corporation, Downingtown, PA) for separate averaging of R of the region, and an additional background area was recorded in each field for on-line subtraction of background fluorescence. R is directly related to $[Ca^{2+}]_c$.[17] Because this study examines ratio changes in R, we did not convert R into actual $[Ca^{2+}]_c$.

*Neuronal Activation*

Two types of stimulation were used to optimally characterize neuronal $Ca^{2+}$ transients in response to activation. In some experiments, transients were elicited by 5 s of superfusion with Tyrode solution containing elevated $K^+$ concentration (50 mM) with reciprocal decrease in $Na^+$ concentration, through a microperfusion system (onset time less than 200 ms) 125 $\mu$m upstream from the imaged field. This frequently used technique maximally activates $Ca^{2+}$ handling processes. It produces a sustained depolarization that is considerably longer than a short train of action potentials, although it could duplicate the $Ca^{2+}$ load provided by a sustained burst of intense pathologic activity. In other experiments, cells were excited by field stimulation to produce more physiologic trains of brief depolarizations.[18] Specifically, current generated by a self-made constant-voltage generator was passed between two 5-mm-long platinum electrodes with a separation distance of 2 mm placed on either side of the target neurons. Pulses (2 ms) of nominal 30 V were delivered at 10 Hz for 3 s. At the end of some experiments, capsaicin was applied through the microperfusion system.

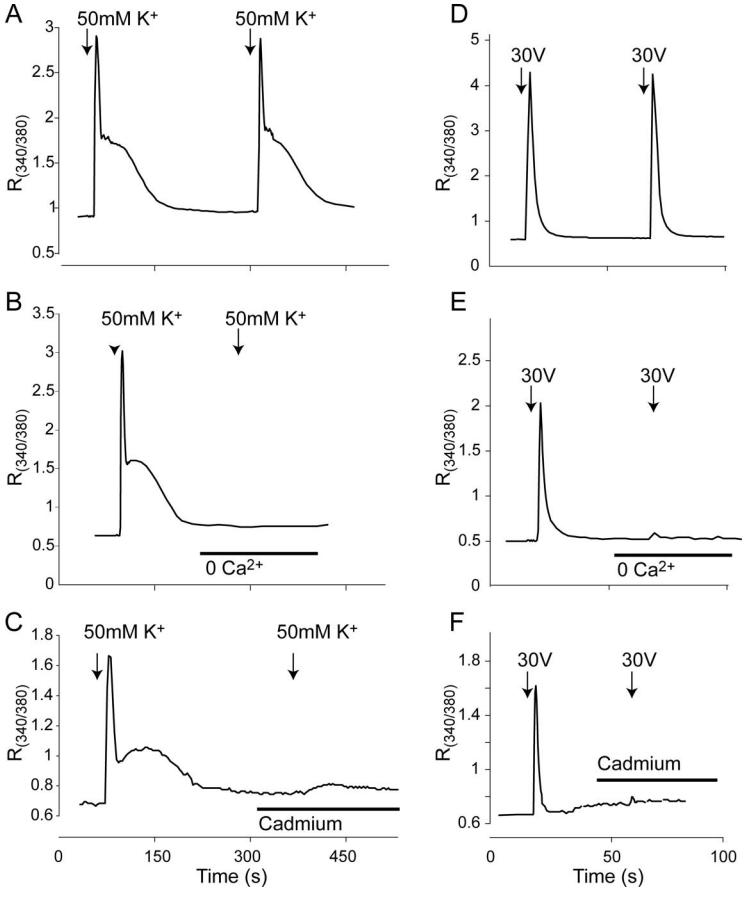


**Fig. 2. (*A–C*) Dependence of $Ca^{2+}$ transients induced by $K^+$ (50 mM) depolarization on extracellular $Ca^{2+}$ and voltage-gated $Ca^{2+}$ channels. (*A*) Repeated $K^+$ stimulation for 5 s produces stable fluorescence ratio ($R_{340/380}$) transients in freshly dissociated dorsal root ganglion neurons (n = 17). (*B*) The transient response requires bath $Ca^{2+}$ (n = 15). (*C*) Transients also require functioning voltage-gated $Ca^{2+}$ channels, as shown by blockade with cadmium (200 $\mu$M, n = 11). (*D–F*) Dependence of field stimulation-induced $Ca^{2+}$ transients on extracellular $Ca^{2+}$ and voltage-gated $Ca^{2+}$ channels. (*D*) Repeated field stimulation (2-ms pulses at 30 V and 10 Hz) produces stable fluorescence ratio ($R_{340/380}$) transients (n = 11). (*E*) Response requires bath $Ca^{2+}$ (n = 13). (*F*) Transients are blocked by cadmium, indicating a requirement for voltage-gated $Ca^{2+}$ channels (n = 13).**

*Analysis and Statistics*

Dimensions of the $Ca^{2+}$ transient induced by neuronal activation were determined by digital trace analysis (Axograph 4.7; Axon Instruments, Foster City, CA). The measurement techniques are detailed in figure 1. The transient following $K^+$ depolarization often includes a sustained plateau after the initial peak. When no plateau was observed, the resolution of the transient was fit by an exponential decay using the analysis program.

Neurons were divided into two groups with diameters above or below 34 $\mu$m.[12,19] This segregates neurons into a population of large neurons with predominantly low-threshold receptive field properties and small neurons predominantly responsive to nociceptive stimuli.[20] Because sensitivity to capsaicin is a feature that distinguishes thermal nociceptors,[21] a subset of neurons were further characterized by their response to capsaicin. Specifically, if R increased by 50% over baseline after bath application of 100 nM capsaicin, the neuron was considered responsive.[12] Data for measurement of transients are expressed as median and 25th and 75th percentile, and main effects were tested by univariate nonparametric Kruskal-Wallis analysis of variance by ranks (Prism 4; GraphPad Software, Inc., San Diego, CA) because the number of neurons in some groups was small. When a significant main effect was confirmed, planned *post hoc* comparisons were performed using the Dunn multiple comparisons test. Data for sequential measurements and for behavior testing are reported as mean ± SEM, and significance was tested by paired or two-sample *t* tests as appropriate. Differences between two groups were considered to be significant when *P* was less than 0.05.

## Results

*Behavioral Responses*

Dorsal root ganglia were removed for study 22 ± 2 days after injury in rats with hyperalgesia after SNL (n = 31). In these rats, hyperalgesia-type responses occurred in 36.6 ± 3.5% of needle applications. Postmortem examination confirmed accurate placement of ligatures and section for all SNL animals. Control rats (n = 27) did not develop hyperalgesic behavior (0 ± 0% hyperalgesic response rate; $P < 0.001$ *vs.* SNL), and DRGs were removed 20 ± 1 day after skin incision surgery.

*Potassium Activation*

Time controls showed stable repetition of transients induced by $K^+$ depolarization (fig. 2A). Specifically, a repeat depolarization produced a reproducible contour with consistent amplitude (the second transient decreased by 3 ± 1%; n = 17) and duration (time for 80% resolution of the transient, increased by 3 ± 2%) and no change in baseline offset in uninjured neurons. Similar experiments in injured neurons produced comparable results (data not shown). Elevation of $[Ca^{2+}]_c$ after $K^+$ depolarization requires inward $Ca^{2+}$ flux, as shown by the absence of a transient in a bath solution with nominally zero $[Ca^{2+}]$ (fig. 2B; 0 ± 0% of baseline response, n = 15 cells). This involves voltage-gated $Ca^{2+}$ channels as indicated by 88 ± 2% (n = 11) blockade by the selective blocker cadmium (200 $\mu$M; fig. 2C).

Cell diameter for small neurons (28.0 ± 0.3 $\mu$m) was not affected by injury, whereas diameters in the large cell groups were smaller in L5 after SNL (37.6 ± 1.0 $\mu$m) compared with SNL L4 and control neurons (46.0 ± 1.7 and 45.3 ± 1.3 $\mu$m; $P < 0.01$ for each). Injury had contrasting effects on the amplitude of $K^+$-induced transients in large and small neurons (table 1). Whereas amplitude was increased in large cells after axotomy, amplitudes decreased in the small cell group in axotomized neurons as well as in the adjacent L4 neurons after SNL. The transient was shortened in the axotomized SNL5 neurons of the small cell group. In the large cell group, there was a degree of inconsistency between measures of duration of the transient, such that duration was not changed, but the time to return to a stable baseline was decreased. The frequency of a plateau during the recovery of the transient was diminished in the SNL5 neurons. For those neurons lacking a plateau, the rate of recovery was accelerated in small SNL5 neurons, as indicated by a decreased $\tau$. For those neurons with a plateau, the level of the plateau was decreased in small SNL4 neurons. Control neurons show an increased $[Ca^{2+}]_c$ after full recovery of the transient. This offset was diminished in axotomized small neurons after SNL.

We separately analyzed responses to $K^+$ in a subset of neurons further characterized by their sensitivity to capsaicin as a means of distinguishing neurons with a thermal nociceptive modality (table 2). Cell diameter for capsaicin-sensitive neurons (28.9 ± 0.5 $\mu$m) was not affected by injury, whereas diameters in the capsaicin-insensitive groups were smaller in L5 after SNL (28.3 ± 0.6 $\mu$m) compared with SNL4 and control neurons (41.0 ± 2.1 and 36.8 ± 1.3 $\mu$m; $P < 0.01$ for each). The changes in sensitive and insensitive neurons largely duplicated the distinctions between small and large neurons described above. Specifically, insensitive neurons showed larger amplitude transients in the SNL5 group, whereas sensitive ones had lower amplitudes, and sensitive SNL4 neurons also had lower amplitudes. Durations of transients in both sensitive and insensitive neurons were shortened by axotomy, and the frequency of a plateau during the transient was decreased ay axotomy in sensitive neurons. Stratification of neurons by capsaicin response in addition revealed a longer transient duration in SNL4 neurons and an increased baseline shift after activation, both in contrast to SNL5 neurons.

Because the neuronal population included cells either with or without a plateau during resolution of the $K^+$-

**Table 1. Measures of Potassium-induced Transients in Neurons Categorized by Size**

| | | Small Neurons | | |
|---|---|---|---|---|
| | Main Effect *P* | Control | SNL4 | SNL5 |
| $R\Delta_{max}$, R.u. | < 0.001 | 1.74 (174) [1.32/2.51] | 1.26 (62) [0.92/1.78]*** | 1.37 (94) [0.68/2.46]** |
| $R\Delta_{max}$, % | 0.002 | 229 (174) [147/333] | 169 (62) [109/241]*** | 219 (94) [95/406]† |
| $T_{80}$, min | < 0.001 | 6.48 (174) [3.28/10] | 10 (61) [5.08/13] | 1.5 (93) [0.37/2.75]***††† |
| $T_{flat}$, min | < 0.001 | 5 (172) [6/10.91] | 8 (55) [5.75/11] | 3 (93) [1.65/4.6]***††† |
| $\tau$, s | 0.005 | 24.0 (11) [11.5/28.0] | 29.2 (6) [8.5/40.6] | 8.6 (35) [4.5/17.2]*† |
| $R\Delta_{pl}$, R.u. | 0.005 | 1.15 (161) [0.83/1.55] | 0.91 (55) [0.67/1.21]** | 1.07 (57) [0.59/1.49] |
| Plateau present | < 0.001 | 161/172, 94% | 55/61, 90% | 57/92, 62%***††† |
| $R_{BL}\Delta$, R.u. | < 0.001 | 0.22 (172) [0.13/0.325] | 0.25 (55) [0.13/0.38] | 0.05 (93) [0.02/0.08]***††† |

Values are expressed as median [25th/75th percentile], except presence of plateau, which is given as number of neurons with plateau/total, followed by percent. Number of cells in parentheses. Not every parameter could be determined for each neuron due to late detachment.

Different from control: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; different from SNL4: † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$.

$R_{BL}\Delta$ = shift in baseline; $R\Delta_{max}$ = transient amplitude, in R.u. and percent change; $R\Delta_{pl}$ = amplitude of plateau above baseline measure at plateau inflection; R.u. = ratio units of fluorescence at 340 nm excitation/fluorescence at 380 nm excitation; SNL4 = fourth lumbar ganglion after spinal nerve ligation; SNL5 = fifth lumbar ganglion after spinal nerve ligation; $\tau$ = time constant for neurons with exponential recovery (no plateau); $T_{80}$ = duration of transient to 80% recovery in neurons with transient plateau; $T_{flat}$ = time to recover to stable baseline.

induced transient, we examined whether this feature was associated with other properties in control neurons. Compared to neurons without a plateau (n = 30), neurons with a plateau (n = 181) have both a greater transient amplitude (1.91 ± 0.08 *vs.* 1.13 ± 0.12 ratio units [R.u.]; $P < 0.01$) and a greater duration (403 ± 16 *vs.* 161 ± 34 s; $P < 0.01$), as well as a greater baseline shift (0.24 ± 0.01 *vs.* 013 ± 0.03 R.u.; $P < 0.01$). Calcium entry across the membrane should be approximately proportionate to cell surface area, whereas $Ca^{2+}$ buffering and sequestration capacity should vary by cell volume. Therefore, we considered the hypothesis that large neurons are better able to accommodate $Ca^{2+}$ loads without participation of mitochondria and thus produce a transient without a plateau. In support of this view, we identified a smaller average diameter (29.9 ± 0.5 $\mu$m) in neurons with a plateau compared to neurons without a plateau (40.0 ± 1.8 $\mu$m; $P < 0.01$).

*Field Stimulation*

Time controls showed stable transients after repetitive field stimulation (fig. 2D). Specifically, a repeat depolarization produced a consistent amplitude (the second transient changed by 0 ± 2%; n = 11) and duration (increased by 5 ± 3%) in uninjured neurons. Similar experiments in injured neurons produced comparable results (data not shown). As with $K^+$ depolarization, elevation of $[Ca^{2+}]_c$ after field stimulation requires inward $Ca^{2+}$ flux, as shown by the absence of a transient in a bath solution with nominally zero $[Ca^{2+}]$ (fig. 2E; 0 ± 0% of baseline response, n = 13 cells), and involves voltage-gated $Ca^{2+}$ channels as indicated by 100 ± 0% (n = 13) blockade by cadmium (fig. 2F). We further examined whether responses during field stimulation are the result of generation of action potentials. Application of stimuli with incrementally greater voltage (fig. 3A) resulted in an essentially all-or-none generation of a tran-

**Table 2. Measures of Potassium-induced Transients in Neurons Categorized by Sensitivity to Capsaicin**

| | | Capsaicin-sensitive Neurons | | |
|---|---|---|---|---|
| | Main Effect *P* | Control | SNL4 | SNL5 |
| $R\Delta_{max}$, R.u. | < 0.001 | 1.82 (118) [1.39/2.56] | 1.29 (49) [1.00/1.81]** | 1.28 (19) [0.61/2.52]* |
| $R\Delta_{max}$, % | 0.002 | 244 (118) [182/350] | 175 (49) [118/246]** | 186 (19) [100/375] |
| $T_{80}$, min | < 0.001 | 7.2 (118) [3.48/10] | 10 (48) [6.7/11.03]** | 1.93 (19) [0.4/4.25]***††† |
| $T_{flat}$, min | < 0.001 | 9.5 (116) [7/10.09] | 9 (43) [6.75/11.45] | 3.75 (19) [1.8/7]***††† |
| $\tau$, s | 0.325 | 17.3 (5) | 8.5 (2) | 15.0 (7) [6.4/30.7] |
| $R\Delta_{pl}$, R.u. | 0.014 | 1.17 (113) [0.86/1.56] | 0.95 (47) [0.71/1.23]* | 0.89 (12) [0.56/1.81] |
| Plateau present | < 0.001 | 113/118, 96% | 47/49, 96% | 12/19, 63%***††† |
| $R_{BL}\Delta$, R.u. | < 0.001 | 0.23 (116) [0.13/0.33] | 0.32 (42) [0.21/0.42]* | 0.06 (19) [0.05/0.10]***††† |

Values are expressed as median [25th/75th percentile], except presence of plateau, which is given as number of neurons with plateau/total, followed by percent. Number of cells in parentheses. Not every parameter could be determined for each neuron due to late detachment.

Different from control: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; different from SNL4: † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$.

$R_{BL}\Delta$ = shift in baseline; $R\Delta_{max}$ = transient amplitude, in R.u. and percent change; $R\Delta_{pl}$ = amplitude of plateau above baseline measure at plateau inflection; R.u. = ratio units of fluorescence at 340 nm excitation/fluorescence at 380 nm excitation; SNL4 = fourth lumbar ganglion after spinal nerve ligation; SNL5 = fifth lumbar ganglion after spinal nerve ligation; $\tau$ = time constant for neurons with exponential recovery (no plateau); $T_{80}$ = duration of transient to 80% recovery in neurons with transient plateau; $T_{flat}$ = time to recover to stable baseline.

**Table 1. Continued**

| Large Neurons | | | |
|---|---|---|---|
| Main Effect *P* | Control | SNL4 | SNL5 |
| 0.003 | 0.84 (39) [0.60/1.32] | 0.95 (27) [0.54/1.30] | 1.64 (15) [1.10/2.14]**†† |
| 0.004 | 98 (39) [72/163] | 115 (27) [63/195] | 231 (15) [140/231]**†† |
| 0.001 | 2.43 (39) [1.27/5.6] | 7.73 (27) [2.73/10] | 1.53 (15) [0.75/3.02]†† |
| 0.004 | 5.5 (39) [3.37/7.15] | 6.81 (27) [3.18/10] | 2.72 (15) [1.9/4.55]**†† |
| 0.950 | 14.8 (20) [10.9/34.0] | 23.7 (12) [13.5/27.0] | 15.0 (3) |
| 0.328 | 0.41 (19) [0.26/1.04] | 0.71 (14) [0.46/0.98] | 0.90 (11) [0.58/1.04] |
| 0.016 | 19/39, 49% | 14/26, 54% | 11/14, 79% |
| 0.043 | 0.09 (39) [0.07/0.17] | 0.12 (26) [0.06/0.20] | 0.08 (15) [0.04/0.09]† |

sient upon reaching a critical voltage, as is expected if field stimulation acts through action potential generation. In addition, lidocaine block of voltage-gated $Na^+$ channels prevents $Ca^{2+}$ transients at a concentration that lacks toxic effects (fig. 3B; 0 ± 0% of baseline response, n = 15 cells), as indicated by recovery of the transient after lidocaine washout. Response to $K^+$ depolarization during lidocaine administration indicates that this concentration of lidocaine does not substantially block $Ca^{2+}$ channels. We conclude that field stimulation provokes $Ca^{2+}$ transients through the generation of neuronal action potentials.

Cell diameters for small neurons (27.2 ± 0.3 μm) and large neurons (40.1 ± 1.0 μm) were not affected by injury. Neuronal activation by field stimulation produced transients with plateaus less commonly than when neurons are activated by $K^+$ depolarization (table 3). In small cells, injury decreased transient amplitude in both axotomized L5 and adjacent L4 neurons and baseline offset in L5 neurons, as it did with $K^+$ depolarization. However, duration of transients decreased in small SNL L4 neurons stimulated by field, for both neurons with plateaus and those without plateaus. In contrast, no change was seen in the much longer transients produced by $K^+$ activation in this group (table 1), and $K^+$ transients were prolonged in SNL L4 neurons grouped by capsaicin sensitivity (table 2).

### *Potassium Depolarization of Neurons from Nonhyperalgesic Injured Rats*

To probe whether $Ca^{2+}$ transient changes after injury are the cause of altered behavior, we examined neurons from a small number of animals (n = 4) that lacked hyperalgesia-type responses to nociceptive sensory stimulation (0.0 ± 0.0% of needle applications) despite anatomically confirmed SNL injury. These were compared with transients in neurons from a concurrent group of rats (n = 4) not included in the results reported above, which demonstrated hyperalgesia (39.2 ± 9.3% of needle applications). The $K^+$ transients in small neurons from nonhyperalgesic animals showed features that are shifted toward the normal pattern in comparison to neurons from the hyperalgesic rats. Specifically, axotomized neurons of L5 from nonhyperalgesic rats have a higher transient amplitude (2.38 ± 0.16 R.u., n = 36) compared with L5 neurons from the hyperalgesic ani-

**Table 2. Continued**

| Capsaicin-insensitive Neurons | | | |
|---|---|---|---|
| Main Effect *P* | Control | SNL4 | SNL5 |
| < 0.001 | 1.05 (71) [0.81/1.55] | 0.74 (34) [0.50/1.22] | 1.55 (73) [0.73/2.55]††† |
| <0.001 | 135 (71) [93/198] | 93 (34) [62/156] | 250 (73) [95/426]**††† |
| < 0.001 | 5 (69) [2.08/10] | 7.03 (34) [2.03/10] | 1.6 (71) [0.45/2.55]***††† |
| < 0.001 | 7.15 (68) [4.83/10] | 5.65 (34) [2/10] | 3 (71) [1.76/5]***†† |
| 0.005 | 16.0 (24) [10.2/34.0] | 23.8 (14) [13.5/29.0] | 9.32 (26) [4.52/17.2]*† |
| 0.006 | 0.78 (47) [0.43/1.14] | 0.56 (18) [0.43/0.88] | 1.05 (45) [0.57/1.45]†† |
| 0.6 | 47/71, 66% | 18/32, 56% | 45/71, 63% |
| < 0.001 | 0.13 (68) [0.08/0.2] | 0.11 (34) [0.06/0.17] | 0.05 (71) [0.03/0.08]***††† |

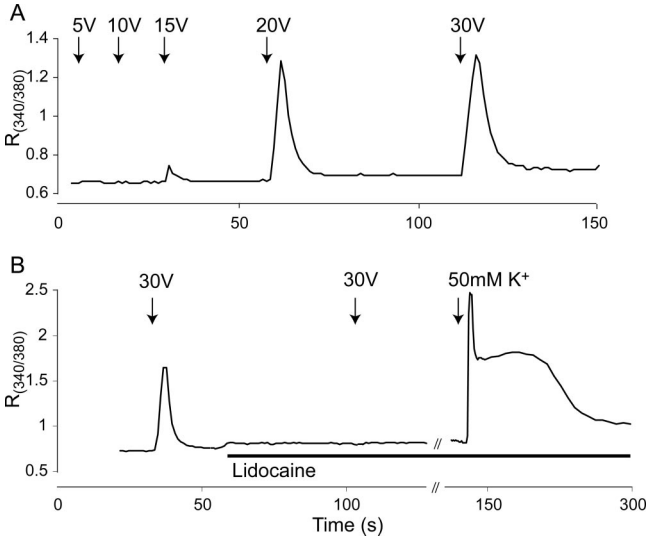


**Fig. 3. Field stimulation evokes $Ca^{2+}$ transients through generation of action potentials. (*A*) Field stimulation (2-ms pulses at 10 Hz) produces $R_{340/380}$ transients in dorsal root ganglion neurons in an all-or-none fashion, indicated by a threshold for eliciting transients with incrementally increasing stimulation voltage (5, 10, 15, 20, 30 V; n = 10). (*B*) Lidocaine (1 mM) reversibly abolishes field stimulation-evoked $R_{340/380}$ transients but does not block $K^+$-induced $R_{340/380}$ transients (note different *time scales*; n = 15).**

mals (1.79 ± 0.13 R.u., n = 39; $P < 0.01$). Neurons from nonhyperalgesic animals had a greater area (0.91 ± 0.12 R.u.) than neurons from animals that developed hyperalgesia after SNL (0.59 ± 0.07 R.u.; $P < 0.05$). Also, nonhyperalgesic animals' L5 neurons have a greater baseline offset after SNL (0.07 ± 0.01 R.u.) compared with L5 neurons from hyperalgesic animals (0.03 ± 0.00 R.u.; $P < 0.01$). In the L4 population, neurons from nonhyperalgesic rats had a shorter transient duration to 80% resolution (61 ± 5 s, n = 53) compared with neurons from hyperalgesic rats (83 ± 7 s, n = 52; $P = 0.01$).

### *Regulation of Transient by $Ca^{2+}$ Flux*

We next examined whether variations of $Ca^{2+}$ load may be a factor accounting for differences in transient measures after injury, using several techniques to alter the inward $Ca^{2+}$ flux during activation of uninjured neurons (table 4). With both field and $K^+$ activation, increased bath [$Ca^{2+}$] (fig. 4) elevated the transient peak amplitude, prolonged the transient, and increased the offset of the resting baseline. Reciprocal changes were seen when bath [$Ca^{2+}$] was lowered, and findings were comparable with both types of neuronal activation. Increasing $Ca^{2+}$ influx by prolonging $K^+$ depolarization to 15 or 30 s (table 4 and figs. 5A and B) substantially increased transient durations, without increasing transient amplitude. The elevation of baseline after the transient was greater after 15 s depolarization compared with 5 s, but did not further increase with 30 s depolarization. The transient amplitude was not affected by prolongation of depolarization beyond 5 s. Finally, increasing the duration of field stimulation with a constant rate (10 Hz; fig. 5C) showed increase of the transient amplitude (1.1 ± 0.3 au for 1 s stimulation, 1.8 ± 0.3 au for 3 s, 1.8 ± 0.3 au for 5 s, n = 8; $P < 0.05$), duration (9.8 ± 1.9 s for 1 s stimulation, 16.4 ± 4.0 s for 3 s, 48.3 ± 13.8 s for 5 s, n = 8; $P < 0.05$), and baseline offset (0.03 ± 0.02 au for 1 s stimulation, 0.05 ± 0.02 au for 3 s, 0.13 ± 0.03 au for 5 s, n = 8; $P < 0.05$). These relations were not altered by changing the sequence of stimulus durations.

### *Regulation of Transient by Intracellular $Ca^{2+}$ Stores*

The $Ca^{2+}$ detected by fura-2 during the activation-induced transient could consist of that which entered through the plasma membrane or $Ca^{2+}$ released from intracellular stores through the process of $Ca^{2+}$-induced $Ca^{2+}$ release (CICR).[22,23] To measure the contribution of

**Table 3. Measures of Electrical Field Stimulation-induced Transients in Neurons Categorized by Size**

| | | Small Neurons | | |
|---|---|---|---|---|
| | Main Effect *P* | Control | SNL4 | SNL5 |
| $R\Delta_{max}$, R.u. | < 0.001 | 0.83 (328) [0.45/1.35] | 0.54 (113) [0.33/1.05]*** | 0.67 (160) [0.30/1.13]** |
| $R\Delta_{max}$, % | < 0.001 | 108 (328) [54/169] | 59 (113) [36/130]*** | 96 (160) [45/174]† |
| $T_{80}$, min | < 0.001 | 0.22 (290) [0.15/0.6] | 0.15 (99) [0.11/0.3]*** | 0.23 (155) [0.18/0.35] |
| $T_{flat}$, min | 0.05 | 0.7 (287) [0.42/1.33] | 0.57 (104) [0.33/1]* | 0.67 (155) [0.37/1.3] |
| $\tau$, s | 0.003 | 4.0 (226) [2.5/6.4] | 2.9 (84) [2.1/5.1]** | 3.6 (132) [2.2/5.5] |
| $R\Delta_{pl}$, R.u. | 0.361 | 0.75 (67) [0.49/1.27] | 0.56 (24) [0.32/1.15] | 0.68 (25) [0.46/0.95] |
| Plateau present | 0.111 | 78/310, 25% | 25/105, 24% | 26/156, 17% |
| $R_{BL}\Delta$, R.u. | < 0.001 | 0.06 (290) [0.03/0.10] | 0.04 (104) [0.02/0.09] | 0.02 (155) [0.00/0.04]***††† |

Values are expressed as median [25th/75th percentile], except presence of plateau, which is given as number of neurons with plateau/total, followed by percent. Number of cells in parentheses. Not every parameter could be determined for each neuron due to late detachment.

Different from control: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; different from SNL4: † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$.

$R_{BL}\Delta$ = shift in baseline; $R\Delta_{max}$ = transient amplitude, in R.u. and percent change; $R\Delta_{pl}$ = amplitude of plateau above baseline measure at plateau inflection; R.u. = ratio units of fluorescence at 340 nm excitation/fluorescence at 380 nm excitation; SNL4 = fourth lumbar ganglion after spinal nerve ligation; SNL5 = fifth lumbar ganglion after spinal nerve ligation; $\tau$ = time constant for neurons with exponential recovery (no plateau); $T_{80}$ = duration of transient to 80% recovery in neurons with transient plateau; $T_{flat}$ = time to recover to stable baseline.

CICR to the $Ca^{2+}$ transient after $K^+$ depolarization, we treated 17 neurons (3 large, 14 small, with comparable results) with ryanodine (10 $\mu$M; fig. 6A). In these neurons, the transient amplitude was decreased by 11 ± 4% ($P < 0.05$), which confirms a minor role for CICR in generating the transient peak. However, we found no changes of the transient duration (5 ± 11%; $P = 0.48$), transient area (−11 ± 9%; $P = 0.16$), or the baseline offset (−0.03 ± 0.02; $P = 0.10$) after blocking CICR by ryanodine.

We also examined whether the failure of the $[Ca^{2+}]_c$ to return to the original baseline level after activation by high $K^+$ might be due to overload of intracellular $Ca^{2+}$ stores. Accordingly, after recovery from activation by $K^+$ depolarization, we exposed control neurons (n = 2 large, 22 small) to caffeine (20 $\mu$M) to release intracellular stored $Ca^{2+}$ (fig. 6B). This resulted in significant ($P < 0.01$) but partial recovery of the baseline toward the original resting $[Ca^{2+}]_c$ level, such that 70 ± 8% of the baseline offset remained. This indicates that only a minor portion of the offset can be attributed to the effect of high $Ca^{2+}$ load in intracellular stores.

## Discussion

In this study, we have identified significant disruption of $Ca^{2+}$ signaling in sensory neurons injured by painful peripheral nerve trauma, summarized by characteristic traces in figure 7. We believe these changes are causally related to hyperalgesia because neurons from animals that do not develop hyperalgesia after SNL have transients distinct from those isolated from hyperalgesic rats. Substantially altered amplitude, duration, and posttransient resting level of $[Ca^{2+}]_c$ evolve after injury. Axotomy-induced changes in the L5 ganglion after SNL contrast with those in neighboring neurons of the L4 DRG that share distal sciatic nerve fascicles with degenerating L5 fibers, which induces inflammation and cytokine production for many weeks.[14,24] These divergent responses indicate distinct mechanisms in these two types of injury. Such effects are clearly intrinsic to the sensory neuron as they survive dissociation. Our study does not allow identification of additional influences that may result from local nonneural tissue damage at the time of SNL injury, although these should be largely resolved by the time of tissue harvest.

Spinal nerve ligation produces distinct effects on $Ca^{2+}$ signaling for neurons of different sensory modality, indicating dissimilar influences of axotomy (L5) and events triggered by inflammation (L4) on inward $Ca^{2+}$ flux or the storage, release, and extrusion processes that shape the $Ca^{2+}$ transient. Presumed nociceptive neurons show largely comparable responses to injury whether characterized by small size or by sensitivity to capsaicin. We do note, however, that capsaicin-sensitive L4 neurons develop a prolonged transient duration when activated by $K^+$, but small L4 neurons show a shortened transient duration when activated by field stimulation. This dissimilarity may be due to the difference in $Ca^{2+}$ load by these two techniques. Specifically, field stimulation that produces 30 APs will depolarize voltage-gated $Ca^{2+}$ channels for a cumulative duration of approximately 60 ms (assuming 2-ms AP duration), which is much shorter than the 5-s duration of high $K^+$ depolarization in our protocol.

A critical factor shaping the $Ca^{2+}$ transient is the magnitude of inward $Ca^{2+}$ flux across the plasma membrane that is then presented to the various buffers and pumps that regulate $[Ca^{2+}]_c$. Specifically, previous research has shown that transmembrane $Ca^{2+}$ flux controls transient amplitude,[8,25] duration,[8,26] and generation of sustained plateaus.[18,27] This is also evident in our study. First, field

**Table 3. Continued**

| Large Neurons | | | |
|---|---|---|---|
| Main Effect *P* | Control | SNL4 | SNL5 |
| 0.920 | 0.70 (47) [0.27/1.83] | 0.63 (25) [0.29/1.42] | 0.52 (17) [0.27/1.56] |
| 0.793 | 87 (47) [38/207] | 78 (25) [30/193] | 74 (17) [45/264] |
| 0.064 | 1.45 (37) [0.2/4.5] | 0.25 (19) [0.15/0.77] | 0.6 (16) [0.17/2.73] |
| 0.930 | 1.12 (37) [0.5/2.15] | 1.2 (23) [0.62/2.37] | 0.73 (17) [0.45/3.15] |
| 0.950 | 10.2 (36) [3.4/19.3] | 4.3 (16) [2.7/8.35] | 8.0 (15) [2.3/61.1] |
| 0.43 | 1.22 (3) | 0.95 (7) [0.37/2.04] | 0.44 (2) |
| 0.05 | 3/40, 8% | 7/23, 30%* | 2/17, 12% |
| 0.293 | 0.11 (39) [0.04/0.35] | 0.05 (23) [0.02/0.29] | 0.03 (17) [0.02/0.20] |

**Table 4. Changes in Transient Measures Induced by Altered Bath $Ca^{2+}$ Concentrations or Duration of Stimulation**

| Activation: | Field Stimulation | | $K^+$ | | $K^+$ | |
|---|---|---|---|---|---|---|
| Duration: | 3 s, 10 Hz | 3 s, 10 Hz | 5 s | 5 s | 5→15 s | 15→30 s |
| Bath $Ca^{2+}$: | 1→4 mM | 4→1 mM | 1→4 mM | 4→1 mM | 2 mM | 2 mM |
| n: | 13 | 6 | 14 | 13 | 11 | 11 |
| $R\Delta_{max}$, % change | 49 ± 20** | −50 ± 7* | 13 ± 6* | −26 ± 5*** | 1 ± 2 | 7 ± 1 |
| $T_{80}$, % change | 274 ± 66** | −59 ± 2** | 161 ± 27*** | −52 ± 8** | 396 ± 86*** | 121 ± 9*** |
| $R_{BL}\Delta$, % change | 134 ± 18*** | −157 ± 70** | 158 ± 88* | −51 ± 10*** | 261 ± 40*** | −4 ± 19 |

Values are mean ± SEM percent change.

Significant change: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

n = number of cells; $R_{BL}\Delta$ = shift in baseline; $R\Delta_{max}$ = transient amplitude; $T_{80}$ = duration of transient to 80% recovery.

stimulation generates transients with lower amplitude, duration, and frequency of plateau compared with $K^+$ depolarization. Further, our experiments varying cytoplasmic $Ca^{2+}$ load through different extracellular bath $Ca^{2+}$ concentrations, duration of $K^+$ depolarization, and number of APs during field stimulation confirm the role of net $Ca^{2+}$ influx in regulating these transient parameters.

After peripheral nerve injury, axotomized neurons show an increased AP duration,[7] which would be expected to admit more $Ca^{2+}$ into the cell. However, we have also demonstrated a depression of $Ca^{2+}$ channel function after axotomy.[11] It seems that the dominant effect of axotomy is a diminished inward $Ca^{2+}$ flux, as our current findings show decreased duration and amplitude of the $Ca^{2+}$ transient in L5 neurons after SNL (fig. 7). However, changes in $Ca^{2+}$ load cannot explain all of our findings, *e.g.*, a faster resolution of transients that lack a plateau in capsaicin-insensitive axotomized neurons despite a larger amplitude, and a lack of correlation of $\tau$ with amplitude (data not shown).[28,29] This indicates that injury alters intracellular processes that concurrently regulate the $Ca^{2+}$ transient. In other studies, duration has been shown to be controlled by mitochondrial $Ca^{2+}$ uptake after large $Ca^{2+}$ loads,[30] whereas small loads are resolved in part by the plasma membrane $Ca^{2+}$ adenosine triphosphatase,[29,31] particularly in nociceptors.[32,33] The plasmalemmal $Na^+$–$Ca^{2+}$ exchanger may also extrude $Ca^{2+}$ from the cell,[34] and the sarco-endoplasmic reticulum $Ca^{2+}$ adenosine triphosphatase clears cytoplasmic $Ca^{2+}$ in large neurons by storage in the endoplasmic reticulum.[28] Transient amplitude is limited by $Ca^{2+}$ uptake into the mitochondria[8,30] and into the endoplasmic reticulum by the action of the sarco-endoplasmic reticulum $Ca^{2+}$ adenosine triphosphatase,[28] whereas amplitude is magnified by release of $Ca^{2+}$ from the endoplasmic reticulum triggered by rising $[Ca^{2+}]_c$ ($Ca^{2+}$-induced $Ca^{2+}$ release).[25,32] Differences in the influence of injury on these complex mechanisms may cause increased amplitude after axotomy in presumed nonnociceptive neurons but decreased amplitude in nociceptive neurons.

The level of the sustained plateau is not a function of $Ca^{2+}$ uptake and release from the endoplasmic reticulum,[35] but rather is set by mitochondrial $Ca^{2+}$ release,[8] through the interactions of intramitochondrial $Ca^{2+}$ concentration and mitochondrial uptake and release pathways.[26] We noted an increase in plateau level in presumed nonnociceptive L5 neurons after SNL, as well as a decrease in plateau level in nociceptors of the L4 group,

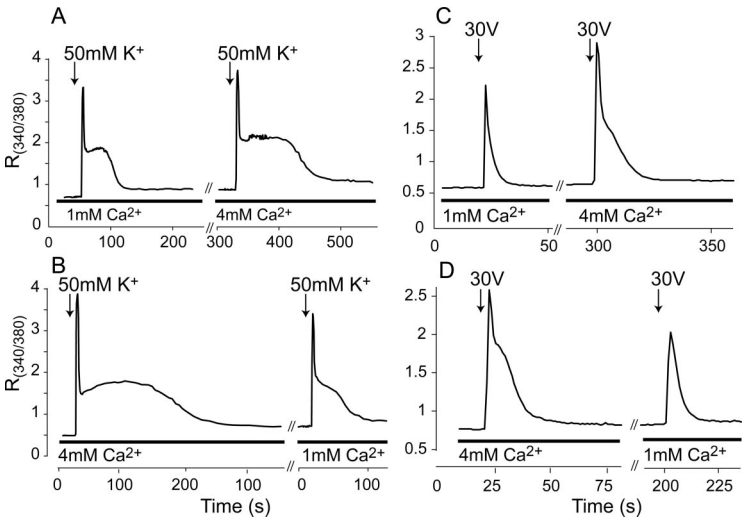


**Fig. 4. Altering neuronal $Ca^{2+}$ load by changing bath $Ca^{2+}$ concentration controls dimensions of $Ca^{2+}$ transients indicated by fluorescence ratio ($R_{340/380}$). During $K^+$ depolarization of dissociated dorsal root ganglion neurons, increasing the extracellular $Ca^{2+}$ concentration from 1 to 4 mM (n = 14) elevates transient peak amplitude, prolongs the transient, and increases the offset of the posttransient baseline (*A*), whereas decreasing bath $Ca^{2+}$ concentration from 4 mM to 1 mM (n = 13) produces reciprocal effects (*B*). Increasing (*C*; n = 13) and decreasing (*D*; n = 6) bath $Ca^{2+}$ concentration has similar effects during neuronal activation by field stimulation.**

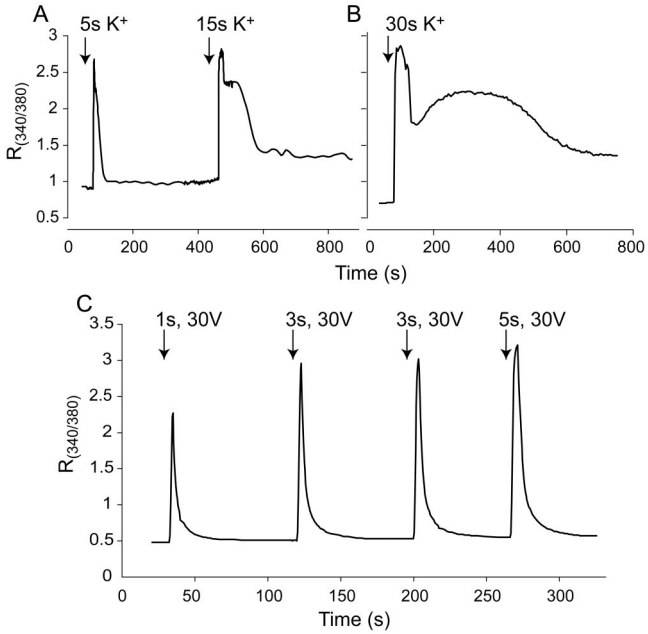


**Fig. 5. (*A* and *B*) Altering the neuronal $Ca^{2+}$ load by changing the duration of $K^+$ depolarization controls dimensions of $Ca^{2+}$ transients indicated by fluorescence ratio ($R_{340/380}$). Higher $Ca^{2+}$ influx produced by prolonging $K^+$ depolarization (5,15, and 30 s) increases transient duration without increasing transient amplitude. The level of the offset did not further increase beyond 15 s of $K^+$ stimulation. The representative trace in *A* is from a different neuron than in *B*. (*C*) Altering neuronal $Ca^{2+}$ load by changing the number of action potentials during field stimulation controls dimensions of $Ca^{2+}$ transients. Increasing the duration of field stimulation impulse trains (2-ms pulses at 30 V and 10 Hz) from 1 to 5 s prolonged the transient duration. Peak amplitude, however, showed no further increase after reaching a neuron-specific maximum (n = 15).**

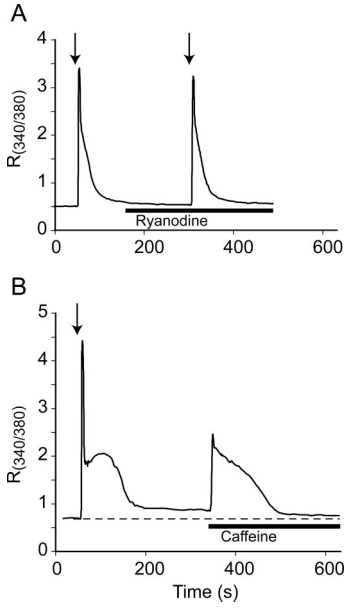


**Fig. 6. (*A*) Blockade of $Ca^2$-induced $Ca^{2+}$ release from intracellular stores with ryanodine (10 $\mu$M, n = 17 neurons) minimally diminishes dimensions of the $Ca^{2+}$ transient (indicated by fluorescence ratio $R_{340/380}$) induced by $K^+$ depolarization (50 mM; *arrows*). (*B*) Overload of $Ca^{2+}$ stores only partially accounts for the sustained offset of the baseline $R_{(340/380)}$ after neuronal activation with $K^+$ depolarization (*arrow*). Caffeine application (20 $\mu$M, n = 24 neurons) transiently elevates cytoplasmic $Ca^{2+}$ concentration but does not return the resting level back to the original baseline (*dotted line*).**

which indicates contrasting and modality-specific influences triggered by axotomy and inflammation on mitochondrial function.

The upward shift in resting $[Ca^{2+}]_c$ after recovery of the $Ca^{2+}$ transient of control neurons is nearly eliminated by axotomy (SNL L5) of both capsaicin-sensitive and -insensitive neurons, whereas this shift is amplified in sensitive neurons of the L4 ganglion. This offset after the transient has not previously been quantified and indicates a prolonged memory-like residual after activation of sensory neurons. We have previously shown that resting $[Ca^{2+}]_c$ is particularly depressed in L5 neurons after SNL,[12] perhaps because of the lack of postactivation offset. The resting $[Ca^{2+}]_c$ is regulated by the balanced operation of endoplasmic reticulum $Ca^{2+}$ release, plasma membrane $Ca^{2+}$ adenosine triphosphatase extrusion, and $Ca^{2+}$ influx through channels sensitive to intracellular $Ca^{2+}$ stores (store-operated $Ca^{2+}$ channels),[27] indicating effects on these processes specific to injury and neuronal types.

We conclude that peripheral nerve trauma causes substantial disturbances in stimulus-evoked $Ca^{2+}$ transients of DRG neurons that are injury and neuronal modality specific. This may be in part due to loss of $Ca^{2+}$ influx through voltage-gated $Ca^{2+}$ channels. Although numerous cellular functions may be affected by the injury-induced changes we have identified, three main consequences may directly alter neuronal activity in sensory pathways. First, DRG neuron secretion[36] and synaptic neurotransmitter release[37] are enhanced by increases in $[Ca^{2+}]_c$, such that the diminished duration of $[Ca^{2+}]_c$ we

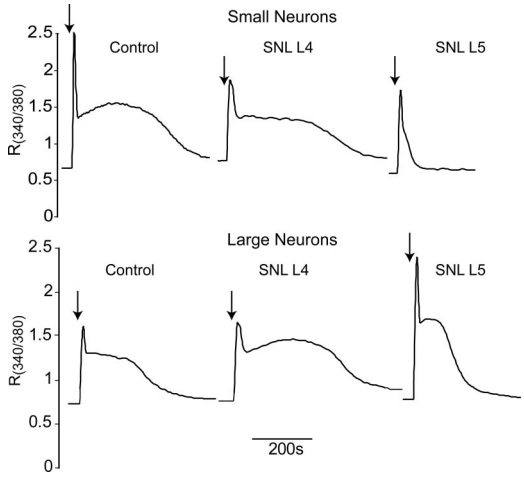


**Fig. 7. Examples of $Ca^{2+}$ transients indicated by fluorescence ratio ($R_{340/380}$), summarizing injury-induced alterations. *Arrows* indicate depolarization by $K^+$ (50 mM). The *top three panels* compare traces for small neurons from the fourth (L4) and fifth (L5) lumbar dorsal root ganglia after spinal nerve ligation (SNL) to a trace from a small control neuron. The *lower three panels* show traces for large neurons.**

have shown in axotomized neurons will result in diminished release, but the prolonged transient and elevated posttransient baseline in small and capsaicin-sensitive DRG neurons may lead to amplified synaptic transmission. Second, inward current through $Ca^{2+}$-activated $K^+$ channels, which set the level for neuronal excitability,[38] is regulated by $[Ca^{2+}]_c$. Our current findings of elevated amplitude and transient plateau in large, capsaicin-insensitive neurons may explain our electrophysiologic observations of decreased repetitive firing of fast conducting Aβ L5 neurons after SNL, whereas decreased transient amplitude and plateau in small, capsaicin-sensitive neurons (presumed nociceptors) may account for increased repetitive firing in Aδ neurons.[7] Finally, kinases sensitive to $[Ca^{2+}]_c$, such as $Ca^{2+}$/calmodulin-dependent protein kinase II, have diverse protein targets that modulate neuronal activity.[39] The observations in this study form a basis for future research determining the influence of injury on the interacting mechanisms that regulate $[Ca^{2+}]_c$ in sensory neurons and their contribution to neuropathic pain.

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