CUTANEOUS AND COLONIC RAT DRG NEURONS DIFFER WITH RESPECT TO BOTH BASELINE AND PGE$_2$-INDUCED CHANGES IN PASSIVE AND ACTIVE ELECTROPHYSIOLOGICAL PROPERTIES

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Running Head: Excitability of Cutaneous and Colonic DRG neurons

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Abstract:

The present study was designed to test the hypotheses that pain syndromes associated with specific body regions reflect unique properties of sensory neurons innervating these regions and/or unique responses of these afferents to tissue damage. Acutely dissociated adult rat DRG neurons retrogradely labeled from either the colon or the glabrous skin of the hindpaw were studied by whole-cell patch clamp recording in current clamp mode. Two populations of colonic afferent neurons were studied: pelvic afferents (arising from L6, S1 and S2 DRG = LS DRG) and hypogastric/lumbar colonic afferents (arising from T13, L1 and L2 DRG = TL DRG). Passive and active electrophysiological properties were studied before and after application prostaglandin E₂ (PGE₂). We observed marked differences between cutaneous and colonic sensory neurons with respect to baseline passive and active electrophysiological properties as well as both the magnitude and pattern of PGE₂-induced changes in excitability, passive and active properties. There were also significant differences between TL and LS neurons with respect to baseline and PGE₂-induced changes in several passive and active electrophysiological properties. Our results suggest that differences between cutaneous and colonic neurons reflect differences in pattern and/or density of ionic currents present in the plasma membrane. More interestingly, the ionic currents underlying the PGE₂-induced sensitization of cutaneous neurons appeared to differ from those underlying the sensitization of colonic neurons. The implication of this observation is that it may be possible, in fact necessary, to treat pain arising from specific body regions with unique therapeutic interventions.
Introduction:

There are a number of pain syndromes such as inflammatory bowel disease (IBD), temporomandibular joint disorder (TMD) and migraine, that are associated with pain arising from specific areas of the body. The basis for this site specificity is largely unknown. One possibility, however is that the restricted distribution of these pain syndromes reflects unique properties of the sensory neurons innervating these body regions.

Consistent with this suggestion, there is evidence that the properties of afferents innervating hollow organ structures are unique relative to those innervating somatic tissue. For example, there are both high and low threshold C- and Aδ-fiber afferents in the pelvic nerve that innervate the colon, yet both of these populations of afferents appear to be nociceptive in that they encode increasing stimulus intensity well into the noxious range and demonstrate sensitization in response to injury (Sengupta and Gebhart 1994; Su et al. 1997). In contrast, C-fibers innervating somatic tissue tend to have a high threshold for activation. While afferent activation thresholds depend on the properties of the innervated tissue (Cooper 1993), there is also evidence that differences between visceral and somatic afferents reflect intrinsic properties of afferent subpopulations (Berkley et al. 1993). However, this issue has not been systematically investigated in DRG neurons.

The excitability of nociceptive afferents may be dynamically regulated. This is particularly true in the presence of inflammation, where the excitability of nociceptive afferents increases (Davis et al. 1993; Sengupta et al. 1996). Inflammation-induced sensitization of afferents is also likely to reflect changes in properties intrinsic to the sensory neurons. Indeed, we (Gold et al. 1996), and others (Baccaglini and Hogan 1983; Nicol and Cui 1994) have previously demonstrated that isolated DRG neurons in vitro may be sensitized following the
application of the inflammatory mediator prostaglandin E₂ (PGE₂). Interestingly, there was significant variability in both the magnitude and the pattern of PGE₂-induced changes in neuronal excitability (Gold et al. 1996). The basis for this variability has yet to be investigated, but if it reflects differences between afferent subpopulations defined by target of innervation, than another explanation for site-specific pain syndromes may be that there are differences in the response to injury between populations of afferents based on target of innervation.

We hypothesize that differences in the expression of pain syndromes in various parts of the body reflect, in part, unique properties of the afferents innervating different structures. Furthermore, we hypothesize that these unique properties influence excitability of afferents innervating naïve tissue as well as inflammation-induced changes in the excitability of these afferents. We have performed the present study in order to begin to test these hypotheses. Specifically, we have recorded from isolated sensory neurons retrogradely labeled from either the glabrous skin of the hindpaw or the distal colon. Because the colon receives innervation from two spinal nerves, the pelvic and the hypogastric/lumbar colonic, we recorded from two distinct populations of colonic afferents. We compared passive and active electrophysiological properties as well as changes in these properties in response to the prototypical inflammatory mediator, prostaglandin E₂.

**Methods:**

Adult (175-300) male Sprague Dawley rats (Harlan Sprague Dawley) were used for this study. Rats were housed in the University of Maryland Dental School Animal Facility in groups of 3 prior to colonic labeling and then individually thereafter. Food and water was available ad
libitum. All experiments were approved by the University of Maryland Dental School Institutional Animal Care and Use Committee.

*Identification of DRG neurons innervating the colon and glabrous skin of the hindpaw: Colonic* DRG neurons were identified by the retrograde administration of DiI into the descending colon. Labeling was performed as described previously (Gold et al. 2002). Briefly, rats were anesthetized with Na-pentobarbital (50 mg/kg IP). The descending colon was exposed by a midline laparotomy and 20 μl DiI (2.5% in methanol) was injected at 10-15 sites into the colon wall with the aid of a dissecting microscope. DiI that visibly leaked from the injection site was wiped away with cotton swabs. The surgical wound was sutured in layers and rats were allowed to recover from anesthesia. Neurons were studied 14 to 21 days after labeling. Cutaneous neurons innervating glabrous skin of the hindpaw were also labeled with DiI, except that the dye was first dissolved in DMSO (170 mg/ml) and then diluted 1:10 in 0.9% sterile saline. Five μl of this solution was injected sub-cutaneously with a 30g injection needle directed into the epithelium, over an area ~5 mm². DiI labeled neurons were easily identified under epifluorescence illumination with a Texas-red/rhodamine filter set.

*Cell dissociation:* The colon receives innervation from two spinal nerves: the pelvic and the hypogastric/lumbar colonic. These nerves arise from lumbosacral (LS: L6-S2) and thoracolumbar (TL: T12-L2) DRG, respectively. The site of injection in the hindpaw receives innervation via the sciatic nerve, which is comprised primarily of axons arising from L4 and L5 DRG. DRG neurons were prepared for recording as described previously (Gold et al. 2002). Briefly, rats were deeply anesthetized with a subcutaneous injection of rat cocktail (1 ml/kg of 55 mg/ml ketamine, 5.5 mg/ml xylazine and 1.1 mg/ml acepromazine); TL and LS or L4 and L5 DRG were removed, and rats were subsequently killed by decapitation. DRG were desheathed in
Ice-cold MEM-BS composed of: 90% minimal-essential-medium (MEM; Gibco BRL, Gaithersburg, MD), 10% heat-inactivated fetal bovine serum (BS), and 1000 units per ml each of penicillin and streptomycin. DRGs were then incubated 45 min at 37°C in 5 ml MEM, to which collagenase P (Roche Bioscience, Palo Alto, CA) had been added to a final concentration of 0.125% and bubbled with carbogen (95% O₂ / 5% CO₂). DRGs were then incubated 5 min at 37°C in Ca²⁺- and Mg²⁺-free Hanks balanced salt solution (GIBCO, BRL) containing 0.25% trypsin (Worthington, Bristol, UK) and 0.025% EDTA (Sigma, St Louis, MO). Trypsin activity was inhibited by the addition of MEM-BS containing 0.125% MgSO₄, and DRG were dissociated by trituration with a fire-polished Pasteur pipette. DRG cells were plated onto glass cover slips, previously coated by a solution of 5 µg/ml mouse laminin (GIBCO BRL) and 0.1 mg/ml poly-L-ornithine (Sigma). The cells were incubated in MEM-BS at 37°C, 3% CO₂ and 90% humidity for 2 hours at which point they were transferred to a HEPES buffered L-15 media containing 10% BS and 5 mM glucose and stored at room temperature. TL and LS DRG were processed in parallel. Colonic and cutaneous neurons were isolated from different rats and therefore studied on different days. All neurons were studied between 2 and 7 hours after removal from the animal.

**Electrophysiology:** Current-clamp recordings were performed using a HEKA EPC9 (HEKA Electonik., Lambrecht/Pfaz Germany). Data were low-pass filtered at 5-10 kHz with a 4-pole Bessel filter and digitally sampled at 25-100 kHz. For voltage clamp protocols, capacity transients were cancelled and series resistance was compensated (>80%). Electrodes (0.7-3 MΩ) were filled with (in mM): 140 K-Methansulphonate, 5 NaCl, 1 CaCl₂, 2 MgCl₂, 11 EGTA, 10 HEPES, 2 Mg-ATP, 1 Li-GTP; pH was adjusted to 7.2 with TRIS-base, osmolality was adjusted to 310 mOsm with sucrose. Bath solution contained (in mM): 140 NaCl, 3 KCl, 2.5 CaCl₂, 0.6
MgCl₂, 10 HEPES, 10 glucose, pH adjusted to 7.4 with Tris-Base, osmolality adjusted to 325 mOsm with sucrose. All salts were obtained from Sigma (St. Louis, MO). Sucrose was obtained from Gibco (Gaithersburg, MD).

**Experimental protocol:** After formation of a tight seal (> 5 GΩ) and compensation of pipette capacitance with amplifier circuitry, whole cell access was established. Cell capacitance was determined with four hyperpolarizing pulses (10 ms) from -60 mV to -80 mV.

Passive electrophysiological properties (resting membrane potential and input resistance) were determined within 2 minutes of establishing whole cell access. Input resistance was determined by assessing current evoked in response to a 10 ms 20 mV hyperpolarizing voltage step from −70 mV. The presence of spontaneous activity was then assessed by observing the neuron under current clamp for 1 min, after which active electrophysiological properties were determined.

In order to assess properties of the action potential waveform (rate of rise and decay, duration, overshoot, afterhyperpolarization magnitude and duration) a single action potential was evoked with depolarizing current injection (4 ms duration rectangular pulse at threshold intensity) (Figure 1). A 500 ms depolarizing current injection was used to assess neuronal excitability (action potential threshold, rheobase and adaptation). Action potential threshold was defined as the maximum depolarization obtained in the absence of an action potential. Rheobase was defined the minimum depolarizing current injection necessary to evoke an action potential (Figure 1). The total number of spikes evoked at threshold was used as a measure of adaptation. Finally, the response to suprathreshold stimuli was assessed by stimulating neurons with depolarizing current inject equal to 1.5, 2, 2.5 and 3 times rheobase (Figure 1). In order to control for the effect of membrane potential on the availability of voltage-gated ion channels and
therefore the secondary influence of membrane potential on active properties, current injection was used to hold neurons between at –55 mV and –60 mV.

At least 3 min of baseline excitability data was collected for each neuron prior to the bath application of PGE₂ (1 μM). Baseline excitability was assessed in 1 of two ways: With “square-wave” or ramp stimuli. The square-wave stimuli were the same as those used to establish action potential threshold and rheobase, except 4 to 6 stimuli were applied during each stimulation series in 1, 5 or 10 pA increments (depending on whether rheobase was <10, <50 or >50 pA, respectively), starting 2 increments below rheobase originally determined. This set of stimuli was applied once per minute over a period of at least three minutes, in order to establish baseline excitability. The ramp and hold stimulus consisted of a 250 ms ramp to an amplitude that was held for an additional 500 ms. The amplitude of the stimulus was adjusted so that an action potential was evoked towards the end of the ramp. Because the latency to the action potential is a measure of both the action potential threshold and rheobase, and subsequent action potentials are a measure of accommodation (Gold et al. 1996), a single protocol could be used to assess several aspects of excitability. After establishing the baseline action potential threshold, rheobase and the response to suprathreshold stimuli with “square-wave” stimuli, the ramp stimulus was used to monitor stability over a period of at least 3 minutes with stimuli applied with an inter-stimulus-interval (ISI) of at least 1-min. The effects of PGE₂ were only assessed in neurons demonstrating stable baseline excitability. Results obtained with both “square-wave” and “ramp and hold” stimuli were comparable. A neuron was considered sensitized by PGE₂ if there was a decrease in threshold or rheobase, or an increase in spike number at least two standard deviations from baseline mean. The effects of PGE₂ were analyzed relative to results obtained with the first application of the square-wave stimulus, either as a difference (i.e., After – Before) or as a
percent of baseline (i.e., (After/Before)\*100). PGE_{2} vehicle (0.1% ethanol in bath solution) had no effect on neuronal excitability in 5 neurons subsequently sensitized by PGE_{2} (data not shown).

The presence of the hyperpolarization activated current I_{h} was assessed in a subpopulation of neurons following completion of current clamp experiments. The amplifier was switched back to voltage-clamp mode. Series resistance compensation (>80%) and whole capacitance compensation was employed via amplifier circuitry. Neurons were held at –60 mV. The presence of I_{h} was assessed with a 500 ms voltage step to –100 mV following a 500 ms pre-pulse to –50 mV. Inward current that developed over the 500 ms step to –100 mV was considered I_{h}. This current was completely and reversibly blocked by the application of 5 mM Cs\(^{+}\) to the bath solution (data not shown).

Data Analysis: Action potential duration was determined at 0 mV. Maximum rates of rise and fall for the action potential were determined by taking the first derivative of the action potential waveform. Magnitude of the afterhyperpolarization was determined relative to the resting membrane potential at the largest potential obtained following the action potential. The afterhyperpolarization (AHP) was fitted with a single exponential equation in order to estimate the decay rate of the AHP. A paired T-test was used to assess the statistical significance of PGE_{2}-induced changes in passive and active properties of colonic DRG neurons, while one- and two-way analyses of variance with a Holms-Sidec post-hoc test were used to assess the statistical significance of differences between cutaneous, TL and LS neurons.

Drugs: PGE_{2} was dissolved in 100% ethanol, stored at a 10 mM stock solution at -20 °C, and diluted in bath solution immediately prior to use. PGE_{2} and all other chemicals employed (unless otherwise stated) were obtained from Sigma.
Results:

Identification of cutaneous and colonic DRG neurons:

We studied 33 cutaneous neurons from 6 rats and 98 colonic neurons from an additional 9 rats. Between 2 and 10 neurons were studied from each rat. Because there is a rough correlation between axon conduction velocity and cell body diameter for afferents innervating cutaneous tissue (Lawson et al. 1993; Lee et al. 1986), and we wished to compare putative nociceptive afferents innervating cutaneous tissue to those innervating the colon, we studied labeled cutaneous neurons with a small cell body size. Because membrane capacitance measured in the whole-cell configuration is an accurate measure of cell body size, we used membrane capacitance to distinguish small from large neurons with a cutoff of 42 pF. This cut-off was chosen because it corresponds with a cell body diameter of ~30µm, based on a specific capacitance of 1.19 µF/cm2 that was determined with a regression analysis of data from over 500 DRG neurons in which cell body diameter and membrane capacitance had been determined. An additional correction factor of 6% was included because of the influence of DiI on capacitance measurements (Gold et al. 2002). The average cell capacitance of cutaneous DRG neurons was 33.2 ± 1.1 pF (mean ± SEM n = 33: range 20.34 to 42 pF). Because the colon is only innervated by afferents with slowly conducting axons (~70% C-fibers and 30% A-delta fibers), we studied all colonic afferents. The average cell body capacitance of colonic DRG neurons was 67.8 ± 2.5 pF (n = 98: range 19 to 131 pF); the difference between colonic and cutaneous DRG neurons with respect to cell body capacitance was statistically significant (p < 0.01). There was no statistically significant difference between TL and LS neurons with respect to cell capacitance; both were ~67 pF (Table 1).
Passive and active properties of cutaneous and colonic DRG neurons:

Cutaneous DRG neurons were significantly different than colonic (both TL and/or LS) DRG neurons in a number of the electrophysiological properties assessed including: resting membrane potential (p < 0.01, cutaneous versus LS only: Table 1), rheobase (p < 0.05, cutaneous versus TL only: Figure 2, although this difference did not reach statistical significance if data were normalized with respect to cell body capacitance), action potential duration (p < 0.01, Table 1), action potential overshoot (p < 0.05, Table 1), maximum rate of action potential fall (p < 0.01, Table 1), and the magnitude of the after-hyperpolarization (p < 0.05, Table 1). Previous studies of somatic afferents indicate that there is a correlation between cell body size, axon conduction velocity and action potential properties: larger neurons tend to give rise to more rapidly conducting axons with narrower action potentials and smaller neurons tend to give rise to more slowly conducting axons with slower broader action potentials (Djouhri and Lawson 1999; Harper and Lawson 1985; Koerber et al. 1995; McCarthy and Lawson 1989; Ritter and Mendell 1992; Villiere and McLachlan 1996; Waddell and Lawson 1990). The differences between cutaneous and colonic neurons observed in the present study are thus consistent with these previous observations of somatic afferents. However, the action potential rate of rise was similar between all three groups of neurons (Table 1) and is much closer to the rates observed in C-fibers (McCarthy and Lawson 1997) then in neurons giving rise to more rapidly conducting axon (Ritter and Mendell 1992). Importantly, the C-fiber-like action potential in colonic neurons was also consistent with our previous results suggesting the majority of DiI labeled colonic DRG neurons give rise to unmyelinated axons (Gold et al. 2002). These observations suggest that we have studied primarily cutaneous and colonic C-fiber afferents that differ with respect to cell body size as well as other properties.
In order to further address the possibility of selection bias in our comparisons between cutaneous and colonic sensory neurons, we sought to determine the extent to which “small” colonic DRG neurons differed from the “medium” diameter population. Therefore, we compared the electrophysiological properties of colonic neurons with a membrane capacitance < 42 pF to those with a membrane capacitance > 42 pF. Only 12 of the 98 colonic neurons studied were < 42 pF (6 LS and 6 TL). For TL neurons alone, or all colonic neurons combined, the only statistically significant difference between small and medium neurons with respect to any of the passive or active properties assessed was with rheobase. Not surprisingly, small neurons had a lower rheobase (with a median of 90 pA) than the medium neurons (with a median of 160 pA; p = 0.02, Rank Sum test). This difference, however, was eliminated if rheobase was normalized for cell body capacitance. Small and medium LS neurons also differed with respect to action potential threshold. However, in contrast to what might be expected for C-fibers, small LS neurons had a significantly lower action potential threshold than medium LS neurons. Thus, we found no compelling reason to use cell body size as a criteria with which to segregate colonic neurons in order to facilitate comparisons with small cutaneous neurons.

In contrast to the marked differences between colonic and cutaneous neurons, TL and LS neurons were similar with respect to the majority of passive and active electrophysiological properties assessed (Table 1). There were, however, several notable exceptions. First, mean R_{in} of TL neurons was significantly larger than that of LS neurons (p = 0.01). Given that a hyperpolarizing current injection was used to assess R_{in}, this difference may reflect differences in the expression of the hyperpolarization activated current I_{h}. Indeed, we assessed I_{h} with a 500 ms hyperpolarizing voltage-step from –50 mV to –100 mV in 20 TL and 18 LS neurons. Consistent with the difference between the two populations with respect to R_{in}, there is a higher density of I_{h}
in LS neurons (2.7 ± 0.5 pA/pF at –100 mV) than in TL neurons (1.3 ± 0.2 pA/pF; p < 0.01). The difference in \( I_h \) may also have contributed to differences in the \( R_{in} \) between cutaneous neurons and LS neurons as the density of \( I_h \) in cutaneous neurons was only 0.5 ± 0.1 pA/pF. Second, action potential threshold of LS neurons was significantly lower than that of TL neurons (Figure 2; p < 0.05). Rheobase in LS neurons was also lower than that observed in TL neurons (Figure 2, p < 0.05). Finally, suprathreshold stimuli resulted in a significantly greater number of evoked action potentials in TL neurons than in LS neurons (Figure 2; p < 0.01) and the median slope of the stimulus response function was significantly greater in TL than in LS neurons (Figure 2; p < 0.01).

**PGE2-induced sensitization of cutaneous and colonic DRG neurons**

Similar to results obtained in previous studies of unlabeled DRG neurons (Baccaglini and Hogan 1983; England et al. 1996; Gold et al. 1996; Nicol and Cui 1994), PGE2 increased the excitability of both cutaneous and colonic DRG neurons (Figure 3). In general, the increase in excitability, or sensitization, was associated with a leftward shift in the stimulus response function, such that lower intensity stimuli were required to evoke action potentials and more action potentials were evoked in response to previously suprathreshold stimuli. Many neurons also demonstrated spontaneous activity.

There were several striking differences between cutaneous and colonic neurons with respect to PGE2-induced changes in excitability. Neurons were considered sensitized if PGE2-induced a decrease in action potential threshold or rheobase, and/or an increase in the response to suprathreshold stimuli greater than 2 SDs from the baseline response. PGE2 was applied to 21 of the cutaneous neurons studied, 10 of which were sensitized. This proportion is significantly smaller than the proportion of colonic neurons sensitized by PGE2 (28/29; p < 0.01 \( \chi^2 \) test).
There were also differences in the nature of the sensitization. More than a quarter of the colonic neurons (8 of 28) became spontaneously active following application of PGE$_2$, while none of the cutaneous neurons demonstrated spontaneous activity. In addition, all but one of the colonic neurons sensitized by PGE$_2$ demonstrated a decrease in rheobase, a decrease in action potential threshold and an increase in the number of action potentials evoked in response to suprathreshold stimuli (Figure 4) whereas only 4 of the 10 sensitized cutaneous neurons demonstrated changes in all of these properties. The remaining cutaneous neurons sensitized by PGE$_2$ demonstrated either a decrease in rheobase (4 neurons) or an increase in action potentials evoked in response to suprathreshold stimuli (2 neurons). Interestingly, sensitization of cutaneous neurons was associated with an increase in the slope of the stimulus response function with little apparent decrease in threshold (Figure 4). This change is analogous to that observed following sensitization of cutaneous nociceptive terminals \textit{in vivo} (Andrew and Greenspan 1999).

Both TL and LS neurons were sensitized by PGE$_2$. The general trend was that TL neurons were sensitized to a greater extent than LS neurons. The difference between TL and LS neurons was statistically significant for both the proportion of neurons that became spontaneously active (7 of 15 TL neurons versus 1 of 13 LS neurons; \(p = 0.03\), Fisher Exact Test), and the shift in the stimulus response function (Figure 4).

\textit{PGE$_2$-induced changes in passive and active properties of cutaneous and colonic DRG neurons}

\textit{PGE$_2$}-induced sensitization of cutaneous and colonic DRG neurons was associated with several significant changes in passive and active electrophysiological properties (Table 2). Interestingly, the pattern and/or magnitude of changes was different among subpopulations of sensory neurons. For example, the increase in input resistance observed in cutaneous and LS colonic neurons sensitized by PGE$_2$ was not associated with a significant change in resting
membrane potential in cutaneous neurons, while it was associated with a small but significant membrane depolarization in LS neurons. In contrast, PGE2 induced a significant decrease in resting membrane potential in the absence of a significant change in input resistance (Table 2). Similarly, there was no significant change in action potential overshoot, rate of rise or rate of fall in cutaneous neurons, while there were significant changes in all three of these properties in TL and LS neurons (Table 2).

**Discussion:**

In the present study, we have tested the hypothesis that differences in the expression of pain syndromes in various parts of the body reflect, in part, unique properties of the afferents innervating different structures. Furthermore, we hypothesized that these unique properties influence excitability of afferents innervating naïve tissue as well as inflammation-induced changes in the excitability of these afferents. Consistent with these hypotheses, we have observed marked differences between cutaneous and colonic sensory neurons with respect to the passive and active electrophysiological properties of these neurons as well as inflammatory mediator-induced changes in their electrophysiological properties.

Both passive and active electrophysiological properties reflect underlying ion channels. Consequently, differences between subpopulations of sensory neurons with respect to passive and active properties indicate that there are differences between subpopulations of neurons with respect to the functional properties of ion channels. For example, the difference between TL and LS colonic neurons with respect to input resistance is likely to reflect, at least in part, differences between these subpopulations of sensory neuron with respect to the density of $I_h$. However, these neurons must also differ with respect to the presence and/or density of at least one other ion
channel active at rest. That is, because $I_h$ is a non-selective cation current that drives membrane depolarization, a higher density of this current, would, if it contributed to resting membrane potential, result in a more depolarized resting membrane potential. In contrast to this expectation, neurons with the highest density of $I_h$ had the most hyperpolarized resting membrane potential.

Similarly, the larger AP overshoot, longer AP duration, slower AP fall and smaller AHP magnitude observed in cutaneous neurons all suggest differences in the activity of at least 1 voltage- and/or Ca$^{2+}$ dependent ion channel in this population of neurons compared to colonic neurons. The presence of a higher density of voltage-gated Na$^+$ channels in cutaneous neurons would be consistent with the larger AP overshoot. However, a more likely explanation is that there is a lower density of a voltage- or Ca$^{2+}$-dependent K$^+$ channel in cutaneous neurons that contributes to AP repolarization. Subclasses of both of these channel subtypes have been shown to function in this capacity (Christian et al. 1994; Rudy 1988). Importantly, such an explanation accounts for all of the differences stated above as well as the lack of difference observed with respect to AP rise time.

Differences in the electrophysiological properties of TL and LS neurons indicated that there are also differences within subpopulations of colonic sensory neurons with respect to ion channels controlling excitability. A higher input resistance should, all else being equal, result in a lower rheobase. That this was not the case in TL neurons suggest that these neurons contain either a higher density of low threshold voltage- or Ca$^{2+}$-dependent K$^+$ or Cl$^-$ channels or a lower density of voltage-gated Na$^+$ channels. This latter possibility is unlikely given our previous (Gold et al. 2002) as well as unpublished observations indicating that there is no difference between TL and LS neurons with respect to the density or biophysical properties of either tetrodotoxin resistant or tetrodotoxin sensitive voltage-gated Na$^+$ current.
That TL neurons may be less excitable in naïve animals than LS neurons was suggested by previous studies performed *in vivo*. In sensory innervation studies of both the cat colon (Janig and Koltzenburg 1991) and the rat uterus (Berkley et al. 1993), afferents in sympathetic nerves (lumbar colonic/hypogastric) had higher thresholds to mechanical stimuli than afferents in the pelvic nerve. Because we have assessed the excitability of colonic sensory neurons with depolarizing current injection, a stimulus that presumably by-passes “natural” transduction processes, our results suggest that at least some of the differences between TL and LS neurons observed *in vivo* reflect differences in the expression of ionic currents. Our observation that TL neurons are intrinsically less excitable than LS neurons may help explain the observation that in the absence of inflammation, there appears to be little TL input into the central nervous system (Traub 2000).

The marked differences between cutaneous and colonic neurons with respect to the influence of the prototypical inflammatory mediator, PGE₂, also suggest that there are differences between these populations of sensory neurons with respect to the underlying mechanisms mediating sensitization. Given the apparent differences between these populations of sensory neurons with respect to ion channels controlling passive and active electrophysiological properties in the absence of inflammation, it is reasonable to expect that PGE₂-induced change in the biophysical properties of a single ion channel will impact the excitability of these different populations differently. However, the pattern of PGE₂-induced changes in both passive and active electrophysiological properties suggests that different mechanisms underlie sensitization of cutaneous and colonic neurons. For example, PGE₂ induced both an increase in input resistance and membrane depolarization in LS colonic sensory neurons, consistent with the closing of a K⁺ leak channel. In contrast, sensitization of cutaneous
neurons was associated with a larger increase in input resistance than that observed in colonic neurons. Yet this change in resistance was associated with no change in resting membrane potential suggesting that either a different channel is closed in cutaneous neurons, or there is an additional channel active at \( V_{\text{rest}} \) in cutaneous neurons that is modified in addition to the channel modified in colonic neurons. Similarly, sensitization of TL and LS colonic neurons was associated with a small but significant increase in AP overshoot and rate of rise and decrease in the AP rate of fall. Given that the presence of TTX-R \( I_{\text{Na}} \) enables AP broadening in sensory neurons (Blair and Bean 2002), these changes in active properties are consistent with the PGE\(_2\)-induced increase in TTX-R \( I_{\text{Na}} \) that we have recently described in colonic sensory neurons (Gold et al. 2002). Furthermore, given that the same mechanism is likely to contribute to the sensitization of cutaneous sensory neurons (Khasar et al. 1998), the absence of similar changes in AP properties in cutaneous sensory neurons suggests that changes in other channels may compensate for the influence of an increase in TTX-R \( I_{\text{Na}} \) on the AP waveform. Thus, sensitization of cutaneous neurons was likely associated with the closing of a leak channel, modulation of a voltage-gated \( \text{Na}^+ \) channel and the inhibition of a voltage or \( \text{Ca}^{2+} \)-dependent channel, while sensitization of colonic neurons was associated with at least the closing of a different leak channel and the modulation of a voltage-gated \( \text{Na}^+ \) channel.

There were also marked differences between TL and LS neurons with respect to the magnitude of PGE\(_2\)-induced changes in excitability. However, the pattern of PGE\(_2\)-induced changes in both passive and active properties was similar between these two populations of neurons. This observation suggests that the underlying mechanisms of sensitization were similar between the two populations and that differences between them with respect to the pattern of
excitability changes reflect differences in density and/or properties of the ion channels active in these neurons in the absence of PGE$_2$.

In summary, we have described striking differences between cutaneous and colonic sensory neurons with respect to the passive and active electrophysiological properties of these neurons as well as changes in these properties in response to the inflammatory mediator PGE$_2$. These differences in electrophysiological properties suggest that the density and distribution of ion channels in sensory neurons varies systemically among subpopulations of sensory neurons defined by target of innervation. That PGE$_2$-induced changes in excitability were associated with different changes in passive and active electrophysiological properties suggest that ion channels underlying sensitization of afferents also varies systematically among subpopulations of sensory neurons defined by target of innervation. This latter observation suggests that it may be possible, if not necessary to treat pain arising from various parts of the body with therapeutic interventions specific for that region of the body.

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Tables:

Table 1: Baseline Passive and Active Electrophysiological Properties of cutaneous and TL and LS colonic DRG neurons

<table>
<thead>
<tr>
<th>Ganglia</th>
<th>Capac. (pF)</th>
<th>Rin (MΩ)</th>
<th>Vrest (mV)</th>
<th>AP Dur. (ms)</th>
<th>AP Over. (mV)</th>
<th>AP Rise (mV/ms)</th>
<th>AP Fall (mV/ms)</th>
<th>AHP Mag. (mV)</th>
<th>AHP decay (τ, ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutan. Baseline (n = 33)</td>
<td>33.2 ± 1.0^a,b</td>
<td>762 ± 121</td>
<td>-55.3 ± 1.1^a</td>
<td>7.7 ± 0.7^b</td>
<td>53.3 ± 1.2^b</td>
<td>87 ± 5</td>
<td>26 ± 1.4^b</td>
<td>14.1 ± 0.7^b</td>
<td>115 ± 15</td>
</tr>
<tr>
<td>LS Baseline  (n = 49)</td>
<td>65.8 ± 3.5</td>
<td>498 ± 68c</td>
<td>-61.0 ± 1.0^c</td>
<td>2.9 ± 0.3</td>
<td>47.8 ± 2.0</td>
<td>108 ± 8</td>
<td>65 ± 5</td>
<td>19.1 ± 1.0</td>
<td>102 ± 9</td>
</tr>
<tr>
<td>TL Baseline  (n = 49)</td>
<td>66.0 ± 3.5</td>
<td>723 ± 99</td>
<td>-57.6 ± 0.9</td>
<td>3.2 ± 0.2</td>
<td>48.5 ± 1.4</td>
<td>98 ± 7</td>
<td>63 ± 3</td>
<td>20.3 ± 1.1</td>
<td>103 ± 9</td>
</tr>
</tbody>
</table>

Cutan.: cutaneous; LS: lumbosacral; TL: thoracolumbar; Capac: Membrane Capacitance; Rin: Input resistance (measured with a 20 mV hyperpolarizing voltage step from –60 mV); AP Dur: Action potential duration (measured at 0 mV); AP Over: Action potential overshoot; AP Rise: Maximum rate of action potential depolarization (determined from the first derivative of the action potential waveform); AP Fall: maximum rate of action potential hyperpolarization (determined with AP Rise); AHP Mag: Magnitude of the afterhyperpolarization (relative to rest) observed following an action potential; AHP decay: decay time constant of the afterhyperpolarization (determined from an exponential fit of the decay phase of the AHP); ^a Statistically significant difference between Cutaneous and LS neurons. ^b Statistically significant different between Cutaneous and TL neurons. ^c Statistically significant difference between LS and TL neurons.
Table 2: PGE₂-induced changes in passive and active properties of cutaneous and colonic DRG neurons

<table>
<thead>
<tr>
<th>Ganglia</th>
<th>$R_m$ %Δ</th>
<th>Vrest Δ (mV)</th>
<th>AP Dur. %Δ</th>
<th>AP Over. Δ (mV)</th>
<th>AP Rise %Δ</th>
<th>AP Fall %Δ</th>
<th>AHP Mag. Δ (mV)</th>
<th>AHP decay %Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutan</td>
<td>78.6 ± 39.8*</td>
<td>0.2 ± 0.3**</td>
<td>2.6 ± 6.5</td>
<td>1.5 ± 1.1</td>
<td>6.2 ± 9.5</td>
<td>-3.0 ± 10.2</td>
<td>-3.4 ± 1.0</td>
<td>-26.3 ± 12.8</td>
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<tr>
<td>(n = 5-13)</td>
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<tr>
<td>LS</td>
<td>64.1 ± 7.8*</td>
<td>6.1 ± 0.5**</td>
<td>9.7 ± 4.5</td>
<td>3.1 ± 1.0**</td>
<td>13.8 ± 5.5*</td>
<td>-13.6 ± 5.5**</td>
<td>-2.2 ± 1.4</td>
<td>13.0 ± 22.8</td>
</tr>
<tr>
<td>(n = 8-14)</td>
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</tr>
<tr>
<td>TL</td>
<td>20.2 ± 10.8</td>
<td>4.2 ± 1.2*</td>
<td>12.4 ± 5.2</td>
<td>4.0 ± 1.2*</td>
<td>18.3 ± 4.8**</td>
<td>-12.8 ± 4.0*</td>
<td>1.9 ± 1.4</td>
<td>8.9 ± 9.1</td>
</tr>
<tr>
<td>(n = 8-15)</td>
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</tbody>
</table>

% Δ: percent change from baseline ([(After PGE₂ – Before PGE₂) / Before PGE₂]*100); Δ: change from baseline (After PGE₂ – Before PGE₂); Not every parameter was assessed in every neuron; at least 5 neurons were studied in each group. Group differences were assessed with a one way ANOVA followed by a Holm-Sidak post-hoc test for pairwise comparison: a Statistically significant difference between Cutaneous and LS neurons. b Statistically significant different between Cutaneous and TL neurons. A paired T-test was used to assess for statistically significant PGE₂-induced changes in passive and active properties within each group of neurons: *Statistically significant difference between before and after PGE₂ application (p < 0.05); ** Statistically significant difference between before and after PGE₂ application (p < 0.01). Other abbreviations are the same as used in Table 1. PGE₂ concentration used was 1 μM.
Figure Legends:

Figure 1: *Passive and active electrophysiological properties of cutaneous and colonic DRG neurons.* Voltage trace was recorded with the whole-cell configuration of patch clamp from DiI labeled sensory neuron. A: Action potential threshold is defined as the greatest membrane potential ($V_m$ (mV)) achieved in response to depolarizing current injection that does not result in an action potential e.g. –27 mV (Top Panel). Rheobase is defined as the smallest amount of depolarizing current (Stim. (pA)) necessary to evoke an action potential e.g. 180 pA (Bottom Panel). B. Many DRG neurons encode suprathreshold stimuli (1x, 1.5x, 2x, 2.5x and 3x rheobase) with increasing number of evoked action potentials. C: Action potential is evoked with 4 ms depolarizing current injection through the recording electrode. Current injection protocol is shown beneath voltage trace. D: Action potential shown in C with an expanded time scale. Trace beneath the voltage trace is the first derivative (dV/dt) of the voltage trace shown above. Letters in C and D refer to passive and active properties to be assessed: a. Resting membrane potential; b. Input resistance (calculated from measured change in membrane potential in response to 20 pA of hyperpolarizing current injection); c. Maximum rate of rise of action potential; d. Action potential over-shoot (mV above 0 mV); e. Action potential duration at 0 mV; f. maximum rate of fall of action potential; g. presence of an inflexion, or “hump” on the falling phase of the action potential; h. Magnitude of afterhyperpolarization in mV below resting membrane potential; i. Rate of decay of afterhyperpolarization, determined with a single exponential fitted to voltage trace.

Figure 2: Active properties of Cutaneous (Cut.) and colonic (lumbosacral (L6-S2: LS) and thoracolumbar (T13-L2: TL)) DRG neurons. A. Action potential (AP) threshold was defined as
described in Figure 1. Values represent mean ± SEM from 33, 43 and 46 cutaneous, LS and TL neurons, respectively. AP threshold was significantly lower in LS neurons than in TL neurons (p < 0.05). B. Rheobase was determined for each cell as described in Figure 1 and Methods and then pooled as in A. Rheobase was higher in TL neurons than in LS neurons (p =0.01) or cutaneous neurons (p < 0.01). C. Stimulus response functions were determined for each neuron as described in Figure 1 and pooled as described in A. Inset: Box plot of the slope of the stimulus response functions for cutaneous and colonic neurons. The median is the center line in the box (which was zero for LS neurons); the lower and upper edges of the box are the 25th and 75th percentiles and the error bars are the 5th and 95th percentiles The median slope of the stimulus response function was significantly greater in TL neurons than that in LS neurons (p < 0.05, Dunn’s post-hoc test).

**Figure 3:** Bath application of PGE2 sensitized both cutaneous and colonic DRG neurons. Sensitization was generally associated with both a decrease in action potential threshold, rheobase (top traces) and an increase in the number of action potentials evoked in response to a suprathreshold stimulus (bottom traces). Voltage traces were evoked from a TL neuron 30 seconds before and 60 seconds after bath application of 1 μM PGE2. Resting membrane potential was –57 mV prior to PGE2 application and hyperpolarizing current injection was applied to maintain the membrane potential at –57 mV after PGE2 application.

**Figure 4:** PGE2 sensitized cutaneous and colonic DRG neurons. A. PGE2 induced a hyperpolarizing shift in action potential threshold in cutaneous, LS and TL neurons. The PGE2-induced change in action potential threshold (Δ in AP Threshold) was significantly greater in TL neurons than in cutaneous neurons (p < 0.05). B. PGE2 induced a decrease in rheobase in
cutaneous, LS and TL neurons. The decrease in rheobase in TL neurons was significantly greater than that in cutaneous neurons. Data were analyzed as a percent of baseline (i.e., (rheobase after PGE₂/rheobase before PGE₂)*100) and plotted as a box plot with: the center line in the box as the median, the lower and upper edges of the box are the 25th and 75th percentiles, the error bars are the 5th and 95th percentiles. The decrease in rheobase was significantly larger in TL neurons than in cutaneous neurons (p < 0.05). C. PGE₂ induced an increase in the number of action potentials evoked (APs / 500 ms) in cutaneous, LS and TL neurons in response to suprathreshold stimulation. The increase in evoked action potentials was significantly greater in TL neurons than in either LS or cutaneous neurons at 2x and 3x rheobase (p < 0.05, two-way analysis of variance follow by Holm-Sidak test for post-hoc comparisons). Filled and open symbols are stimulus response data prior to and after application of PGE₂, respectively: Circles are cutaneous neurons, triangles are LS colonic neurons and squares are TL colonic neurons.
Figure 1:
Figure 2:

- **A**: AP Threshold (mV) for Cutaneous (Cut.), LS, and TL.
  - Cutaneous: -36 mV
  - LS: -32 mV (***), -36 mV (*)
  - TL: -32 mV

- **B**: Rheobase (pA) for Cutaneous (Cut.), LS, and TL.
  - Cutaneous: 0 pA
  - LS: 100 pA
  - TL: 200 pA

- **C**: Spikes / 500 ms for Cutaneous (Cut.), LS, and TL.
  - Cutaneous: 0 Spikes
  - LS: 1 Spikes (***), 2 Spikes (**)
  - TL: 2 Spikes

Legend:
- Cutaneous (n = 21)
- LS (n = 28)
- TL (n = 30)
Figure 3:

Before 1 \( \mu \text{M PGE}_2 \) | After 1 \( \mu \text{M PGE}_2 \)

Rheobase

150 pA

300 pA

2x Rheobase

300 pA

30 pA

40 mV

150 ms
Figure 4

A

Δ in AP Threshold (mV)

-12 -8 -4 0

Cut. LS TL

* *

B

Rheobase (% of Baseline)

0 20 40 60 80 100 120 140

Cut. LS TL

* *

C

Stimulus Intensity (x Rheobase)

Spikes / 500 ms

0 2 4 6 8 10

1.0 1.5 2.0 2.5 3.0

Cut. TLLS

Rheobase (% of Baseline)

0 20 40 60 80 100 120 140

Cut. LS TL

*