Tensile and Compressive Responses of Nociceptors in Rat Hairy Skin

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Khalsa, Partap S., Robert H. LaMotte, and Peter Grigg. Tensile and compressive responses of nociceptors in rat hairy skin. J. Neurophysiol. 78: 492–505, 1997. Mechanically sensitive nociceptor afferents were studied in a preparation of isolated skin from rat leg. Each neuron was studied while the skin was subjected to tensile and compressive loading. The experiment was designed to create highly uniform states of stress in both tension and compression. Tensile loads were applied by pulling on the edges of the sample. Applied loads were used to determine the tensile stresses. Surface displacements were used to determine tensile strains. Compressive loads were applied by indenting the surface of the skin with flat indenter tips applied under force control. The skin was supported by a flat, hard substrate. Compressive stresses were determined from the applied loads and tip geometry. Compressive strains were determined from skin thickness and tip excursions. All nociceptors were activated by both tensile and compressive loading. There was no interaction between the responses to compressive and tensile stimuli (i.e., the responses were simply additive). Responses of nociceptors were better related to tensile and compressive stresses than to strains. Nociceptors responded better to tensile loading than to compressive loading. Response thresholds were lower and sensitivities were higher for tensile stress than for compressive stress. The response to compression was better related to compressive stress than to other stimulus parameters (i.e., load/circumference or simply load). Indentations of intact skin over a soft substrate such as muscle would be expected to cause widespread activation of nociceptors because of tensile stresses.

INTRODUCTION

Mechanosensitive nociceptors undoubtedly play an important role in the genesis of mechanically evoked pain. Yet, quantitative empirical and theoretical studies of the biomechanical basis for their activation are lacking. Cutaneous mechanonociceptors have received the greatest attention from neurophysiologists primarily because of greater accessibility and greater ease in relating nociceptor functional properties to sensations of mechanically evoked pain in humans. The most common method of stimulation is to vary the force of an indenting probe, or probes of differing diameter, and obtain either the threshold for evoking a response or a stimulus response function relating magnitude of discharge to force. Nociceptors with myelinated or unmyelinated axons (A or C fibers) are typically responsive not only to mechanical stimuli but also to noxious heat (termed AMHs and CMHs) and/or to cold (similarly, AMCs and CMCs) or irritant chemicals. As well, they have force thresholds that are roughly similar and generally exceed those of sensitive low-threshold mechanoreceptors with myelinated axons (e.g., Adriaensen et al. 1983; Bessou and Perl 1969; Burgess and Perl 1967; Campbell et al. 1979; Georgopoulos 1977; Handwerker et al. 1987; Kumazawa and Perl 1977; Lynn and Carpenter 1982; Perl 1968; Van Hees and Gybels 1972). Both A and C fiber types exhibit increased discharge rates as a function of force, although the A fibers generally exhibit higher discharge rates in response to a given force than the C fibers (Garell et al. 1996; Handwerker et al. 1987; Reeh et al. 1987) and greater differentiation in their responses to small probes of different diameter (Garell et al. 1996).

There are considerable individual differences in the sensitivities of cutaneous nociceptors to mechanical stimuli (e.g., Burgess and Perl 1967; Cooper et al. 1991; Garell et al. 1996; Lynn and Carpenter 1982; Meyer et al. 1991). It is controversial as to whether such differences imply the existence of separate subpopulations of mechanonociceptors or just a single population with a wide range of response sensitivities (e.g., Burgess and Perl 1967; Cooper et al. 1991; Garell et al. 1996; for review, see Lynn 1992). The major problem one faces in addressing this question is that the nociceptor does not respond directly to the externally applied stimulus itself (e.g., indentation force or probe geometry) but to the changes in the internally produced stresses and strains in the tissue brought about by that stimulus. These changes depend in large part on the biomechanical properties of the tissue, such as its compliance. For example, tensile stresses and strains (in the plane of the skin) will be relatively low in relation to compressive stresses and strains (normal to the skin) for indentations of skin lying directly over a hard substrate such as bone. In contrast, both tensile and compressive stresses and strains may be significant when indenting skin lying over soft substrates such as fat and muscle.

Although there has been speculation in the literature that nociceptors might differ in their sensitivities to compressive versus tensile stresses (e.g., Cooper et al. 1993; Handwerker et al. 1987; Reeh et al. 1987), there have been no tests of the hypothesis because there were no practical means of obtaining separate stimulus control of these two mechanical quantities. Now, however, a method has been developed in which tensile and compressive loading can be independently applied to an isolated patch of skin (Grigg 1996) or joint capsule (Khalsa et al. 1996) and the resulting stresses and strains directly measured during simultaneous recording of evoked discharges in single low-threshold mechanoreceptive afferent nerve fibers. The purpose of the present study is to determine the sensitivities of nociceptive mechanoreceptive afferents to tensile and compressive stresses and strains with the use of the same methodology.
METHODS

Preparation

Experiments were performed with the use of isolated skin-nerve specimens that were obtained from the ventral surface of the hindlimb of adult Sprague-Dawley rats of either sex. The location of the specimen on the leg is shown in Fig. 1A. This preparation has been described in detail elsewhere (Grigg 1996). Briefly, rats were anesthetized with pentobarbital sodium (50 mg/kg ip). The hair on the hindlimb was clipped and removed with a chemical depilatory (Nair). The area to be studied was marked by gluing an array of nine surface markers (0.3-mm black disks) on the skin (Fig. 1A). The markers in the array were 7 mm apart, and the selected area was 14 mm square. In addition to defining the area selected for study, the markers were also used during the experiment to track surface displacements associated with stretching the skin. The orientation of the sample was defined by a 10th marker that was placed along the X direction (along the axis of the tibia, Fig. 1A). The locations of the markers were recorded so that the in situ geometry of the specimen could be reproduced when it was excised and in vitro (Fig. 1B). The edges of the specimen, along which it would be cut, were then drawn on the skin. The edges were laid out as tabs (7 × 10 mm) that were subsequently used to couple the skin to actuators that applied tensile loads. The skin was excised from the hindlimb by cutting along the marked boundaries. The nerve branches innervating the specimen were identified, dissected centrally to the sartorius nerve, and cut in the inguinal fossa. The isolated skin-nerve specimen was removed to an apparatus (Fig. 1B), where it was maintained (epidermis up) in artificial interstitial fluid (Bretag 1969). The fluid was kept at room temperature (20°C), gassed with 95% O₂-5% CO₂, and circulated with a pump.

Stretching and compression apparatus

Tensile loads were applied to the skin specimen with the use of an apparatus (Fig. 1B) consisting of 12 linear actuators and load cells, arranged three along each side of the skin specimen. The apparatus has been described in detail previously (Khalsa et al. 1996). Each actuator/load cell was coupled to a skin tab via a length of suture with a miniature fishhook at the end. Each hook was engaged in a hole punched in the end of a tab. Thus, when an actuator was operated, a load was developed at the point of application of its hook. When loaded, the skin tabs had an aspect ratio (length/width) > 2, so that the applied point loads were approximately uniformly distributed at the end of the tab (Khalsa et al. 1996). The skin was stretched until the distances between the markers closely approximated their in vivo configuration. Typically, this resulted in an initial preload of ~0.01 N per tab.

When the skin was mounted in the apparatus, it was preconditioned to establish a pseudoeelastic state. Preconditioning was done by stretching the sample with the use of uniform tab loads of 0.08 N per tab. The stretch was maintained for 10 s, and the sample was relaxed for 4 min. This paradigm was repeated 10 times. The specimen was then returned to its initial geometry, and all experimental trials were performed starting from this reference state.

Tensile loading of the sample was accomplished by actuating the tabs until predetermined loads were achieved. All tensile loading paradigms used uniform loads (equal on each tab) so as to minimize in-plane shear stresses and strains. Compressive loads were applied by compressing the skin between a flat-tipped indenter and a flat platform located under the skin. The indenter tip was actuated by a force controlled DC motor (Series 300B Lever System, Cambridge Technology, Watertown, MA). The motor rotated a 55-mm-long arm with the indenter tip at its end (Fig. 2). Loads were determined from the torque at the motor shaft; displacements were determined with the use of the angular displacement of the shaft. The indenter arm bent slightly under load, confounding displacement readings. However, its compliance was found to be highly linear (r > 0.999), so that its contribution to displacement recordings could be calculated and subtracted. In compressing the skin, vertical displacements of the indenter tip were <0.5 mm; thus while the indenter moved along a circular path, the small displacements meant that the tip displacement was essentially linear. The orientation of the indenter apparatus was carefully adjusted so that the excursion of the tip would be normal to the surface of the skin and the platform. However, the indenter was not perfectly normal to the plane of the skin, so that off-axis loads were gener-

FIG. 1. A: area of skin studied. Rat is ventral side up; sample was cut from right hindlimb along the line drawn. Three tabs are cut along each edge. Dots: markers glued on skin surface. B: skin sample mounted in apparatus. Each tab is coupled to a linear actuator and a load cell (L1–L12). Rotary actuator (C) applies compression loads by actuating the arm (A). N, nerve is threaded into chamber for recording.
Nerve recording, identification, and classification

The nerve was dissected into a compartment for recording. Loose connective tissue was dissected free, and the nerve was immersed in a 1.5% solution of collagenase (Worthington Biochemical). After 30 min, the collagenase was removed and the nerve was rinsed with artificial interstitial fluid. The nerve was then dissected into small filaments with the use of fine forceps. Individual filaments were placed on a fine gold wire electrode for recording. An indifferent electrode was located in the saline bath.

Neural signals were amplified and filtered with the use of conventional methods. Action potentials were identified as unitary spikes on the basis of a template matching paradigm (SPS Systems, Prospect, South Australia). Times of occurrence of action potentials were recorded. The neural response to a stimulus 10 s in duration was characterized with the use of the total number of evoked action potentials. This single measure of the neural response was chosen because of the high variability of the response. The total number of action potentials represented a measure of response common to all afferents.

Conduction velocity was measured by applying electrical stimuli to the skin surface at the location of the receptive field and measuring the latency of the evoked action potential. The conduction distance was measured with a ruler from the receptive field location to the recording electrode. We increased the conduction velocities by 45% (Petajan 1968) to correct for the slowing effect of the relatively cold preparation. We classified afferents with corrected conduction velocities <2.0 m/s as C fibers and faster afferents as A fibers.

In sampling afferents, the principal search stimulus was electrical stimulation of the skin surface with the use of bipolar electrodes with polished tips 2 mm apart. Only mechanically sensitive neurons were selected for study. The response of a neuron to mechanical stimuli was qualitatively assessed by reducing tensile forces on the skin and by compressing the receptive field with the use of both a fire-polished glass rod with a 1.5-mm-diam tip and various von Frey filaments. Sensitivity to heat was assessed by placing a servo-controlled thermal probe on the receptive field for 5 s. Two temperatures were used: 38°C, which felt warm to the experimenters, and 55°C, which was immediately painful. Cold sensitivity was assessed by placing ice chips on the skin surface for 10 s.

Experimental procedure

When a suitable afferent was found, it was studied while the skin was mechanically loaded with the use of tensile stimuli alone, compressive stimuli alone, or combinations of tensile and compressive stimuli. Three levels of tensile load (0.01, 0.04, and 0.08 N) were used for any given trial. First, the indenter was driven into the skin under constant load, a significant creep response was observed. Second, the skin thickness did not fully recover during the intertrial interval; at the beginning of the next trial there was a residual indentation (a “crater”) present in the skin. This presented a problem because 1) the skin was in a non-steady state and 2) it was not possible to accurately measure the local thickness of the skin in the resulting crater. Therefore a Lagrangian formulation [ε = (L1 − L0)/L0] was used for strain calculation. In this method, all strain measures are referenced to the skin thickness (L0) measured in the first trial. L0 was measured by lowering the indenter to the skin surface until it measured a minimal load (0.001 N), and recording its location. Later, after all indentation trials were completed, the skin was moved aside, and the location of the platform surface was recorded. The difference between the skin surface and the platform surface was the value of thickness used for L0. The value of L1 used for any given trial was determined from the difference between the location of the platform and the location of the indenter during the trial. L1 was measured during the last 0.5 s of the 10-s static load, when the creep response was at a minimum.

Nerve recording, identification, and classification

The nerve was dissected into an oil-filled compartment for recording. Loose connective tissue was dissected free, and the nerve was immersed in a 1.5% solution of collagenase (Worthington Biochemical). After 30 min, the collagenase was removed and the nerve was rinsed with artificial interstitial fluid. The nerve was then dissected into small filaments with the use of fine forceps. Individual filaments were placed on a fine gold wire electrode for recording. An indifferent electrode was located in the saline bath.

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the three left actuators while relaxing equally with the three right actuators. This translated the skin while maintaining the tab loads. The location of the marker at the receptive field was tracked in real time, and translation continued until the marker was returned to its initial location. At this point, the motors were stopped, and the final position of each motor was recorded. Later, driving the motors to those positions would create the desired tensile loads while maintaining the receptive field under the indenter. This procedure was repeated three times, once for each of the tensile load levels to be used in the experiment. The marker directly overlying the receptive field was removed, and the loading trials were begun.

In the experimental runs, the skin was first loaded in tension, running the motors to the positions determined as above. The ramp up to the positions had a duration of 0.9–1.5 s depending on the magnitude of the load. When the application of the tensile load was finished, the compression stimulus was applied. Both stimuli were maintained for 10 s, during which time both load and neuron response data were collected (Fig. 3). At the end of the run, the indenter was lifted off and the tensile loads were relaxed. To optimize the likelihood that the receptive field would be under the indenter, the indenter tip used in these trials was ≥2.0 mm diam. The intertrial interval was 4 min, based on previous observations that C mechanoreceptors in skin had stable responses when repeatedly stimulated at this or shorter intervals (Bessou et al. 1971; Garell et al. 1996).

Data analysis

The three-dimensional state of stress and strain was evaluated at the mechanoreceptor location. Plane (tensile) stresses were estimated from the tab loads and the cross-sectional area of the tabs. Tab cross-sectional area was calculated from the widths, measured from a video image of the preparation, and with the use of a value of 0.3 mm for skin thickness (Grigg 1996). Tensile stresses were measured with high accuracy (Khalsa et al. 1996). Plane (tensile) strains were calculated from the displacements of the array of surface markers (Hoffman and Grigg 1984). Each marker formed a node of a quadrilateral. Tensile strains were calculated at each node. Tensile strains at the location of the mechanoreceptor ending were linearly interpolated from the nodal strains of the quadrilateral encompassing the mechanoreceptor ending. Nodal strains were measured with an accuracy of ±0.002. Compressive stress was calculated from the indenter load and the tip geometry and was accurate to within ±1%. Compressive strain was calculated from indenter displacements and was accurate to within ±0.001.

In addition to measuring the magnitudes of the components of stresses and strains along the axes of the apparatus (i.e., the components of the stress and strain tensors), we also computed the magnitudes of coordinate-invariant quantities (i.e., variables whose magnitude is independent of the orientation of the coordinate system in which they are measured). Because the orientation of collagen fibrils varies throughout the skin, and because the response of some mechanoreceptors has been shown to be determined by the local orientation of the fibrils (Khalsa et al. 1996), it was surmised that neural response might be related to a mechanical quantity whose magnitude is not dependent on the orientation of an arbitrary coordinate system. Thus relationships were sought between neural response and coordinate-independent variables. For example, strain energy density is a scalar quantity that is the sum of the products of stress and strain along each of the three principle axes. Its magnitude is independent of the orientation of the coordinate system in which it is expressed. The other coordinate-independent quantities that we used were similar to those described for the two-dimensional situation in Khalsa et al. (1996). The expressions used to compute these quantities in three dimensions are given in the APPENDIX.

Estimation of the responses of a population of nociceptors due to skin indentation in the intact limb

The section of skin studied in these experiments overlies muscle. Thus, when it is indented in situ, large indentations can result, with the development of both tensile and compression stresses and strains. We wished to determine the relative magnitudes of the compression and tensile stresses caused by indentations in situ to estimate how a population of nociceptors located in the skin would respond to the indentation.

In five experiments, rats were anesthetized and maintained supine and ventral side up, as in Fig. 1A. The indenter was applied to the skin of the hindlimb, in the center of the array (Fig. 1A), and compressive loads similar to those used in vitro were applied. The resulting compression stresses under the indenter could be measured directly from the applied load and the indenter geometry. Tensile stresses, however, could only be estimated on the basis of measurements of tensile strains. Tensile strains were measured by determining the three-dimensional surface profile of the skin along two (i.e., the X and Y) directions while it was indented in situ. A line of five markers (~2 mm apart) was arranged along the X and the Y direction in the center of the specimen. The indenter was placed over the center of the specimen. The markers were visualized with a stereoscopic microscope equipped with dual beamsplitters and two charge-coupled device cameras. The indenter was actuated, creating an indentation and causing the mark-
ers to move in the Z direction. A stereo pair of images was taken during the indentation. Subsequently, the stereo pairs were processed so as to yield the X, Y, and Z location of each marker (Yakimovsky and Cunningham 1978). In addition, the depth of the indentation directly under the indenter tip was measured directly from the actuator. From the resulting data we determined the uniaxial tensile strains between each pair of markers as well as between the edge of the indenter and the first marker.

In each in vitro experiment, the biaxial relationship between stress and strain was measured during skin stretching. These relationships were modeled with nonlinear curves and fit to the data with a least-squares error method. The in vivo tensile stress was calculated from the measured tensile strains and the above relationship. The tensile stress directly underneath the indenter was estimated from the stress in the adjacent segments, taking into account the angle of the skin relative to the indenter (Grigg and Hoffman 1996).

The spatial distribution of responses of a population of nociceptors to an in situ indentation was estimated in the following way. The neural responses peripheral to the indenter, where there were tensile but minimal compressive components of stress, were modeled from the estimated tensile stress in conjunction with the thresholds and sensitivity data that were measured in the in vitro experiments. The response under the indenter was modeled in the same way except that a component of response from compression was included.

RESULTS

Recording of neurons

Ninety-eight C or Aβ-afferent neurons were recorded in 25 successful experiments. Corrected conduction velocities averaged 0.3 and 3.7 m/s for the C and Aβ-afferents, respectively. Afferents that lacked mechanical sensitivity were not suitable for this investigation, and many units that were mechanically sensitive were not recorded for long enough to allow for them to be adequately characterized. Thus the results are based on recordings from 37 mechanically sensitive afferents (Table 1) for which sufficient data were available to undertake an analysis of their mechanical sensitivity. The neural response in each trial, changes in firing rate, and adaptation of response was analyzed for attributes that might be related to the stimuli. No relationship was found, so the total number of action potentials that occurred was used as the response measure for each trial.

Responses to tensile loading

Each neuron was studied while the tabs were actuated, creating in-plane stresses and strains. An example of data recorded during a single trial is shown in Fig. 3. In this trial, the neural response averaged ~5 imp/s, which was typical for the C afferents. As can be seen, all tab loads were approximately equal. Every neuron was tested with the use of uniform tab loads of 0.00, 0.01, 0.04, and 0.08 N per tab, although some were studied with the use of more load levels. Because applied loads were the controlled variables, the tensile stresses were approximately equal in both directions.

However, because the skin in this region of the leg is slightly orthotropic (Grigg 1996), strains were not equal in both directions. Both stresses and strains varied slightly between experiments because of differences in geometry of the specimens. Every mechanically sensitive afferent was activated by tensile loading. Figure 4, A and B, shows the averaged response of six C mechanoreceptor not sensitive to cold or heat (CM) afferents to tensile loading along the X direction. Because the loading was uniform and biaxial, similar plots could also be constructed for stress and strain along the Y direction. Because stress was the controlled variable in these experiments, there was little variability in the stress levels used with different neurons, resulting in the narrow horizontal error bars of Fig. 4A. In contrast, the (resulting) tensile strains that were observed in those experiments were quite variable, resulting in the broad horizontal error bars of Fig. 4B. Because of limitations of the apparatus, we never achieved tensile stresses >30 kPa. Reflecting this low level of loading, saturation of neural response was never observed with the use of tensile loading.

Responses to compression loading

All mechanically sensitive afferents were activated by compressive stimuli. When afferents had multiple receptive fields in the skin, we used the most excitatory spot for compressive stimuli; AMC, A and C mechanoreceptor sensitive to cold.

Quantitative relationships between neuronal response and the magnitudes of mechanical states in the skin

Each experimental trial resulted in stresses and strains in both tension and compression. It was of interest to determine the relationship between the neural response and the magni-
tudes of those variables. We first wished to identify those variables that were most closely related to the response of the neuron. For example, either stress or strain might be considered to be the independent variable in exciting neurons. Relationships between the response of a neuron and the magnitudes of various mechanical states were explored with the use of correlation analysis. In these analyses, correlation coefficients were calculated between neuronal response levels and the magnitude of various candidate mechanical variables. The resulting correlation coefficients were lumped together across all neurons (Figs. 5 and 6). As was previously found for other mechano-sensitive neurons (Fuller et al. 1991; Grigg 1996; Khalsa et al. 1996), neuronal response levels were more highly correlated with stress variables than strain variables. Overall, the highest correlation was with the compressive stress, followed by the tensile stresses and then tensile strains. There was essentially no correlation between the neural response and shear strains. The neural response was more highly correlated with the coordinate invariant mechanical quantities composed solely of stress tensor components, termed "stress invariants," than with those composed solely of strain tensor components (Fig. 6). The neural response was approximately as well correlated with the strain energy density as with the stress invariants.

Because the neuronal responses were better correlated with stress variables than strain variables, in the rest of this communication we describe the responses of afferents with the use of stress variables as the independent variables.

Thresholds and sensitivity to tensile and compression stresses

Of the 37 neurons, 33 yielded both threshold and sensitivity values in compression, and 16 yielded both threshold and sensitivity values in tension. Afferents were characterized with the use of a minimum of three levels (4, including 0) of pure tension or compression loading (i.e., not combination trials). The resulting data (i.e., see Fig. 4) allowed us to estimate both the threshold and sensitivity (i.e., the slope of the curve in Fig. 4, A and C) of each neuron for both tensile and compression stresses. The thresholds for tensile stresses were much lower than those for compressive stress (Table 2). In addition, the sensitivity to tensile stress was much higher than for compressive stress (Table 2).

The thresholds were significantly lower (Fig. 7), and the sensitivities were significantly higher, for tension than for compression loading (Fig. 8). There were no significant differences in thresholds or sensitivities between different submodalities of nociceptors for tension and compression (P = 0.65 and 0.52, respectively, for thresholds; P = 0.32 and 0.35, respectively, for sensitivities). Tensile thresholds were very similar to those reported for stretch-sensitive neurons in feline joint capsule (Khalsa et al. 1996) and slowly adapting type II (SAIIs) and C mechanoreceptors in rat hairy skin (Grigg 1996).

Dependence of neuronal response on compressive stress versus compressive load

We wished to determine whether the neural response to compressive loading was best related to the compression load, the resulting compressive stress, or the load/circumference of the indenter (cf. Garell et al. 1996). Eight neurons were studied during indentation trials in which the diameter of the indenter tip was varied between 1.4 and 3.0 mm. The compressive load was varied so that approximately equiva-
lent stress levels were generated with each tip. No tensile loads were used in these trials. Results from one neuron are shown in Fig. 9. The strength of the relationship between the neural response (total number of action potentials per 10 s) and each variable was determined by calculating the correlation coefficient, $R$, between the neural response and the variable. $R^2$, which expresses the percentage of variance in the dependent variable that is accounted for by the independent variable, was calculated for each variable for each neuron (Table 3).

Interaction between tensile and compressive responses

To determine whether there was any interaction between the responses to tensile and compressive loading, each neuron was studied with the use of combinations of compressive and tensile loadings. We attempted to record the responses of each afferent while presenting all combinations of the set of tensile and compressive loads that were used with that neuron. Thus, ideally, each neuron was studied with the use of 15 experimental runs that represented each combination of tensile and compressive loading. Results were obtained from 37 afferents. Data obtained from one neuron are shown in Fig. 10. To test whether there was a significant interaction between the responses to tension and compression, an analysis of variance (ANOVA) was performed, in which tensile and compressive stresses were independent variables. The resulting ANOVA revealed that the tension $\times$ compression interaction was not significant ($P > 0.50$) for any type of afferent. Thus the

![Figure 5: Mean value, across all neurons, of correlation coefficient between neuronal response level and magnitude of individual components of stress and strain tensors. Sx and Sy: tensile strength in the X and Y directions. Sz: compressive stress. Ex and Ey: tensile strains in the X and Y directions. Ez: compressive strain. Exy: in-plane shear strain.](image-url)

![Figure 6: Mean value, across all neurons, of correlation coefficient between neuronal response level and magnitude of variables whose magnitude is independent of orientation of coordinate system. I1–I3: 1st–3rd invariants, respectively, of stress tensor. J2 and J3: 2nd and 3rd invariants of stress deviatoric tensor. MSS, maximum shear stress; SED, strain energy density. E1–E3: 1st–3rd invariants, respectively, of strain tensor. F2 and F3: 2nd and 3rd invariants of strain deviatoric tensor. MSSr, maximum shear strain.](image-url)
TABLE 2.  Mean thresholds and sensitivities

<table>
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<th>Tension</th>
<th>Compression</th>
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<td>Mean threshold, kPa</td>
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<td>46</td>
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<td>Sensitivity, Aps/10 sec/kPa</td>
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<td>0.36</td>
<td>&lt;0.001</td>
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<tr>
<td>N</td>
<td>16</td>
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Mean thresholds and sensitivities of all neurons with submodalities combined (i.e., AMC, CM, CMH, CMC, CMHC) to tensile and compressive stresses.

Responses to simultaneously applied tension and compression are simply additive.

In vivo tensile and compressive stresses caused by indenting intact skin over muscle

In five experiments, the indenter was used to apply compressive loads to the middle of the sampled area (Fig. 1). The loads used were in the same range as those used in the in vitro experiments. Because the skin overlies muscle, large indentations resulted. With an applied load of 0.3 N, the indentation was ~12 mm. The tensile strains observed along the X and Y directions were summarized in Table 4. They were greatest near the indenter, and decreased with distance from the indenter. The depth of the indentation was determined mainly by the magnitude of the indenting load; changing the diameter of the indenter had only a minor effect on the depth of the indentation. The profile of this indentation is shown three-dimensionally in Fig. 11. The asymmetry of the skin indentation profile resulted at least in part from the skin anisotropy (i.e., the skin was stiffer along the Y-axis than the X-axis) (Grigg 1996).

From these tensile strain measurements, tensile stresses were estimated along the segments and beneath the indenter. Segment stresses were calculated with nonlinear curves fit to the biaxial stress-strain data with the use of a least-squares error method for a power curve (Fig. 11). The equations for these curves were $\sigma_X = 105\varepsilon_X^{1.7}$ and $\sigma_Y = 155\varepsilon_Y^{1.4}$, where $\sigma$ is the stress and $\varepsilon$ is the strain, respectively, for the X and Y directions. Tensile stresses underneath the indenter were calculated from the stresses in segment 1 (Table 4) and the geometry of the skin next to the indenter, which formed angles of 0.94 and 0.34 rad along the X and Y directions, respectively (Fig. 12).

From the stress estimates, the level of neural activity was estimated for a uniformly distributed population of nociceptors on the basis of the thresholds and sensitivities described earlier. These neural activity estimates are superimposed on the three-dimensional spatial indentation profile and are shown in color in Fig. 12. This figure illustrates the estimated spatial extent of nociceptor activation following an 0.3-N compression stimulus. For this patch of rat skin, the model predicts that the greatest neural response would occur just next to, rather than underneath, the compressive probe. This is due to the combination of the nociceptor’s greater sensitivity to tensile versus compressive stress and the largest tensile stresses occurring just next to the indenter.

DISCUSSION

Others (Bove and Light 1995) have shown that nociceptors are sensitive to stretch. This is the first report that shows that nociceptors have a significant and substantial sensitivity to tensile loading of skin. In experiments in which both tensile and compressive stress and strain were measured, we found that all nociceptors were better activated by tensile than by compressive loading. They had lower thresholds and higher sensitivity to tensile stress than compressive stresses. These results have important ramifications in the study of mechanical nociception. If compressive stimuli are applied to skin that lies over soft tissue such as muscle, substantial indentations can result. Large indentations can cause substantial tensile loading around the indenting stimulus. In previous studies, any such tensile component developed during mechanical loading could not be measured or controlled. Thus, when nociceptors have been activated by compressive stimuli applied to skin overlying soft tissue, any neural response would be confounded by (and potentially even dominated by) the response...
to the resulting tensile stresses. The higher responsiveness to tensile as compared with compressive components of loading, was observed in all submodalities of nociceptors.

Assuming that nociceptors in human and cat are also responsive to tension, our results may account for one of the findings of Greenspan and McGillis (1991) and Garell et al. (1996). They found that the thresholds for pain in humans, and responses of nociceptors in cat hairy skin, were better related to the applied force/circumference than to applied force/area (i.e., compressive stress) of the stimulus. We find those results difficult to interpret, because compressive stress scaled according to the circumference of the indenter has no physical meaning and would be unique to any particular indenter geometry. However, the above findings could be accounted for by the tensile component of nociceptor responses, which were not accounted for in either study.

Both Garell et al. and Greenspan and McGillis indented skin overlying soft tissue, so that tensile stresses were created during the trials. Our experiments showed that the depth of the indentation, and thus the magnitude of tensile stresses, was determined mainly by the magnitude of the applied load, with little effect of tip diameter. Thus, for the 90-g compressive load used by Garell et al., the tensile component of response would be similar for all groups. The component of response resulting from compressive stress, however, would be inversely proportional to tip area. Thus, when large tips were used, the tensile response constituted a larger fraction of the response than when small tips were used. Therefore, when the data are plotted against compression stress, the groups for larger tips have a higher response than those for smaller tips (cf. Garell et al. 1996, their Fig. 7), where the response magnitude is ordered directly in inverse order of tip size. A similar outcome is shown in Greenspan and McGillis (1991, their Fig. 3a), in which the pain threshold is lower with larger indenter tips. In our experiment, when the tensile component of the response was eliminated by applying only pure compression, the resulting neuronal response was closely related to the compressive stress.

Greenspan and McGillis also observed that tips with a common area but a different shape led to different thresholds for pain, and they considered this as further evidence that factors other than compressive stress are important for nociceptor activation. However, compressive stresses would be differentially distributed under tips with differing geometry. For example, a right cylinder with a tip area of 0.05 mm$^2$ elicits pain with lower applied loads than does a 120° cone with a truncated tip with an area of 0.05 mm$^2$. However, the cone distributes the load over a greater area, reducing the compressive stress under the tip. Thus, unless the actual stress at the neuron location is known, no conclusions can be drawn about the stress sensitivity of the nociceptor.

With regard to the issue of determining stresses at the location of a neuron’s receptor ending, we attempted to create uniform states of stress by the use of large, flat indenter tips, and compressing the skin against a hard, flat surface. Furthermore, because the skin was flat during uniform tensile loading, tensile stresses were also uniform throughout the sample. This design created a uniform state of compressive and tensile stress under the indenter. Other experimental paradigms, in which the skin is compressed against soft substrates and/or compressed with a nonflat indenter, will result in nonuniform states of stress throughout the skin (Grigg and Hoffman 1996). Very small tips also create problems for stress determination, because shear stresses and strains will be present around the edges. These stresses and strains cannot be evaluated, and the neuron ending may be close enough to the edges to be influenced by them. However, by controlling for the effects of tensile loads and by avoiding problems associated with the above types of tips, we have shown that responses to compressive loading are caused by the resulting compressive stress.

It is unclear what compressive strain the nerve ending actually experienced during indentation, because the skin did not fully recover its thickness between trials. This viscous deformation resulted in a crater whose diameter was essentially that of the indenter tip. Given sufficient time between trials (>15 min), the skin would have fully recovered; however, this would have made performing the experiment pro-

![Table 3](attachment:image.png)

**TABLE 3. $R^2$ values**

<table>
<thead>
<tr>
<th>NR vs. Load</th>
<th>NR vs. Load/Circumference</th>
<th>NR vs. Compressive Stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R^2 = 0.08$</td>
<td>$R^2 = 0.29$</td>
<td>$R^2 = 0.66$</td>
</tr>
</tbody>
</table>

Mean values of $R^2$ calculated between neuronal response (NR) and applied load, load/circumference, and load/area (compressive stress). *$P < 0.01$ (statistically significant).
TENSILE AND COMPRESSIVE RESPONSES IN NOCICEPTORS

FIG. 10. Interaction between responses for 1 neuron when subjected to pure tensile loading, pure compressive loading, and combinations of tensile and compressive loading. Surface representing neural response was forced to pass through data points (symbols on surface) and was interpolated between these points.

hibitively time consuming. Thus we expressed the strain with the use of the Lagrangian method in which the strain is expressed as a fraction of the original thickness before indentation (see METHODS). Other methods of describing strain could have been used (e.g., Euler, Cauchy, engineering, logarithmic, etc.). However, given the design of the experiment, the Lagrangian method was the most amenable to the displacement measurements available to us. Although our displacement measures were quite accurate, we recognize that other methods of describing strain may be more relevant to the physiological state experienced by the nociceptor ending.

By closely measuring and/or controlling stresses and strains in three directions (i.e., both in and normal to the plane of the skin), we were able to observe several parallels between the properties of nociceptors and those of other mechanically sensitive afferents. First, we found that the response of nociceptor units was more closely related to tissue stress variables than to strain variables. Although the experimental design was not specifically intended to separate stress and strain variables, these variables were in fact substantially decoupled from each other in these experiments. Neural responses were found to be better related to stress tensor components than they were to strain tensor components. They were better related to invariant quantities composed of stress variables than they were to strain variables. Thus these findings are consistent with earlier findings from this laboratory (Fuller et al. 1991; Grigg 1996; Khalsa et al. 1996) showing that stretch-sensitive afferents appear to function as tensile stress sensors. Second, it was observed that the threshold for activation by tensile stress in nociceptors (~5 kPa) is similar in magnitude to that observed for SAI afferents and C mechanoreceptors in rat hairy skin (Grigg 1996) and stretch sensors in cat knee capsule (Khalsa et al. 1996).

<table>
<thead>
<tr>
<th>TABLE 4. Tensile strains measured along the X and Y directions during an 0.3-N compressive load</th>
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<tbody>
<tr>
<td><strong>X direction</strong></td>
</tr>
<tr>
<td><strong>Stress, kPa</strong></td>
</tr>
<tr>
<td>Segment 1</td>
</tr>
<tr>
<td>46</td>
</tr>
<tr>
<td>148</td>
</tr>
</tbody>
</table>

Tensile stresses were calculated the use of the best fit power relationship shown in Fig. 11. Tensile stresses under the indenter were calculated the use of the geometry of the skin next to the indenter as shown in Fig. 12 and the stresses in segment 1.
The compressive stimuli in these experiments were clearly noxious in that a long-lasting crater was formed with notable redness associated with the indentation site. These experiments were not designed to assess whether these stimuli would cause aversion behavior in rats that would suggest that the stimuli were painful. Psychophysical studies in human volunteers in which indenters of similar diameter were used have demonstrated that pain would be expected for our smallest-diameter indenter (1.6 mm) at forces exceeding ~100 g (Garell et al. 1996; White et al. 1991). Thus, at the highest compression loads for the smallest-diameter indenters, these stimuli would be noxious and, most likely, also painful.

The finding that nociceptive afferents have thresholds to stretch that are similar to those of SAI afferents is unexpected. In other locations and in other animals, SAI afferents are spontaneously active, respond to innocuous limb movements, and are thought to contribute to proprioception (Burgess et al. 1968; Gynther et al. 1992; Jarvilehto et al. 1981; MacWhinney et al. 1990; Vallbo et al. 1995). These would be unprecedented roles for nociceptive afferents. However, the above finding may be unique to the skin studied in this experiment. In the region of the rat leg that we studied, neither SAI afferents nor nociceptors were spontaneously active, and neither would be activated during any rotations of the leg (Grigg 1996). The tensile threshold would, we estimate, be reached only when large tractions are applied or when large indentations are made into the skin. The fact that nociceptors and SAI afferents in the hindlimb had similar thresholds for stretch should not, we feel, be generalized to other sites or other animals without circumspersion, and the sensory role played by the stretch responses of nociceptors should be evaluated exclusively for any specific site. It is unclear whether the properties that we describe would apply (for example) to the cat hindlimb, where there might be substantial differences in the mechanical properties of the skin and possibly phenotypic differences in the properties of neurons. In the rat hindlimb, at least, there is no evidence of a proprioceptive role for nociceptors. However, the stretch responses caused by indentations would serve to amplify the population response of nociceptors to a strong indenting stimulus that could cause a penetrating injury. Furthermore, the strains caused by the stress levels that we employed were well beyond the range of what might be called “physiological” (Grigg 1996).

The substantial difference in the threshold and sensitivity to tensile versus compressive loading raises the issue of the mechanism through which applied stimuli might be coupled to the transducer mechanism of the receptor ending of the neuron. Skin can be considered to be a fiber-reinforced composite material (Ault and Hoffman 1992). In contrast, our measures of stress and strain are based on modeling the skin as a continuum material. However, notwithstanding the shortcomings of using a continuum model for determining skin stresses and strains, these variables were highly predictive of neuronal responses. The difference in threshold and sensitivity between tensile and compressive stimuli may be coupled to a single transducer mechanism through different constituents of the skin.

The same difference in sensitivities might also arise from neurons that have multiple endings. If these endings were far enough apart, the compression stimulus would possibly act on a single ending while the tension stimulus would act on all endings. If the responses from separate endings summed together, then the tensile stimulus would be more effective than the compressive one. This is a possibility we cannot discard and will require further experiments to decisively determine.

The small sample size made it infeasible to draw conclusions about the differences between specific subcategories of nociceptors. In particular, we encountered a low number of afferents in the AMC, CMH, and heat- and cold-sensitive C mechanoreceptor (CMHC) subcategories. It will be very important for future studies to resolve the issues revolving around the possible differences in these subcategories.

Several investigators (Dandekar and Srinivasan 1995; Grigg and Hoffman 1984; Khalsa et al. 1996; Srinivasan and Dandekar 1996) have postulated that strain energy density is the local mechanical state that is encoded by mechanically sensitive afferents. If a uniform stress or strain field is applied to a material like skin that has structural components with spatially varying orientations, stress or strain vectors would differ in various regions according to the local orientations of collagen fibrils. The magnitude of such a stress or strain field could be signaled by neurons that encode coordinate-independent variables (e.g., strain energy density). However, we found no evidence to support such a model. On the other hand, the magnitude of such a spatially varying state would also be encoded by neurons that signal stress and are not directionally tuned. The nociceptors in our study appeared to be indistinguishable from the C mechano-
receptors observed by Grigg (1996), which were not directionally selective. Our data would then support this latter model.

**APPENDIX: DESCRIPTION OF THE IN Variant MECHANICAL QUANTITIES**

The three-dimensional stress ($\boldsymbol{\sigma}$) and strain ($\boldsymbol{\epsilon}$) at the location of a terminal ending of a nociceptive afferent ending can be described with the use of tensor notation as:

$$
\boldsymbol{\sigma} = [\sigma_{ij}] = 
\begin{bmatrix}
\sigma_{11} & \sigma_{12} & \sigma_{13} \\
\sigma_{21} & \sigma_{22} & \sigma_{23} \\
\sigma_{31} & \sigma_{32} & \sigma_{33}
\end{bmatrix}
$$

(1)

and

$$
\boldsymbol{\epsilon} = [\epsilon_{ij}] = 
\begin{bmatrix}
\epsilon_{11} & \epsilon_{12} & \epsilon_{13} \\
\epsilon_{21} & \epsilon_{22} & \epsilon_{23} \\
\epsilon_{31} & \epsilon_{32} & \epsilon_{33}
\end{bmatrix}
$$

(2)

In this study, the components of the stress and strain tensor were referenced to the right-handed coordinate system where the $Y$- (or "2") axis corresponded to the long axis of the femur. The $X$- (or "1") axis was perpendicular to the $Y$-axis, and the $Z$- (or "3") axis was orthogonal to the $XY$ plane. Given the design of the study, the only stresses reported were those along the diagonal of the stress tensor (i.e., $\sigma_{11}$, $\sigma_{22}$, and $\sigma_{33}$). In addition to the strains along the diagonal of the strain tensor (i.e., $\epsilon_{11}$, $\epsilon_{22}$, and $\epsilon_{33}$), we were also able to measure one of the planar shear strains (i.e., $\epsilon_{12}$).

From the stress and strain tensors, it was possible to calculate a number of scalar quantities that were invariant to the orientation of the external imposed coordinate system. Individual mechanoreceptors have been shown to align themselves to the local orientation of collagen fibers (Halata et al. 1985). Therefore a population of receptors within a large enough volume of tissue would be expected to have different orientations. Thus a uniformly applied stress (or strain) field would result in different stresses (or strains) depending on the orientation of the receptors. However, the magnitude of the tensor invariants would be the same irrespective of receptor orientations. Thus the tensor invariants were attractive candidates for being the mechanical quantity(s) that might be encoded by mechanically sensitive nociceptors.

Stress tensor invariants are commonly defined by first describing the principal stresses. The same process is directly used to described strain tensor invariants and will not be repeated for sake of brevity. Principal stresses are defined as those, for a particular coordinate system orientation, in which the shear stresses are nonexistent (i.e., $\sigma_{ij} = 0$, for $i \neq j$). The determination of the principal stresses and their directions is solved by the standard calculation of the eigenvalues and eigenvectors. Not all of the invariant quanti-
ties that arise from the characteristic equation that is used to solve the eigenvalues and eigenvectors have direct relationships with
easily described physical states. However, they form the mathematical
basis of much of material failure and plasticity theories (Chen
and Han 1988). The following is a description of the invariants
evaluated in this study.
$I_1$, $I_2$, and $I_3$ are the first, second, and third invariants, re-
spectively, of the stress tensor. $I_1$ is related to the stresses responsible
for pure dilation of an elastic volume. $I_1$ and $I_3$ do not have easily
identifiable physical correlates. Their equation are given as follows

$$I_1 = \sigma_{11} + \sigma_{22} + \sigma_{33}$$

(3)

$$I_2 = (\sigma_{11}\sigma_{22} - \sigma_{12}^2) + (\sigma_{11}\sigma_{33} - \sigma_{13}^2) + (\sigma_{22}\sigma_{33} - \sigma_{23}^2)$$

(4)

$$I_3 = (\text{the determinant of the stress tensor})$$

(5)

The maximum shear strain ($\text{MSS}_{\text{r}}$) is directly analogous to the
MSS and is given by

$$\text{MSS}_{\text{r}} = \frac{1}{2} \left( \epsilon_{11} - \epsilon_{22} \right)$$

(15)

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REFERENCES

ADRIENNESS, H., GYBELIS, J., HANDWERKER, H. O., AND VAN HEES, J. Res-
ponsibility of thin myelinated (A-delta) fibers in human skin

AULT, H., AND HOFFMAN, A. H. A composite micromechanical model for

BURGESS, P. R., PERL, E. R., AND TAYLOR, C. B. Dynamic
properties of mechanoreceptors with unmyelinated (C) fibers. J. Neuro-

BURGESS, P. R., AND PERL, E. R. Response of cutaneous sensory units with
unmyelinated fibers to noxious stimuli. J. Neurophysiol. 32: 1025–1043,
1969.


BOVE, G. M. AND LIGHT, A. R. Unmyelinated nociceptors of rat parapinal

BRETAG, A. H. Synthetic interstitial fluid for isolated mammalian tissue.

BURGESS, P. R., AND PERL, E. R. Myelinated afferent fibers responding speci-
fically to noxious stimulation of the skin. J. Physiol. Lond. 190: 541–562,
1967.

The principal axes are aligned with the global coordinate system, the maximum
of these shearing stresses is

\[ \text{MSS}_{\text{r}} = \max \left\{ \left| \frac{1}{2} (\epsilon_{11} - \epsilon_{22}) \right| \right\} \]

(8)

The incremental strain energy density ($\text{SED}$) is the elastic
energy stored in a material when it is deformed. The Biot (1965)
formulation was used, which takes into account the stresses present
in an initially stressed material such as skin in vivo, where $\sigma_{i}$
are the initial stresses

\[ \text{SED} = \frac{1}{2} \sigma_{ij} S_{ij} \epsilon_{ij} \]

(9)

There are three invariants ($E_1$, $E_2$, and $E_3$) of the strain tensor, which are analogous to the invariants of the stress tensor described previously. Their equations are given by

\[ E_1 = \epsilon_{11} + \epsilon_{22} + \epsilon_{33} \]

(10)

\[ E_2 = (\epsilon_{11}\epsilon_{22} - \epsilon_{12}^2) + (\epsilon_{11}\epsilon_{33} - \epsilon_{13}^2) + (\epsilon_{22}\epsilon_{33} - \epsilon_{23}^2) \]

(11)

\[ E_3 = \left| \epsilon_{ij} S_{ij} \epsilon_{ij} \right| \] (the determinant of the strain tensor)

(12)

The strain tensor, like the stress tensor, can also be decomposed into two components: a hydrostatic and a deviatoric tensor. The deviatoric strain invariants ($F_1$, $F_2$, and $F_3$) are related to the strains that deform the material into a skew form. The two nontrivial invariants ($F_2$ and $F_3$) of the strain deviator tensor are given by

\[ F_1 = \frac{1}{3} (2E_1^2 - 9E_2 E_3 + 27E_3) \]

(14)

\[ F_2 = \frac{1}{2} \left( (\epsilon_{11} - \epsilon_{22})^2 + (\epsilon_{22} - \epsilon_{33})^2 + (\epsilon_{33} - \epsilon_{11})^2 \right) + \frac{1}{2} (E_3^2 - 3E_2) \]

(13)

\[ \text{MSS}_{\text{r}} = \max \left\{ \left| \frac{1}{2} (\epsilon_{11} - \epsilon_{22}) \right| \right\} \]

(15)


