Encoding of Location and Intensity of Noxious Indentation Into Rat Skin by Spatial Populations of Cutaneous Mechano-Nociceptors

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Khalsa, Partap S., Ce Zhang, and Yi-Xian Qin. Encoding of location and intensity of noxious indentation into rat skin by spatial populations of cutaneous mechano-nociceptors. J. Neurophysiol. 83: 3049–3061, 2000. The ability of a spatial population of cutaneous, Aδ, and C mechano-nociceptors to encode the location and intensity of a noxious, cutaneous indentation was examined using an isolated preparation in a rat model. Skin and its intact innervation were harvested from the medial thigh of the rat hindlimb and placed in a dish, with the corium side down, containing synthetic interstitial fluid. The margins of the skin were coupled to an apparatus that could stretch and apply compression to the skin. The skin was suspended on top of a deformable platform whose bulk, nonlinear, compressive compliance emulated that found in vivo. The isolated preparation facilitated examination of the spatial population response by eliminating the nonlinear geometry and inhomogeneous compressive compliance present in vivo. Spatial population responses (SPR) were formed from recordings of single neurons that were stimulated by compressing the skin with an indenter (flat cylinder, 3-mm diam) at discrete intervals from the center of their receptive fields. SPR were composed of the neural responses (z axis) at each indentation location (x, y plane), and were analyzed quantitatively using nonlinear regression to fit an equation of a Gaussian surface. Both Aδ and C SPR accurately encoded the location and intensity of noxious indentation. The intensity of the stimulus was encoded in the peak neural response of the SPR, which had a nonlinear relationship to the compressive force. The location of the stimulus was encoded in the x, y position of the peak of the SPR. The position of the peak remained constant with increasing magnitudes of compressive force. The overall form of the SPR also remained constant with changes of compressive load, suggesting a possible role for encoding in the SPR some aspects of shape of a noxious stimulus.

INTRODUCTION

Noxious indentation of skin is transduced by cutaneous mechano-nociceptors that can lead to the percept of pain. Pain, because of acute mechanical stimuli, is not perceived until mechano-nociceptors respond at firing rates substantially greater than threshold (Garell et al. 1996; Greenspan and McGillis 1991). It is questionable whether suprathreshold firing of single mechano-nociceptors is sufficient to be perceived as painful (Greenspan 1997; Greenspan and McGillis 1991; Heppelmann et al. 1991). This implies that it requires a population of nociceptors to accurately encode the location and intensity of noxious mechanical stimuli.

Neuron population studies have been used widely to exam-
gerpad produced an asymmetrical spatial population response (from low-threshold mechanoreceptors) biased along the long axis of the finger (Khalsa et al. 1998). Indentation with a symmetrical object (e.g., a flat tipped cylinder) would produce different mechanical states distal to the contact site. Third, the bulk compressive compliance of a large enough region would not be spatially homogeneous because of the different tissues involved, their dimensions, and their potential interactions with hard tissues like bone. Hence the same magnitude stimulus at different spatial locations could produce different mechanical states in the skin. Fourth, the receptive endings of single nociceptors are often multiple (particularly for Aδ fibers) and can extend over many square millimeters of skin (Lynn and Carpenter 1982). Hence different terminal endings of a single parent axon presumably could be subjected to different mechanical states even with a single stimulus.

To overcome these difficulties, an isolated skin-nerve preparation, suspended directly on the surface of a compliant platform, was developed. The platform was constructed to emulate the nonlinear, bulk compressive compliance of the substrate underlying the skin in the intact animal. However, its compliance was spatially homogenous and its surface was linear (i.e., flat). Hence indentations of in vitro skin overlying the compliant platform produced consistent mechanical stimuli to the nociceptors regardless of their location within the skin. Thus a spatial population response could be constructed from the neural responses of single mechanonociceptors and used to examine the ability of a nociceptor population to encode location and intensity of noxious indentation.

METHODS

Preparation

Experiments were conducted using an isolated skin-nerve preparation similar to that previously reported in detail (Khalsa et al. 1997). Briefly, hair was removed from the medial thigh of an anesthetized rat (Sprague-Dawley, 250 g, either sex), and small markers (0.5-mm diam) were glued to the depilated skin. Dots were spheroids (0.5 mm diameter) glued to the depilated skin. Drawn. Dots were spheroids (0.5 mm diameter) glued to the depilated skin. A syringe containing a denervating solution was introduced through the incision and the nerve was cut. The incision was closed with 10-0 nylon, and then gassed (100% O2) rodent, synthetic interstitial fluid (Koltzenburg et al. 1997). Tabs (7 mm wide × 14 mm long) were cut into the margins of the skin (3 tabs per side, total of 12) and coupled to force transducers mounted on the ends of linear actuators (3 actuators per side, total of 12; Fig. 1B). The skin then was stretched until the markers closely approximated their in vivo locations.

Pure compressive and pure tensile loads were applied as previously reported (Khalsa et al. 1997). Briefly, tensile loads were applied to the skin by actuating the tabs until predetermined loads were achieved. Compressive loads were applied by indenting the skin with a flat tipped cylinder (3-mm diam) between a compliant or a hard platform. The cylinder was actuated with a force controlled DC motor (model 305B, Aurora Scientific, Aurora, Canada) mounted on a three axis positioning stage (resolution 0.1 mm and range 40 mm on each axis). Actuator control and data acquisition was accomplished via a laboratory computer, A/D and D/A converter, and custom software. Loads (12 tensile and 1 compressive) were sampled at 500 Hz.

Compliant platform

To emulate the in vivo response of indenting the skin overlying a compliant substrate, a platform was constructed with nonlinear compliance similar to the in vivo, bulk-compressive compliance of the rat medial thigh. The approach we adopted was to develop a platform composed of multiple layers of silicone rubber varying the compliance and thickness of each layer appropriately. Thus while the material properties of each layer were intrinsically linear, the bulk compliance of the multilayered platform to indentation was nonlinear for large deformations. Four sequential steps were required to construct the platform that have been reported previously in abstract form (Qin and Khalsa 1999).

First, the in vivo, compressive compliance of the skin/muscle of the medial thigh region was measured by indenting the region in intact Sprague-Dawley rats. A rat was anesthetized with an intraperoneal injection of pentobarbital sodium (25 mg/kg), laid on its back, and its right hindlimb was externally rotated (so that the medial thigh was “up”) and clamped to a hard platform. Anesthesia was maintained by periodic supplemental injections of pentobarbital sodium (5 mg/kg) to keep the animal areflexic to noxious stimuli. The skin of the medial thigh, overlying muscle and not the bone, was indented using the same indenter system as employed in the neurophysiological experiments. Compressive “creep” tests were performed as follows. Under force feedback control, the indenter was lowered to the skin until a minimal force was registered and held at this position for 0.5 s. Then a step indentation was performed to the desired force, the force maintained for 10 s, and then unloaded. Intertial intervals were 5 min to allow the skin and underlying substrate (principally muscle) to fully recover. Force and displacement were recorded at 100 Hz with the same equipment used for the neurophysiological experiments and were reported as the means of the last 0.5 s of the indentation. Each
indention was performed three times, and the results were averaged. Subsequently the compliance of just the underlying substrate was tested by excising the patch of skin being indented and repeating the same series of tests. No significant differences (P > 0.05) were found between the two sets of data, indicating that the bulk-compressive compliance of the hindlimb was determined by the underlying substrate and not the skin. By visual inspection, it was observed that the compliance curve could be represented by three linear regions. This suggested that the deformable platform could be constructed of three layers of silicone rubber with different thicknesses and different linear compliances, and thus reproduce the empirically determined nonlinear bulk compliance.

Second, the thicknesses of three layers of different linear compliances necessary to produce a nonlinear bulk compliance were calculated using finite element analysis with commercially available software (ABAQUS version 5.6, HKS, RI) (see Appendix for a description of finite-element analysis). Because the compliant platform was designed to be axially symmetric, it was sufficient to model it in two rather than three dimensions (Fig. 2). The compliant material was represented by 300 four-noded quadrilaterals (336 nodes), with dimensions of 35 (wide) × 17 (thick) mm. The model used a large strain, elastic formulation (hyperelastic) under plane stress. The constitutive behavior of the material was defined by the strain energy density. The elasticity was based on the Ogden (n = 2) form of total strain and total stress relationship. In the model, the material was compressed by a rigid indenter (3-mm diam) using the ABAQUS nonlinear geometry parameter. The geometry and the mesh refinement were biased toward the center of the specimen where the largest deformation occurred (Fig. 2). Bottom and side boundaries were constrained. A contact pair was defined between the surfaces of the indenter and the silicone in the contact region. Loading was applied through downward displacement of the indenter by a distance of 10 mm. Different combinations of the thicknesses of three layers were used in the model. Material properties for each silicone layer were defined from the linear regions of measured in vivo compliance curve and for rat skin came from those previously reported (Khalsa et al. 1997).

The nonlinear, compressive compliance of the intact skin-muscle was well fit with a power function (Fig. 3, solid line overlying crosses: $y = 1.544 \times x^{0.3836}$, $R^2 = 0.997$). The finite element model (FEM) produced a close fit to the in vivo data using three layers of silicone rubber with different thicknesses and compliances (from the bottom to the top layer: 2, 3, and 12 mm; and 0.036, 0.092, and 0.389 mm/g, respectively). The compliance was largest for the most superficial layer because the rat thigh became stiffer with greater indentation.

Third, pure silicone rubber (rubber compound, RVT615A; curing agent, RTV615B; courtesy of GE Silicones, Waterford, NY) was made with different compliances by varying the ratio of the compound to the curing agent. After curing for 24 h, the compressive compliance of the silicone rubber was measured in the same manner as described for the skin/muscle. Once the correct ratios of compound to curing agent were determined, the layers could be formed inside a rigid cylinder (35-mm diam and 17-mm height). To assure continuity between the three layers in the multilayer silicone platform, the bottom layer was poured first and allowed to cure for 24 h, followed in similar fashion by the middle and top layers. The bulk-compressive compliance of the platform then was measured as described previously to verify that it adequately emulated the in vivo nonlinear compliance (Fig. 3). It was not required that it match it exactly, only that it produced a similar form, as undoubtedly, the actual bulk compliance would vary by location of indentation in the hindlimb and in different animals.

Finally, validation that indentation of the in vitro preparation was similar to in vivo indentation was performed as follows. A rat was anesthetized and his hindlimb clamped as described previously. Hair was removed from the medial hindlimb with a chemical depilatory (Nair). Small flat reflective markers (1-mm diam) were glued on the skin, forming two lines orthogonal to one another with the ends of the lines meeting in the center of the patch of skin covering the medial thigh. The thigh was indented as described previously while optically tracking in three dimensions the positions of the markers with a kinematic analysis system (model 50, Qualisys, Glastonbury, CT). Subsequently, the same skin patch was excised from the rat, placed in the saline dish, coupled to the actuators, and suspended above the compliant platform. The in vitro skin then was indented as described previously while again optically tracking the displacements of the markers. Displacements of the in vivo and in vitro preparation were compared and found to be similar (Fig. 4).

Neuron recording, classification, and receptive field mapping

Neural recording and classification has been reported previously in detail (Khalsa et al. 1997). Briefly, the nerve was threaded from the saline compartment through a hole into an adjacent oil-filled chamber. Bundles of nerve filaments were teased apart until the neural response of single neurons could be discriminated. Neural responses were monitored on a digital oscilloscope, over an audio speaker, and by a template matching system (Spike2, Cambridge Electronic Design, UK). Only neurons responsive to mechanical stimuli and with conduction velocities (corrected for the room temperature saline bath) in the Aβ- and C-fiber range were included in this study. Neurons also were categorized by their response to noxious heat (35 and 55°C for 5 s.) and cold (ice chips on the skin for 10 s). The response of a neuron to the mechanical stimulus (10 s) was reported simply as the total number of action potentials that occurred (Khalsa et al. 1997).

The “compressive receptive field” (RFc) of some afferents were mapped using calibrated monofilaments (Stoelting, Chicago, IL) with the skin overlying a hard platform. A video camera imaged the skin onto a monitor over which was placed a clear acetate sheet. A dot was marked onto the acetate sheet at each location where indentation with the monofilament produced a neural response. The threshold response
reasonable, though arbitrary, choice of 10 s during each indentation. Values for indentations ranging from 10 to 80 g-f. Differences (residual displacements) between the in vivo and in vitro displacements were compared. Any neurons the sensitivity of which did change were no longer included in this study.

Experimental procedure

Once a suitable afferent was identified, its neural response first was calibrated to pure compression (i.e., compression of the skin overlying a hard platform) and, for some afferents, pure tension. Compressive loads were applied at the MSS by first lowering the indenter to the surface of the skin until a minimal force (3 g-f) was detected, maintaining this position for 0.5 s, step indenting to a predetermined force, maintaining this load for 10 s, and then unloading (Fig. 5). Intertrial intervals were 3 min to allow the skin to recover its prestimulus state and to allow the mechano-nociceptors to have stable responses for repeated stimulations (Garell et al. 1996; Grigg 1996). Ranges of loads were applied to encompass the threshold to saturation level for compression for each neuron. Compressive sensitivity was defined as the slope of a linear regression of the compression response curve. The hard platform then was replaced with the compliant platform centered under the MSS of the neuron. Indentation was performed in the same manner as described previously; however, because of the substantial compliance of the platform, the indenter would displace more than with the hard platform and because of the platform’s viscoelasticity, the indenter would “creep” during the 10-s stimulus (Fig. 5). The compressive force of the indentation was selected by choosing the value at or just below the saturation level determined from the sensitivity trials using the hard platform. Indentation first was performed at the MSS and then at consecutive 1-mm intervals, circling the MSS in an expanding clockwise fashion until an outer boundary was reached where there was no neural response. The intertrial interval was maintained at 3 min. Neurons the MSS of which was located at the periphery of the demarked central 14 mm square were stimulated only up to the margin of the square. The total number of trials for each neuron varied depending on the spatial extent of its response and whether it was possible to indent completely around the MSS. Hence for a neuron centrally located in the skin and with a large response area, to fully explore its response could require in excess of 150 trials to be performed. With a 3-min intertrial interval, this resulted in recording times that could, and did, exceed 7 h for some neurons. To verify that a neuron was not changing its sensitivity during these long recording periods, indentations at the MSS were repeated periodically (~1-h intervals) and the neural responses compared. Any neurons the sensitivity of which did change were no longer stimulated, and only the data from the earlier verified periods were used.

Spatial population response

An interpretation of the data collected as described in the preceding text was that it estimated, using the responses of a single neuron to stimulation at spatially discrete locations, how a uniformly distributed population of neurons with the same thresholds and sensitivities would respond to a single stimulus (Goodwin et al. 1995). Such a uniform spatial population response (SPR_u) was represented by plotting, for each nociceptor, the neural response (No. of action potentials/10-s stimulation) versus the x, y location of each indentation. The SPR_u was depicted as two-dimensional contour and/or three-dimensional (3D) surface plots; each method used a Kriging algorithm (Surfer, Version 6.02, Golden Software, Golden, CO), which is a “best-estimate method” of interpolating unknown data points of a surface while not altering the known data (Davis 1973). A few of the

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**FIG. 4.** Kinematic comparison of in vivo indentation of skin from the medial thigh of the rat with same area of skin indented in vitro over the compliant platform. Skin was indented with a cylinder (3-mm diam) under servo-force control. See METHODS for indentation protocol. The system was axi-symmetric, and hence the results for markers 7–11 were virtually the same as for markers 1–5. For clarity, results for only markers 1–6 are shown. A: in vivo indentation of 50 g-f. Because of their visco-elastic nature, the skin and its underlying substrate (i.e., principally muscle) continue to displace during the indentation. This is called “creep” in biomechanics. B: in vitro indentation of 50 g-f showing a similar rate and magnitude of indentation and creep. C: differences (residual displacements) between the in vivo and in vitro displacements for indentations ranging from 10 to 80 g-f. Values were compared at a reasonable, though arbitrary, choice of 10 s during each indentation.
neurons had MSS near the edge of the allowable stimulation area of the skin and hence were not able to be stimulated completely around the MSS. The SPRu for these neurons were estimated by reflecting only the zero-responses of the stimulated region to the nonstimulated region and allowing the Kriging algorithm to interpolate the unknown data from the recorded responses.

A spatial population response at a given stimulus intensity (SPRi) was developed from the SPRu as follows. The sensitivity and threshold (compressive load above which the neuron began to respond) data for each neuron were used to linearly scale the neural responses of its respective SPRu in 10-g-f increments (forming SPRu,i’s for each neuron) from the actual recorded values down to a minimum of 40 g or its threshold, whichever came first. For example, for a C fiber that was stimulated at 80 g-f (i.e., this value was at, or just below its saturation level) and had a threshold of 30 g-f, SPRu,i could be estimated at 70, 60, 50, and 40 g-f as well as the SPRu that was recorded at 80 g-f. Each SPRu,i was regressed nonlinearly (SigmaPlot 5.04, SPSS), using an equation for a Gaussian surface (Khalsa et al. 1998) of the form

$$z = f(x, y) = a \exp\left\{-0.5 \left(\frac{x-x_0}{b}\right)^2 + \left(\frac{y-y_0}{c}\right)^2\right\}$$

where x and y were the spatial coordinates, z was the neural response, and the rest were parameters to be fit to the surface (i.e., a was the peak magnitude; x0 and y0 were the offsets from 0 for the x and y axes, respectively, and, b and c were the widths of the surface at 60.7% of the peak magnitude along the x and y axes, respectively). At each stimulus intensity for Aδ- and C-mechano-nociceptor subpopulations, the means and standard errors of the fit parameters were reported.

The population responses at each stimulus intensity (i.e., 40–90 g-f) were displayed by the following procedure. First, for a given stimulus intensity and Aδ or C subpopulation, the neural responses were averaged at each spatial location. Second, the averaged spatial population response (SPRα,i) was nonlinearly regressed in the same manner as described for the SPRu,i. Third, the SPRα,i was displayed as a surface plot. The horizontal extent (i.e., the widths and areas of the “base” of the SPRα,i) was calculated at a reasonable, though arbitrary, value chosen at 10 spikes/10 s. The area of a slice was calculated by integrating over one of the horizontal parameters (e.g., xmin to xmax) the equation for a Gaussian surface solved for the remaining horizontal parameter (e.g., y) (Khalsa et al. 1998).

**Statistics**

Significance of the surface fit parameters was evaluated by one-way ANOVAs with repeated measures or Friedman’s one-way ANOVAs (if the data failed a normality test). Student’s t-tests were used to assess differences between individual groups. All statistical tests were done with a probability criterion for significance of $\alpha = 0.05$.
RESULTS

Seventy-eight afferents, either A\textsubscript{\textdelta} or C fibers, were isolated during 33 successful experiments. Afferents that did not display mechanical sensitivity were inappropriate for this investigation (i.e., they were either sympathetic efferents, chemosensitive nociceptors, or so-called “silent nociceptors” requiring chemical sensitization before they exhibit any mechanical sensitivity). Some afferents had mechanical thresholds too low to be considered nociceptors; and, some afferents had high mechanical thresholds but stopped responding prematurely to the stimulation protocol. Hence the results are based on recordings from 23 mechanically sensitive nociceptors (13 A\textsubscript{\textdelta} and 10 C) (Fig. 6). Corrected conduction velocities averaged 4.6 and 1.4 m/s for the A\textsubscript{\textdelta} and C afferents, respectively, and within each group, were not significantly different based on their ability to respond to noxious heat and cold (Fig. 7).

Nociceptor receptive fields

Compressive receptive fields (RF\textsubscript{c}), mapped using Semmes-Weinstein monofilaments, for A\textsubscript{\textdelta} and C mechano-nociceptors (n = 9) averaged 4.51 mm\textsuperscript{2} for suprathreshold stimuli and 1.60 mm\textsuperscript{2} at threshold (Fig. 8), slightly smaller than those reported in the monkey hairy skin (Treede et al. 1990) and much smaller than those in human hairy, leg skin (Schmidt et al. 1997). The mean threshold and suprathreshold RF\textsubscript{c} were larger for the A\textsubscript{\textdelta} (1.80 and 5.05 mm\textsuperscript{2}, respectively) than the C population (1.20 and 3.43 mm\textsuperscript{2}, respectively), but these differences were not significant (ANOVA, P > 0.05). For both A\textsubscript{\textdelta} and C nociceptors, receptive fields typically were composed of spot-like areas of response rather than a contiguous area to which the neuron responded. Hence the margins of the RF\textsubscript{c} were composed of the outer-most spots that responded to the monofilaments (either at threshold: median 36 mN, range 15–116 mN; or suprathreshold: median 150 mN, range 54–751 mN). Using monofilaments with larger compressive forces (i.e., greater than suprathreshold) did not increase the area of the suprathreshold RF\textsubscript{c}. Although threshold and suprathreshold RF\textsubscript{c} occasionally shared a boundary, the threshold RF\textsubscript{c} always were enclosed by the suprathreshold RF\textsubscript{c}. The mean compressive stress at threshold produced by the monofilaments was 34 kPa (range: 21–59; SE: 6.3), but given the small cross-sectional area of the filament, substantial, and immeasurable, out-of-plane shear stresses undoubtedly also were present.

Nociceptor threshold and sensitivity to compression

Nociceptor compressive thresholds (mean: 45 kPa) were the same whether indenting the skin over the hard or the compliant platforms and were not significantly different for the A\textsubscript{\textdelta} or C mechano-nociceptor populations (Fig. 9). This mean was only slightly larger than the compressive threshold calculated using the monofilaments. Compressive sensitivity, however, was substantially and significantly greater using the compliant versus the hard platform (Fig. 10). The increased sensitivity was undoubtedly related to the more complex stimulus (combined compression, tension, and shear) that developed during indentation using the compliant platform (Fig. 11).

Population encoding of noxious mechanical stimuli

Uniform spatial population responses (SPR\textsubscript{u}) were estimated from each nociceptor, and two representative nociceptors are shown using contour and surface plots (Fig. 12) and fitted with Gaussian surfaces (Fig. 13). Whereas most SPR\textsubscript{u} demonstrated a single peak at, or near, the location of the indentation, three exhibited more than one distinct peak (Fig. 14) and five others...
exhibited multiple, but very small, peaks. Averaged spatial population responses (SPR\(_{a,i}\)) for the \(A\delta\) and \(C\) subpopulations at compression stimulus intensities ranging from 40 to 90 g-f are shown fitted with Gaussian surfaces (Fig. 15). The intensity of the stimulus was represented by the peak neural response of the SPR\(_{a,i}\), which increased monotonically over the range of compressive stimuli (Fig. 15). The relationships between peak neural response was greater for the \(C\) than the \(A\) subpopulation.

The center location of the stimulus was represented by \(x_0\) and \(y_0\), the offsets from 0 along the \(x\) and \(y\) axes for the

**DISCUSSION**

Vallbo et al. (1993) were the first to report a spatial event plot for a mechanically sensitive \(C\) afferent; however, they stated that the afferent was not nociceptive as it had a threshold of \(<4\) g-f. The current study is the first report of a SPR of mechanically sensitive nociceptors to noxious indentation. The population response was created by combining the data obtained from individual mechano-nociceptors from different rats, though from the same area of skin. This method of sampling likely introduced variability in the data that would not have been present had an actual population of mechano-nociceptors been recorded. Further, the protocol required obtaining the data over long time periods (\(\geq 7\) h) to represent how a population would respond to a single indentation (lasting only 10 s). However, our data indicate that there was no
significant difference in spatial extent of the population response from different rats. In addition, the sensitivity of nociceptors to mechanical stimuli has been shown to be relatively constant over long recording periods in cats (Garell et al. 1996), rats (Khalsa et al. 1997), and in the current study.

The type of indenter used in this study also contributed somewhat to the variability of the data. Rather than a linear actuator, indentation was performed using a force-controlled torque motor that actuated a lever arm. For small displacements, like occurred during indentations of the skin overlying the hard platform, off-axis loads have previously been shown to be insignificant (Khalsa et al. 1997). However, for indentations of skin overlying the compliant platform, the displacements of the lever arm would be relatively large and the resultant force vector would develop an increasing angle proportional to the displacement. This was partially compensated for by allowing the indenter tip to be “self-centering” relative to the force vector (Khalsa et al. 1997). The angle of the resultant force vector may partially explain the offset of the SPR along the y axis. However, such a resultant force angle vector would only occur in the yz plane and should not have influenced the observed x axis offset.

The location of the noxious indentation was unambiguously encoded in the SPR. The x, y position of the peak magnitude of the SPR always was located underneath the indenter, although it did exhibit an offset from the center of the indenter. An explanation for this offset, in addition to experiment variability discussed previously, may be that it was encoding the most noxious portion of the indentation. From a mechanical perspective, the most damaging or noxious state would be where there was the highest energy. Strain energy density (SED) represents the energy developed in tissue due to an applied load, and it has been proposed as a mechanical quantity encoded by low-threshold mechanoreceptors (Dandekar and Srinivasan 1995; Grigg and Hoffman 1984; Srinivasan and Dandekar 1996) and mechano-nociceptors (Khalsa et al. 1997). The finite-element model of the in vitro skin and compliant platform predicted that, during indentation, the highest SED in the skin occurred near the edge, rather than in the center, of the indenter (Fig. 11). If mechano-nociceptors are responding to SED, then this would tend to shift the peak of the SPR from the center toward the edge of the indenter.

The intensity of the noxious indentation also was encoded in the SPR. Increasing compressive load was encoded by increasing peak neural response of the SPR, and this relationship was observed even with nociceptors of different sensitivities, thresholds, and saturations and in both the Aδ and C subpopulations. For both subpopulations, the peak neural response

**FIG. 12.** Uniform spatial population responses (SPR<sub>u</sub>) represented as contour and surface plots for representative A and C mechano-nociceptors. A: contour plot for AMC. B: surface plot for AMC. C: contour plot for CM. D: surface plot for CM. + locations of all the indentations performed for that nociceptor.
tended to saturate approaching a compressive force of 70 g-f (or, given the area of the indenter tip, a compressive stress of 221 kPa). This stimulus magnitude was considerably lower than that which produced suprathreshold pain rated as moderate to intense in human dental mucosa (Cooper et al. 1993). Unfortunately, there are few other studies of mechanically evoked suprathreshold pain (cf. Greenspan and McGillis 1991) and none that have examined it in hairy skin. Hence, we can only speculate on an explanation for this difference. One possibility is that it simply reflects a species difference. Another possibility is that it was due to the large deformations produced during the indentation that resulted in a three-dimensional combination of tension, compression, and shear. Hence, while the compressive force (or stress) may have been smaller than that necessary to produce moderate pain in humans (dental mucosa), the total state of stress and strain would have been relatively large in magnitude. The neural responses of single nociceptors in rat, hairy skin have been shown to correlate well with strain energy density, a scalar quantity reflecting the total energy developed in a volume of material during loading (Khalsa et al. 1997). Thus in the current experiments, the SPR may likely have been responding to the strain energy density, or a similar quantity, rather than simply to the compressive force (or stress).

The peak neural responses of the SPR were higher in the C than in A\(\delta\) subpopulations. This corresponded to the average higher compressive sensitivities of the C mechano-nociceptors that comprised the population studied. Other studies have found that A\(\delta\) mechano-nociceptors respond to indentation at higher rates than do C mechano-nociceptors (Garell et al. 1996; Handwerker et al. 1987). However, when the effects of tension were removed explicitly from those of compression, some classes of C mechano-nociceptors were more sensitive to compression and tension than A\(\delta\) mechano-nociceptors (Khalsa et al. 1997). Hence combinations of tensile and compressive stimuli could produce higher response rates in C than A\(\delta\) mechano-nociceptor populations, as was found in the current study.

The overall “form” (peak, slopes, and base area) of the SPR was comparable with that previously predicted in a first-order model (Khalsa et al. 1997). All three form features would be scaled by the sensitivities of an actual population. For example, a reduced tensile sensitivity would decrease the peak neural response and the areal extent of the base of the SPR as was seen in the current study. The areal extent, representing the portion of a population of neurons responding to a given stimulus, of any SPR will be dependent not only on the relative tensile compliance of the skin region, but also the bulk compliance and geometry of the underlying tissue (e.g., muscle).

The SPR had a central high point (i.e., a peak), and the neural responses at all other locations were lower. This contrasts with the prediction of Khalsa et al. (1997) of the largest neural responses being next to the edges of the indenter along the x axis rather than toward its center. That prediction was based on measurements of high tensile sensitivity of nociceptors and the highest tensile strains occurring next to the indenter. The finite-element model in the current study also estimated that the maximum tensile stresses occurred next to the edge of the indenter along the x axis rather than toward its center. That prediction was based on measurements of high tensile sensitivity of nociceptors and the highest tensile strains occurring next to the indenter. The finite-element model in the current study also estimated that the maximum tensile stresses occurred next to the edge of the indenter (Fig. 11). A few SPR\(_u\) did exhibit bilateral peaks next to the edge of the indenter (Fig. 14), although more commonly only a single peak was observed and on average it was offset from the center almost 1 mm. Another explanation for the phenomena (shown in Fig. 14) would be that shear stress (or strain) caused the high neural response at the edge of the indenter. The finite-element model estimates that both shear stress and strain are maximal at the edge of the indenter (Fig. 11). For the SPR\(_u\) (shown in Fig. 14), the edge of the indenter would be positioned, in principle, over the most sensitive spot of the neuron’s receptive field when the indenter was centered at ±1.5 mm along the x or y axes. If there was a
symmetrical response to the indentation, this would produce a SPR formed similar to a “volcano” with the maxima along the edges of the central crater. The data from this study do not allow making a determination as to whether the observed phenomena was due to high tensile stress, high shear stress, or some combination of both. Finally, multiple terminal endings of single nociceptors also would tend to broaden the SPR and make the peak solitary rather than annular.

The SPR suggests a possible mechanism for encoding some aspects of shape of a noxious mechanical stimulus. Not only was the SPR well fit with a Gaussian surface, but the widths of SPR (i.e., the $b$ and $c$ parameters and hence area) remained unchanged, as was the area of the indenter, with increases of compressive load. Evidence to support this conjecture comes from two different venues. First, Vallbo et al. (1999) recently have demonstrated that mechanically sensitive C afferents can encode nonnoxious tactile stimuli. Low-threshold C mechano-nociceptors encoded dynamic loads during indentation using probes with both rounded and sharp tips. In both cases, there was a noticeable time lag between the stimulus and the neural...
response, but the response was proportional to the stimulus. High-threshold C mechanosensitive afferents, presumably mechano-nociceptors, showed proportional response to noxious indentation. They also documented that low-threshold C mechanoreceptors can encode movement of objects, although the correlation was better at low than high velocities. Second, population responses of Aβ, low-threshold, slowly adapting mechanoreceptors have been shown to encode object curvature during indentation in monkey fingerpad (Goodwin et al. 1995; Khalsa et al. 1998), human glabrous skin (Goodwin et al. 1991, 1997) and during stroking in the monkey fingerpad (Friedman et al. 1998). All these population responses were also well described by Gaussian surfaces. Thus taken together, these previous studies and the current study suggest a possible role for C mechano-nociceptors to encode some aspects of object shape during noxious stimulus and particularly during noxious indentation.

This study did not measure the actual number of mechano-nociceptors innervating the region of skin (i.e., the innervation density) that was mechanically stimulated nor the size and extent of overlap of their receptive fields. Our estimate of a SPR to noxious indentation is based on an assumption of relative uniform distribution of the terminal endings of mechano-nociceptors. If an actual population was distributed nonuniformly, our data suggest that noxious indentation by a symmetrical object would result in a SPR the form of which would be skewed and its peak location significantly offset from the actual site of indentation. However, the effect of innervation density should only be to improve the accuracy of encoding magnitude and location with increasing density. Similarly, increasing innervation density also should improve the postulated ability to encode object shape.

In conclusion, this study found that the location and intensity of noxious, compressive stimuli were encoded in the spatial population responses of Aδ and C mechano-nociceptors.

Appendix

The finite element method (FEM, also termed finite element analysis—FEA) is a general-purpose numerical procedure for analyzing structures and continua. In simple terms, the FEM solves a complex problem by subdividing it into a collection of smaller and simpler problems that can be solved using computational techniques. Although originally developed to solve stress-distribution problems in aircraft frames, it has since been evolved to analyze heat transfer, strain energy, fluid flow, electrical fields, and cellular mechanics in biological tissues (Grandin 1991). It was used in the current experiments in two fashions: to predict the number, thickness, and compliances of different layers of silicone rubber comprising a deformable platform used for in vitro experiments to emulate experimentally measured in vivo, bulk-compressive compliance of the rat medial thigh and to estimate the spatial distribution of mechanical stress and strain energy density developed during indentation of rat skin.

Conceptually, to implement the FEM two separate but related processes are undertaken: the geometry of the tissue is mathematically described by a finite number of small “elements” (i.e., connected regions or volumes) and a mathematical expression is formulated that describes the stiffness (i.e., resistance to deformation) of the elements so that when loaded, the displacements of various points within the
global structure can be calculated. The stiffness of each element is determined from both its geometric properties (shape) and from the material behavior that is assigned to each element. Mechanical stresses and strains then can be calculated for each element from the structural displacements. When the formulation that describes the material is the same function as the shape function used to define the element geometry, as was done in these experiments, this function and the family of elements are called isoparametric.

Soft tissues, like skin, derive their tensile strength primarily from collagen fibers, and secondarily from elastin, embedded in the extracellular matrix (Lanir 1976, 1979). The collagen and elastin fibers are individually linearly elastic, but the bulk-stress-strain relationship of skin is nonlinear due to the inhomogeneous composite nature of its structure (Maurel et al. 1998). After preconditioning (Fung 1993), the short-time stress-strain relationship is pseudoelastic, meaning that one can model the response ignoring its viscous components. However, skin undergoes relatively large deformations for small loads. In the FEM, a material exhibiting nonlinear, large deformations not dependent on the rate of loading is termed “hyperelastic,” and its constitutive behavior is defined as a total stress–total strain relationship as follows (Maurel et al. 1998):

Writing the current position of a material point as \( x \) and the reference position of the same point as \( X \), the deformation gradient is

\[
F = \frac{\partial x}{\partial X}
\]  
(A1)

Then \( J \), the total volume change at the point, is

\[
J = \det (F)
\]  
(A2)

For simplicity, we define

\[
\tilde{F} = J^{-1/2} F
\]  
(A3)

as the deformation gradient with the volume change minimized. Then the deviatoric stretch matrix of \( F \) (i.e., the change in shape without change in volume) can be defined as

\[
\tilde{B} = \tilde{F} \cdot \tilde{F}^T
\]  
(A4)

From the deformation matrices, two quantities, invariant with respect to an arbitrary choice of axes, can be defined as the first and second strain invariants \( I_1 \) and \( I_2 \), respectively as

\[
I_1 = \text{trace} \tilde{B} = 1: \tilde{B}
\]  
(A5)

\[
I_2 = \frac{1}{2} (\tilde{I}_1^2 - \text{trace} (\tilde{B} \cdot \tilde{B})) = \frac{1}{2} (\tilde{I}_1^2 - 1: \tilde{B} \cdot \tilde{B})
\]  
(A6)

where \( I \) is a unit matrix.

A hyperelastic material strain-stress relationship can be derived from an internal strain energy function. For purpose of experimentation, the strain energy functions are more appropriate expressed using the strain invariants \( I_1 \), \( I_2 \), and \( I_3 \), respectively as

\[
I_1 = \lambda_1^2 + \lambda_2^2 + \lambda_3^2 = I_1
\]

\[
I_2 = \lambda_1 \lambda_2^2 + \lambda_2 \lambda_3^2 + \lambda_3 \lambda_1^2 = I_2
\]

\[
I_3 = \lambda_1 \lambda_2 \lambda_3 = I_3
\]  
(A7)

In an undeformed state, \( I_1 = I_2 = 3 \), and \( I_3 = 1 \). The general form of the strain energy may then be written as an infinite series in power of \( (I_1 - 3) \), \( (I_2 - 3) \), and \( (I_3 - 1) \) (Ogden 1984)

\[
W(I_1, I_2, I_3) = \sum_{p,q,r=0}^\infty c_{pqr}(I_1 - 3)^p(I_2 - 3)^q(I_3 - 1)^r
\]  
(A8)

where \( c_{000} = 0 \), and \( p, q, r \) are integers. Or

\[
W = \sum_{p,q,r=0}^\infty a_{pqr} \left[ \lambda(I_1 + \lambda^2) + \lambda^2(I_1 + \lambda^2) + \lambda^4(I_1 + \lambda^2) \right] (\lambda_1 \lambda_2 \lambda_3)^{-6} - 6
\]  
(A9)

where \( c_{000} = 0 \).

It is convenient to describe \( W \) in terms of distortional and dilatational energies as

\[
W(I_1, I_2, I_3) = W_1(I_1, I_2) + W_2(I_3)
\]  
(A10)

\[
W_1(I_1, I_2) = \sum_{p,q,r=0}^\infty c_{pqr}(I_1 - 3)^p(I_2 - 3)^q(I_3 - 1)^r
\]  
(A11)

Skin, however, can be considered virtually incompressible (North and Gibson 1978) and for such materials, we note that \( (\lambda_1 \lambda_2 \lambda_3)^2 = 1 \) and \( (\lambda_1 \lambda_2 \lambda_3)^n \) tends to 1 (for \( n = 1, 2, 3, \ldots \)),

\[
W_1 = \sum_{p=1}^\infty 2a_{p12}(\lambda_1^2 + \lambda_2^2 + \lambda_3^2 - 3)
\]

allowing \( W_2 \) to effectively be zero. Thus for this case, the Ogden form of the strain energy function depends only on \( I_1 \) and \( I_2 \) and is described as

\[
W = \sum_{i=1}^N \frac{2\mu_i}{\alpha_i^2} (\lambda_1^2 + \lambda_2^2 + \lambda_3^2 - 3)
\]  
(A12)

where \( N \) is a parameter chosen to describe the degree of nonlinearity in the strain-stress relationship, and \( \mu_i \) and \( \alpha_i \) are material parameters determined by the specific form of the stress-strain curve. Ogden’s energy function cannot be written explicitly in terms of \( I_1 \) and \( I_2 \). However, it is possible to obtain closed form expressions for the derivatives of \( W \) with respect to \( I_1 \) and \( I_2 \).

Once this strain energy function is formed, then the principal components of the Lagrangian stress tensor \( \sigma \) are derived as (Allaire et al. 1977):

\[
T_{ii} = 2 \frac{\mu_i}{\alpha_i^2} \left[ \lambda_1^2 \frac{\partial W}{\partial I_1} + \lambda_2^2 \frac{\partial W}{\partial I_2} + I_1 \frac{\partial W}{\partial I_3} + I_3 \frac{\partial W}{\partial I_3} \right] i = 1, 2, 3
\]  
(A13)

For the current experiments, parameters \( \mu_i \) and \( \alpha_i \) were determined from uniaxial tests of strips of skin. Because the deformable platform was axisymmetric, it was sufficient to model it as a central plane along the longitudinal axis, hence in two dimensions.

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