Rat Cutaneous RA Afferents Activated by Two Dimensional Skin Stretch.

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Abstract.

Skin develops biaxial stresses and strains when stretched. Rapidly adapting cutaneous mechanoreceptor neurons are known to be stretch sensitive, yet in the past they have been studied using stretch stimuli applied along only a single direction. In this study, cutaneous rapidly adapting mechanoreceptors were studied in preparations of isolated skin in which the skin was stretched dynamically using biaxial stretch stimuli and in which loads and displacements were measured along 2 directions. Stretch stimuli followed a pseudo Gaussian waveform, and were applied along either one or two directions simultaneously. Associations between spikes and mechanical variables were determined using multiple logistic regression. When the skin was actuated along a single direction, holding the orthogonal axis fixed, spike responses were strongly associated with mechanical variables along the actuated direction. The variables were stress and its rate of change, the rate of change of strain, and the product of stress and its rate of change, which is proportional to strain energy density. When the skin was stretched along a single direction, spikes were very poorly associated with stress variables measured along the direction orthogonal to the stretch. Afferents showed weak directional selectivity: they were slightly more responsive to the variable, stress, along the circumferential direction of the hindlimb. When the skin was stretched biaxially (i.e., along both directions simultaneously) with identical pseudo Gaussian noise stimuli, neuronal responses were associated with the same variables as above, but the associations were weaker.
Introduction

Skin stretch is a powerful stimulus for many cutaneous mechanoreceptor neurons. Skin is commonly approximated as a 2-dimensional structure, so the stimulus a cutaneous mechanoreceptor neuron experiences in relation to stretch may be approximated as a 2-dimensional state of stress or strain. These stresses and strains may include tensile, compressive, and shear components. Therefore, in characterizing the way mechanoreceptor neurons respond to stretch stimuli, it is important that stimuli be 2-dimensional. In previous studies from this laboratory, the properties of stretch-sensitive mechanoreceptor neurons were studied during 2D skin stretch (Del Prete et al. 2003; Grigg 1996). Those studies subjected an in-vitro preparation of isolated specimens of rat skin to planar biaxial stretch stimuli. Spike responses were recorded from cutaneous afferent neurons. The first of those studies (Grigg 1996) used an apparatus capable only of slow movements; only static stimuli could be created, and only slowly-adapting afferents (SA2 and C mechanoreceptor afferents) were studied. SA2s had strongly preferred directions for stretch. It was possible to design biaxial stretch stimuli in which stress and strain were varied independently of each other along the preferred direction of SA2 afferents. The neural response of SA2s was found to be strongly related to tensile stress, and weakly associated with tensile strain. A limitation of those experiments was that although most cutaneous mechanoreceptors are rapidly adapting (RA), it was not possible to study them because the applied stimulus was too slow. In subsequent experiments, Del Prete et al. (2003) and Robichaud et al. (2003) studied the stretch responses of cutaneous RA afferents. They used 2-dimensional, isolated skin specimens and stimulated them using dynamic stretch. The skin sample was held by all its edges but was actuated along only one direction; it was simply fixed along the orthogonal direction. Stress and strain were measured along the actuated direction, and relationships were sought between spike responses and uniaxial measures of tensile stress and strain variables. However, stretching skin along one direction also produces loads and displacements along the orthogonal direction because of the Poisson effect. It was a limitation of those experiments that while the stimulus was biaxial, only uniaxial variables were analyzed.
The current studies were designed to overcome the limitation of the previous experiments. Here, we study cutaneous mechanoreceptors using an apparatus that allows for controlling dynamic stretch stimuli along two orthogonal directions in biaxially loaded skin samples.

**Methods**

With a few exceptions, the preparation, apparatus and experimental methods for recording afferent neurons follow those described in the recent communication from this laboratory (Del Prete et al. 2003).

Experiments used an in vitro preparation of innervated skin samples from adult Sprague-Dawley rats of either sex. The Institutional Animal Care and Use Committee approved all procedures involving animals.

Rats were anaesthetized with sodium pentobarbital (IP, 45 mg/kg). The hair on the inner surface of the hindlimb was clipped with electric shears, and was removed with a commercially available chemical agent (Nair). The outline of the sample (a cross shape, approximately 15 mm from end-to-end) was marked on the skin (Figure 1A). A thin, 5mm wide plastic tab was glued at each end of the sample, using cyanoacrylate adhesive. The skin along with the tabs was then cut around its margins and excised along with the cutaneous nerve innervating it. The resulting skin-nerve specimen was then removed to an apparatus designed to stretch the skin dynamically along two directions (Figure 1B).

In the apparatus, the skin specimen was bathed in Hepes-buffered (pH 7.4) artificial interstitial fluid (Koltzenburg et al. 1997). The plastic tabs were used to couple the skin to the apparatus. Two tabs were coupled to the lever arms of two Aurora Scientific model 300B lever systems, which were used to actuate the specimens. The Aurora lever system is a rotary DC servomotor with a lever on its shaft. The motor can be operated in force or position control mode. Small rotary displacements of the shaft cause approximately linear displacements at the end of the lever. A string, with a small hook at its end, was attached to the end of each lever. The hooks were engaged in a hole at the end of the tabs. The other tabs were coupled via similar strings to the sides of the apparatus (Figure 1B).
Neurons were sampled only in the center of the sample (the grey area depicted in Figure 1). Stress was determined from the applied loads and tissue geometry. Loads were measured directly from the Aurora actuators. The cross section area was calculated from the width of the tabs, measured from digital photographs made of specimens when mounted in the apparatus, and using 0.3 mm (Grigg 1996) as thickness.

Strain was measured with 2 methods. The first method relied on actuator displacements measured with the Aurora actuators. However there were several problems associated with measuring strain from actuator displacements. First, when a skin sample is actuated along some direction as depicted in Figure 1B, local strain in the central region of the skin is smaller than in the tabs. Thus strain can not be directly determined using actuator displacements. For this reason, recordings of actuator displacements could be used only to calculate a pseudostrain, using the expression \( E = \Delta L / L_0 \) where \( \Delta L \) was the actuator displacement and \( L_0 \) was the initial length of the sample between the plastic tabs. The second problem with using actuator displacements was that in experiments where the margins of the skin were fixed along one direction, the actuator displacements (and therefore the pseudostrains) along that direction were zero. In contrast, it was anticipated (and in fact we showed) that the Poisson effect would cause strains to be finite and negative along that direction. In order to determine the magnitude of the problems that were caused by the above limitations, we measured actual tissue strain in several initial experiments. We used a method based on tracking surface markers with a video system. Four black markers were fixed on the central region of the skin (Figure 1B), and their locations were determined from video images taken while the skin was stretched. The video system used a UNIQ UF-1000 camera fitted to a dissecting microscope mounted over the apparatus. Images were taken at 500 /s, and were synchronized with the acquisition of other data. The data stream from the camera was managed by a dedicated PC equipped with a Coreco PC-Dig Frame Grabber and running Video Savant (IO Industries) software. Each image was time-stamped and binarized in real time, and was stored sequentially on two SCSI hard drives. The images were post-processed to determine the location of the centroids of the four markers. Displacements of centroids were calculated and used to compute normal and shear strains (Hoffman and Grigg 1984). This method proved to be too complex for routine use, but it was used
successfully in several experiments to validate the use of actuator displacements to measure strain. Pseudostrain calculated from actuator displacements was found to be different from the true strain. However, stress and strain data were normalized before logistic regression analyses were done, so that it was possible to use the actuator displacement method for determining strains. The experiments in which one boundary was fixed constitute a different problem since true strains were small and negative while the actuator recorded zero strain. However, because we found no relationship between neuronal responses and true strains along the fixed direction, we ignored those strains.

The nerve innervating the specimen was drawn into a small recording chamber filled with mineral oil. It was treated with a collagenase solution (Worthington, CLS1), rinsed off, and then dissected into filaments with sharpened tweezers. Individual filaments were placed on a recording electrode made from platinum wire; the indifferent electrode was placed in the bath. Rapidly adapting afferents were identified by stroking the skin with a blunt glass probe or by pulling on individual tabs. It was not possible to obtain estimates of conduction velocity. The short length of the nerve and the fact that the specimens were wetted with saline meant that stimulus artifacts overwhelmed the relatively small spike potentials in the 0 – 3 msec latency period during which evoked spikes were expected. Neural activity was amplified with an EG&G PARC Model 113 preamplifier; noise in phase with the 60 Hz line frequency was removed with a Riverbend Learning Filter. Individual neuron activity was discriminated using a template matching algorithm (SPS; Prospect, S. Australia). Neuronal recordings were classified as arising from single neurons based on the constant size and shape of the action potential. The SPS system outputted a TTL pulse to signal the presence or absence of a spike, which matched the template. This output was recorded along with the load and displacement data from the two servomotors.

Specimens were subjected to two different forms of biaxial stretch stimuli, referred to as protocols. Figure 2 shows examples of loads and displacements measured in each protocol.

In the ‘uniaxial actuation’ protocol (Figure 2A), the sample was actuated along one direction in force control with a PGN waveform; both load and actuator displacement were measured along this direction. The motor in the orthogonal direction was operated
in position control mode; its position was fixed and loads were measured. The pseudo Gaussian noise (PGN) signal was created with a computer program, using LabView software. The frequency bandwidth for all PGN signals was 0-60 Hz. PGN signals were outputted to the appropriate motor. Two tests were performed along each direction: one with a low stress PGN (mean amplitude = 10 kPa) and another with a high stress PGN (mean amplitude = 40 kPa). Uniaxial actuation tests were done along both X and Y directions. Thus in this protocol each neuron was studied using four separate runs.

In the ‘symmetrical biaxial’ protocol (Figure 2B), the skin was stretched with force-controlled PGN stimuli along both axes simultaneously. Differently scaled versions of the same PGN signal were used to control the two motors. In all cases, one motor was actuated with a 40 kPa amplitude PGN. In successive runs, the mean amplitude of the control signal to the orthogonal motor was increased from 10 kPa to 40 kPa. The stimulus to the skin thus ranged from asymmetrical to increasingly symmetrical stretch. Asymmetrical biaxial runs were done in order to explore the possibility that spike responses might be caused by shear stress. The maximal values of shear stress, along directions other than the directions of stretch, is proportional to the difference in the magnitude of the 2 normal stresses. Thus shear stress would be maximal in uniaxial actuation trials, minimal in symmetrical biaxial trials, and intermediate in asymmetrical biaxial trials.

In each protocol, neuronal activity was recorded along with load and displacement data from both motors, at 2 ms intervals. Data collection runs were 30 seconds in duration, and analyses were based on data collected during the entire 30 sec period. There was a 3 minute rest period after each run.

We used multiple logistic regression (MLR) analysis to determine the strength of association between mechanical variables and spike discharge for each run. MLR is a multiple correlation method used in situations where there are multiple predictor variables and a binary outcome event (Hosmer and Lemeshow 1989). Its use in determining the relationship between multiple mechanical inputs and spike responses of neurons is described in detail in Del Prete et al. (2003). The methods used here follow exactly those described in Del Prete et al. (2003). All predictor variables were normalized to a mean = 0 and standard deviation = 1 before performing MLR analyses.
Memory effects, whereby a stimulus applied at a particular time can have an effect observed later in time, were quantified using ‘lag’ analysis. The outcome of MLR analyses is an odds ratio, whose magnitude reflects the strength of the association between predictors and the binary spike events.

The goal of multiple regression is to determine the association between multiple predictors and a common outcome variable. However as the number of predictors in a multiple regression model increases, the model can become overfitted and numerically unstable (Hosmer and Lemeshow 1989, Chapter 4). Our strategy in previous experiments (Del Prete et al. 2003) was to use a model, which included every scientifically relevant factor. Since we had little a priori information about RA afferents, that meant including all factors measured in the experiment. However, the number of factors in those experiments was relatively small. There were 4 main factors: stress (σ), its time rate of change (dσ/dt), strain (ε) and its time rate of change (dε/ dt), and there were 6 interaction terms, for a total of 10 factors in the model.

However in the current biaxial experiment, the number of main factors is 8 (σ, dσ/ dt, E and dE/ dt in each direction), and the number of first-order interaction terms is 28. When this many factors are included in the model, considerable confounding is present. This resulted in overfitting of the model, and numerical instability of solutions. Very large odds ratios were obtained and odds ratios for a particular factor could be very different in successive runs. For this reason we restricted the dimensionality of the model by selectively including and excluding factors. Our main strategy was to perform separate analyses for factors along the two axes of the sample. We used separate analyses to test the strength of association between a set of spikes and the predictors measured along the X direction, and, in separate analyses, the Y direction. In order to determine whether there were any interactions between predictors along the X and Y directions, we followed the guidelines for model building outlined in Hosmer and Lemeshow (1989): we tested for interactions using analyses in which we used all the variables along one direction while variables measured along the orthogonal direction were included one at a time.

Results.
Experiments were performed using 9 adult rats of either sex, average body mass = 200 grams. Not every experimental protocol was used with each isolated neuron. Twenty neurons, from 9 experiments, were tested using the uniaxial actuation protocol; 11 neurons, from 7 experiments, were tested using the symmetrical biaxial protocol.

The dot-tracking method was used to measure strain in several of the initial experiments. Tensile strains were approximately half the magnitude of the pseudostrains obtained using motor displacements (Figure 3). Pseudostrains were, however, closely ($r^2 = 0.79$) related to the true strains. Shear strains were very small; in the run depicted in Fig.3, the mean magnitude of shear strain was 0.012. Analysis of strains using marker displacements also allowed us to determine the true biaxial strain in uniaxial actuation runs in which one actuator was fixed. Since the position of one motor was fixed, pseudostrains calculated from motor displacements along that direction yielded a value of zero. The video method, however, revealed small negative strains along that direction, (e.g., see Figure 2A, right panel).

Initial MLR analyses were done using both true strains and pseudostrains. There were no differences between odds ratios calculated using pseudostrains and those calculated using true strains. This is because the pseudo strains are essentially a scaled version of the real strains (Figure 3). Because all variables were normalized prior to doing MLR analyses (Del Prete et al. 2003), scaling the strain values had no effect on the outcome of the MLR analyses. Strains measured along the fixed direction in uniaxial actuation trials were different. Since the actuator along that direction was fixed, pseudostrain along that direction was zero. The actual strains, measured with the video system, were small and negative (Figure 2). However, odds ratios calculated using the true strains revealed no association between spikes and strains. Because it was much simpler to measure motor outputs than to use the video method, and because the outcome was the same in either case, MLR analyses were routinely performed using pseudostrains determined from actuator outputs.

Using the data from each run, odds ratios were calculated between spikes and the following mechanical variables (also referred to as predictors, for their role in logistic regression analyses): stress ($\sigma$), pseudostrain (E), their time rates of change ($d\sigma/dt$ and $dE/dt$), and six first-order interactions between those factors, along each direction of the
sample. In testing for memory effects, spikes were shifted with respect to the predictors in increments of 2 ms for lags between 0 and 50 ms. Thus 26 MLR analyses were done using mechanical variables measured along the X direction and 26 more were done using mechanical variables measured along the Y direction. Figure 4A shows results obtained from a single uniaxial actuation run. Averaged results from all 20 neurons that were studied the same way are shown in Figure 4B. These results are similar to those obtained previously with uniaxial stretch (Del Prete et al. 2003; Robichaud et al. 2003). There was a strong association between spike response and dσ/dt, with a peak at memory times ranging from 10 to 14 ms, and there was a weaker association with σ. There was also a weak association with the interaction dσ/dt x σ, which is proportional to incremental strain energy. There was no apparent relationship between spikes and E.

We wished to know whether, in uniaxial actuation runs, neuronal responses were influenced by the stress variables observed along the direction orthogonal to stretch. Figure 5 illustrates the absence of such an effect: spikes were only weakly associated with those variables. The left panel shows the strength of association between the spikes and the variables measured along the direction of stretch, and the right panel shows the much weaker association between the same spikes and the stress variables measured along the (fixed) orthogonal axis. The same result was observed irregardless of whether the stretch was applied along the X or the Y direction.

In order to determine whether neurons had a preferential response to uniaxial actuation along the X or Y directions, we compared neural responses to X and Y direction stretches using the uniaxial actuation protocol. Odds ratios were calculated between spike responses and the predictors along the direction of stretch, for both X and Y directions. There were 4 predictors with significant odds ratios; namely σ, dσ/dt, dE/dt, and dσ/dt x σ. The odds ratios for these predictors are compared in Figure 6. An effect of direction was observed in the association between spikes and σ (Figure 6B): odds ratios between spikes and σ were significantly higher for Y direction stretches than for X direction stretches. Otherwise, there was no directional preference in the odds ratios for any of the other predictors.

The symmetrical biaxial trials were initially done in order to determine whether the responses to X and Y direction stretch were additive. It was found that simultaneous
stretch along both directions actually resulted in a smaller response than either X or Y direction uniaxial actuation (Figure 7). Spikes were associated with the same predictors as in the uniaxial actuation runs, although the odds ratios were smaller than in the uniaxial actuation runs, and there was no evidence for any directional selectivity in the response.

The finding that symmetrical biaxial stretch led to lower odds ratios than were observed with the uniaxial actuation paradigm, led to the hypothesis that shear stress might be a factor in eliciting activity form neurons. For that reason we undertook trials in which the degree of symmetry of biaxial loading states was systematically varied. However, the degree of asymmetry in these trials did not match the magnitudes of the odds ratios that were observed (Figure 8). The peak value of the odds ratios for \( \sigma \) and \( d\sigma/dt \) were consistently smaller than in the uniaxial actuation runs, and did not increase with the degree of asymmetry.

**Discussion**

A concern with previous studies of RA afferents was that while the stimuli caused biaxial states of stress and strain, the analyses were strictly one-dimensional. The skin was stretched along a single direction and analyses were based on variables measured along just that direction. What was left unclear by those experiments was (1) whether variables other than the (uniaxial) ones measured in that experiment might be contributing to the response and (2) whether neurons might respond differently if they were stretched along a direction other than that single direction. Here we show that afferents’ response to stretch is not highly directional. In uniaxial actuation trials, the only variable for which there was directional selectivity was stress. Afferents were more sensitive to stress along the Y direction (i.e., the circumferential direction on the leg) than the X direction. Furthermore, in uniaxial actuation runs, there was little evidence of a contribution from predictors along the direction orthogonal to stretch. When we stretched the skin along one direction while the orthogonal boundary was fixed or actuated in position control, spikes were associated with the same predictors along both
directions. The small odds ratios between spikes and stress variables along the fixed direction (i.e., Figure 4) likely reflect the small magnitude of the stresses present along that direction. In this light, it should be noted that a variable that is a strong predictor may possibly have a low odds ratio in a particular analysis, if its amplitude is small.

Biaxial loading allowed us to determine how a neuron’s response to X and Y loads interact when those loads are presented simultaneously. The symmetrical biaxial experiments revealed that simultaneous X and Y direction stretch resulted in a reduction of response compared to X or Y loading alone. This finding suggested a dependence of neuronal responses on shear stress, since the maximal value of shear stress along planes other than those actuated is proportional to the difference in the magnitudes of the 2 normal components. However, when the degree of symmetry of biaxial loads was manipulated, with the intent of systematically varying shear stress, the findings did not support a shear stress model (Figure 8). Thus, the potential role of shear stress in activating these neurons is unclear and will require further study.

All of the reported results are based on MLR analyses in which the number of predictors was restricted in order to avoid overfitting. A limitation of the logistic regression method is that it was not possible to consider the effect of all the predictors (i.e., along both directions) in a single analysis. Our approach was to break the analyses down into two components, involving predictors measured along the X and Y directions respectively. The potential error in this approach arises if there are interactions between X and Y direction predictors. For example, the association between spikes and some X direction variable might depend on the level of a particular Y direction variable. We tested for such interactions using analyses in which we included all the variables along one direction (say, X) while including Y direction variables one at a time. These analyses did not reveal any significant interactions between the X and Y predictors, suggesting that our basic strategy was acceptable.

A potential concern is the limitation in accuracy of estimates of stresses and strains. First, measuring strains by tracking surface markers has been shown to be very accurate (Grigg & Hoffman, 1984), and the pseudostrains we used were closely related to the true strains ($r^2 = 0.79$). Pseudostrains were greater than the true strain by a factor of 2 (Figure 3). However, since the values of all the predictors were normalized to a mean = 0
and SD = 1 before MLR analyses, any prior scaling would be without effect on the outcome of those analyses. Stresses were based on the assumption that the applied loads were distributed uniformly through the area within which neurons were sampled. This assumption is based on the fact that we applied loads through solid tabs, to skin tabs that had an aspect ratio of approximately 2. Prior analyses of the distribution of loads in tabs (Flynn 1998) suggests that the resulting stresses should be quite uniform. The uniformity of the strains that were observed with the dot tracking system also suggests a uniform loading state.

We have used the magnitude of odds ratios to draw conclusions about the relationship between spike responses and predictors. In interpreting these findings, it is important to recognize that the numerical value of an odds ratio is tied to the units of the predictor variable. In our analyses we normalized the values of each independent variable to have a mean = 0 and standard deviation = 1.0. An odds ratio of 8 means a stimulus that is 1 standard deviation greater than the mean is 8 times more likely to elicit a spike than a stimulus whose magnitude is equal to the mean.

The large difference in odds ratios seen between uniaxial trials and symmetrical biaxial trials suggested a potential role for shear stress in activating neurons. When a tissue sample is loaded biaxially, increments in the magnitude of shear stress are determined by the difference in the magnitude of the normal stresses applied along the 2 directions. Increments in shear stress would be greatest when the degree of asymmetry in biaxial loading was greatest. In contrast, applying equal stresses along each direction would create zero increments in shear stress. However, when we systematically altered the degree of asymmetry in biaxial trials, it was not reflected in the odds ratios between spikes and $\sigma$ and $d\sigma/dt$. Further experimentation in which shear stress is directly controlled will be required to resolve whether spike responses are associated with shear stress.

Our finding that RA afferents have limited directional selectivity is generally consistent with the findings of Birznieks et al. (2001), and Grigg (1996) who reported that cutaneous RA afferents were not directionally selective.

We were unable to obtain measures of conduction velocity for these afferents. While we do not have evidence to positively identify them, we have reasonable evidence
that they are not A-δ or C afferents. While it is difficult to measure conduction times for fast-conducting axons in these preparations, it is relatively easy to measure conduction velocities in slower conducting, A-δ and C afferents (Khalsa et al. 1997; Zheng et al. 2002). None of the filaments tested in these experiments showed spike responses with conduction times in the A-δ or C range.

Our findings are consistent with earlier findings, which indicate a relatively small role for strain energy density in determining responses in this population of neurons. The interaction σ x E reflects the level of SED, and was not significantly associated with spikes in any analyses. The interaction term σ x dσ/dt reflects the time rate of change of incremental strain energy, and was modestly associated with spikes (Figures 4, 5, 6). Both of these findings are consistent with previous reports of the properties of RA afferents (Robichaud et al. 2003). They are also consistent with findings from other mechanoreceptors that show that the association between spike responses and SED was less than that with individual tensor components of mechanical stimuli (Khalsa et al. 1996; Khalsa et al. 1997). It is not clear how these findings relate to those of Dandekar et al. (2003) who reported close correspondence between modeled values of SED and neuronal responses in SA1 endings in monkey fingertips. However, one should note there are differences in the type of skin (glabrous vs hairy), the type and location of the endings, and the species.

Hindlimb skin is stretched during locomotion, which raises the issue of how the stretch stimuli that we used might relate to those that occur in normal locomotion. In a previous experiment from this laboratory (Grigg 1996), skin strains were measured while the rat hindlimb was manually moved in flexion and extension. Rotation the leg into full extension resulted in positive strains along the X direction (i.e., along the direction of the leg) up to 0.13 in magnitude. We used X direction strains that were somewhat comparable, up to 0.08 in magnitude. However, it should be emphasized that the stresses, with which neuronal responses are associated, are unknown in situ. In particular, limb extension caused the orthogonal Y direction strains to be strongly negative, which would lower any stresses along the X direction. Therefore, while the present results are of interest from the standpoint of understanding the encoding of
mechanical variables, caution should be used in extending these findings to the situation in normal locomotion.
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Figures.

Figure 1.

A. The area on the rat’s hindlimb from which the skin sample (shaded gray) was harvested. The darker grey square represents the area within which stresses were estimated, and neurons were sampled. The coordinate axes refer to the X and Y directions of the apparatus in which the skin was studied.

B. Apparatus for 2 dimensional stretching. S: skin sample. Dots on the skin surface are used for video tracking system (not shown); R: recording chamber; T: plastic tab (one of 4, that couple the skin to the actuators); MX, MY: Aurora actuators for stretching in the X and Y directions.
Figure 2.
Segments of stress and strain data records, to illustrate the 2 experimental paradigms.
A. Uniaxial actuation paradigm. A force-controlled PGN stretch stimulus is applied along one axis while the orthogonal axis is fixed.
   Left panel: stress and strain measured along the direction in which the skin is stretched.
   Right panel: stress and strain along the orthogonal, fixed, direction. The strain data is recorded by the video tracking system; the negative strain is due to compression from the Poisson effect.

B. Symmetrical biaxial paradigm. Force-controlled stretch stimuli are applied to both axes simultaneously. In the run illustrated, the stimulus applied along the Y axis was a scaled version of that applied along the X axis.
   Left panel: X-axis. The applied PGN stretch has a mean amplitude of 40 kPa.
   Right panel: Y-axis. The applied PGN stretch is a scaled version of that on the X axis, and has a mean amplitude of 10 kPa.
Figure 3. Relationship between pseudostrains (E) measured from actuator displacements and true strains (ε) measured with video system. Slope = 0.475; \( r^2 = 0.79 \). Data were taken from a 10 kPa PGN uniaxial actuation run.
Figure 4. Associations between spike responses and predictors using logistic regression.

A. Analysis of data from a single uniaxial actuation run. Odds ratios are shown for each main factor (left panel) and all the first-order interactions between them (right panel), for memory times between 0 and 40 msec.

B. Mean values (+ SEM) of odds ratios averaged across 20 neurons. Same format as above.
Figure 5. Results from uniaxial actuation trials. Left panel: odds ratios for predictors measured along the actuated direction. Right panel: odds ratios for stress predictors measured along the fixed direction. Odds ratios are not shown for strain related variables because analyses are based on pseudostrain determined from actuator displacements, which were zero along the fixed direction.
Figure 6. Directionality of response of afferents. Each panel shows odds ratios for $\sigma$, $d\sigma/dt$, $dE/dt$, and $\sigma \times d\sigma/dt$, for uniaxial actuation runs along the X and Y directions. Note that the scales of the vertical axes differ. The variables plotted in each panel are:

A. $d\sigma/dt$
B. $\sigma$
C. $dE/dt$
D. $\sigma \times d\sigma/dt$
Figure 7. Results from symmetrical biaxial trials. Skin was stretched along both directions using identical stresses. Left panel: association between X direction variables and spikes. Right panel: association between simultaneously-recorded Y direction variables and the same spikes.
Figure 8. Effect of symmetrical vs. asymmetrical biaxial loads. The horizontal axis labels represent the degree of asymmetry of biaxial loads. ‘Uniaxial’ is data from uniaxial actuation runs, in which applied loads are maximally asymmetrical: The mean stress was 40 kPa along one direction and 0 along the other. 10:40 represents trials in which the mean value of stress was 10 kPa along one axis and 40 kPa along the other. 40:40 indicates trials with identical stresses along both axes.
References.


