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THE RESPONSE OF MECHANORECEPTORS IN RAT SKIN TO BIAXIAL LOADING: A PRELIMINARY STUDY

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ABSTRACT

Mechanoreceptor neurons were studied in an isolated rat skin preparation subjected to dynamic biaxial stretch. The strength of the relationship between neuronal responses and mechanical variables was determined using multiple logistic regression. The experimental protocol allowed the normal stresses as well as the maximum shear stress to be manipulated. In n=4 neurons, response was associated with the time rate of change of normal stress and this response was affected by the direction of loading. There was no relationship between response and the value of maximum shear stress. There was a strong association with the rate of change of maximum shear stress.

INTRODUCTION

Mechanoreceptors are sensory neurons that are activated by mechanical stimulation of soft tissues. There are large numbers of mechanoreceptors in skin, and virtually all of them can be activated by stretch stimuli. Stretching skin alters the states of both stress and strain. Work in our laboratory has focused on determining the coupling between stretch-evoked responses in cutaneous neurons and the stress and strain components of stretch stimuli. Recently, (Del Prete et al., 2003) showed that the responses of rapidly-adapting (RA) cutaneous mechanoreceptors were more closely coupled to stress-related variables. In that experiment, uniaxial stretch stimuli were applied to an in vitro preparation of mouse skin and nerve. Multiple logistic regression analysis was used to seek associations between spike responses in RA neurons and tensile stresses and strains. It was found that spikes were strongly associated with stress variables and were very weakly associated with strain variables. However, there were serious limitations to that study. The tissue sample was mounted biaxially but was only stretched uniaxially. The boundary orthogonal to the direction of stretch was fixed. Thus the stresses in the orthogonal direction resulted from the Poisson effect rather than being directly controlled. Furthermore, the loads along that boundary were not measured. In the experiments reported here, skin specimens were actuated biaxially using pseudo Gaussian (PGN) stress inputs with different mean values. Loads and displacements were measured along both directions, and spike responses of single mechanoreceptor neurons were recorded. This protocol allowed the tissue shear stress to be varied on planes that were not aligned with the principal stress directions.

METHODS

Skin specimens, along with the sensory nerve that innervates them, were excised from the hind limb of adult Sprague Dawley rats. The samples had the shape of a Maltese cross, whose axes were aligned parallel (X direction) and perpendicular (Y direction) to the long axis of the limb. The skin and nerve were studied in vitro in an apparatus (Fig. 1) in which the skin could be stretched biaxially while responses of individual neurons were recorded. Since the neurons were rapidly adapting, dynamic stimuli were required to activate them. Pseudo Gaussian (PGN) force-controlled inputs (0-100 Hz bandwidth; 30 second duration) were simultaneously applied along both axes of the specimen (Hoffman and Grigg, 2002). The amplitude of the waveform was twice the mean value. Both axes of the apparatus were actuated using the same waveform; the amplitudes of the waveform were scaled so that different stresses could be applied to each axis. Loads and displacements were measured along each direction using the outputs from the actuators. These were used to calculate normal stresses and strains as well as maximum shear stress ($\tau = (\sigma_x - \sigma_y)/2$). Stresses were calculated using 0.3 mm as an estimate of dermal thickness. Shear strains were measured by placing surface markers on the skin and tracking their locations with a video system (Hoffman and Grigg, 1984). The experimental design was to apply unequal normal stresses to the specimen. A 56 kPa (mean value) PGN stress stimulus was applied to one axis while in successive trials PGN stress stimuli with means of 14, 28, 42 and 56 kPa were applied to the orthogonal axis. This procedure varied both normal stresses and the magnitude of the maximum shear stress. The stresses (σ, τ) were differentiated to determine their time rates of change ($d\sigma/dt$, $d\tau/dt$). The relationship between spikes and each variable was determined using multiple logistic regression. Strength of association is expressed as an odds ratio. The magnitude of the odds ratio is a measure of the strength of the association between two variables. An odds ratio of 1.0 reflects no relationship. The effect of system memory was accounted for by shifting the time of occurrence of spikes relative to the stimuli (Del Prete et al., 2003).

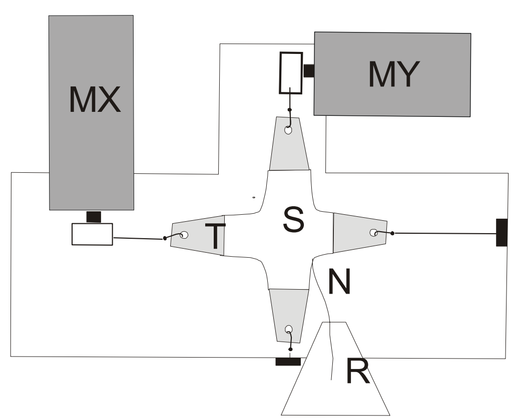

Figure 1. Tissue stretching apparatus. S: skin; N: nerve; R: recording chamber; T: tabs attached to skin; MX, MY; DC servo motors (Aurora Scientific 300B) for stretching tissue along X and Y directions.

RESULTS

Results are reported for 4 neurons from 4 skin preparations that were studied using biaxial stretch stimuli. Odds ratios were calculated between stress variables and spike responses for each run with each neuron. The results were aggregated so as to show the average response (Fig 2). All neurons were well activated by biaxial stretch stimuli, and responses were associated with $d\sigma/dt$ along either direction, at a memory time of 10 - 12 ms (Fig. 2A, 2B). However, when the magnitudes of the mean normal stresses were varied between the X and Y axes the response differed depending on the direction of the loads (Fig. 2A, 2B). In Figure 2A, σ_x was always 56 kPa (all values refer to mean stress), while σ_y was either 14 kPa or 56 kPa. The value of σ_y had very little effect on the odds ratio for $d\sigma_x/dt$. In Figure 2B, σ_y was always 56 kPa, while σ_x was either 14 kPa or 56 kPa. The odds ratio for $d\sigma_y/dt$ was greater when the biaxial stresses were not equal. Consistent with earlier studies, the components of stress: σ_x (Fig. 2A), σ_y (Fig. 2B) and τ (Fig. 2C) showed minimal or no association with neuronal response. Neural responses were most strongly associated with $d\tau/dt$ (Fig. 2C).

DISCUSSION

Results show that RA cutaneous afferents in rat skin are biaxially activated, and are somewhat directional dependent in their response to normal stresses. Associations with the rates of change of the normal components of stress are similar to those in mouse skin that resulted from uniaxial activation (Del Prete et al., 2003). The results also suggest a strong relationship between neuronal response and $d\tau/dt$. However the present experiments utilized a pure biaxial loading and shear stresses were not directly applied to the specimen. The measured values of shear strain (ε_{xy}) were all less than 0.021. The relationships between spike responses and $d\tau/dt$ are based upon calculation of the maximum shear stress along planes oriented at 45 degrees to the X and Y axes. Shear stresses on planes with different orientations would be less. Due to these factors and the small number (4) of neurons studied, the findings with regard to the rate of change of shear stress should be taken as suggestive but very preliminary. Future experiments will investigate the relationship between neuronal response and $d\tau/dt$ in an apparatus where dynamic shear stresses can be directly applied.

ACKNOWLEDGEMENTS

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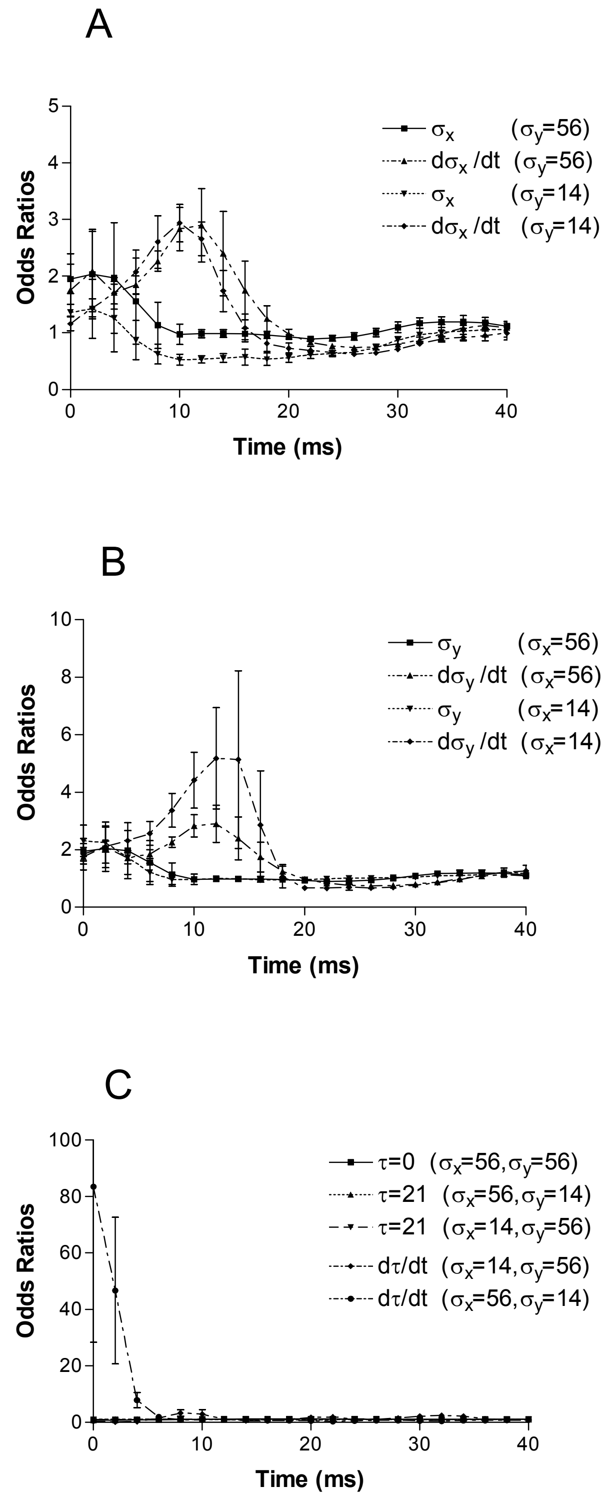

Figure 2. Odds ratios between stress variables and neuron spikes. (A) σ_x = 56 kPa, σ_y = 14 or 56 kPa. (B) σ_y = 56 kPa, σ_x = 14 or 56 kPa. (C) Maximum shear stress τ = 0 or 21 kPa.