Encoding of Compressive Stress During Indentation by Slowly Adapting Type I Mechanoreceptors in Rat Hairy Skin

WEIQING GE AND PARTAP S. KHALSA
Department of Biomedical Engineering, State University of New York, Stony Brook, New York 11794-8181

Received 21 May 2001; accepted in final form 20 November 2001

Ge, Weiqing and Partap S. Khalsa. Encoding of compressive stress during indentation by slowly adapting type I mechanoreceptors in rat hairy skin. J Neurophysiol 87: 1686–1693, 2002; 10.1152/jn.00414.2001. The mechanical state encoded by slowly adapting type I mechanoreceptors (SAI) during 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. Using a standard teased nerve preparation, the neural responses of single SAI were identified. SAI were stimulated, using controlled compressive stress while simultaneously measuring displacement, by compressing the skin between indenters (flat cylinders) of different diameters and a hard platform. SAI were subcategorized according to whether their neural response saturated above or below 10 kPa compressive stress (SAI-H or SAI-L, respectively). Linear regression was used to evaluate the relationships between neuron response and stress and force and displacement. For all SAI, the mean neural response was significantly and substantially more highly correlated with compressive stress than force or displacement. For the SAI-L subcategory, the mean correlation coefficient was significantly and substantially greater for stress than for force but not significantly different for displacement. The data from this study support the hypothesis that SAI mechanoreceptors stimulated by indentation encode compressive stress rather than force, displacement, or strain.

INTRODUCTION

Slowly adapting type I mechanoreceptors (SAI) play an essential role in the sensation of touch. While characterized by a sustained neural response during constant compression (i.e., slow adaptation) (Adrian and Zotterman 1926; Iggo and Muir 1969), SAI are also responsive to compressive vibration with a minimal threshold at approximately 20 Hz (Bolanowski et al. 1988) as well as to the velocity of indentation (Pubols 1982a). However, they also respond proportionally (power function) to the velocity of a stimulus stroked tangentially through their receptive fields (Greenspan 1992). SAI population studies have demonstrated their ability to reliably encode implicit object parameters such as shape (LaMotte and Srinivasan 1993; LaMotte et al. 1994) and texture (Blake et al. 1997; Johnson and Hsiao 1992) and external stimulus parameters of location and intensity (Friedman et al. 1998; Khalsa et al. 1998). However, there is controversy regarding the mechanical state, or “adequate stimulus,” encoded by SAI. The mechanical state is characterized by the internally developed local stress (related to force) and/or strain (related to displacement), rather than the externally applied force or displacement.

Early functional studies of SAI response to indentation were performed using displacement or force control but did not determine stress or strain. Using displacement control and measuring force, Werner and Mountcastle (1965) indented SAI in rat and monkey hairy skin and found that the neural response was more linearly correlated to force than to displacement. In raccoon skin, Pubols (1982a) controlled for both static force and displacement but was unable to conclude which was the “critical variable.” SAI in raccoon and monkey glabrous skin were found to be more sensitive to variations in dynamic displacement than to variations in dynamic force (Pubols 1990). A key confounding variable is that skin is viscoelastic, exhibiting creep (increasing displacement during constant force) or relaxation (decreasing force during constant displacement). Skin viscoelasticity can profoundly influence the time necessary for repeated trials to be independent (Pubols 1982b). A further complication is created while indenting mechanoreceptors in vivo if the skin overlies soft tissues instead of bone. In this common, if not typical, case, indentation produces tension as well as compression and hence combined (and confounded) tensile and compressive stresses and strains (Khalsa et al. 1997).

The first published study to explicitly examine SAI response to stress and strain used a linear, elastic continuum model of skin in primate fingertip (Phillips and Johnson 1981). The neural response of SAI to indentations was correlated to strain (calculated from plane stress, which was derived from applied forces) and found to be proportional to the maximum compressive strain. However, the validity of this model is questionable because fingertip skin is nonlinear, viscoelastic, and anisotropic in contrast to the model. Using a finite-element analysis of the primate fingertip to compare SAI response and mechanical states, Srinivasan and Dandekar (1996) found that maximum compressive strain and strain energy density best fit the neurophysiological data from Philips and Johnson (1981). Using a three-dimensional model of primate fingertip, Dandekar and Srinivasan (1995) estimated mechanical states at a hypothetical SAI location. They found that the mean stress had an even higher correlation with SAI response, although the maximum...
compressive strain and strain energy density (a scalar quantity representing the energy stored in a material during loading) were also well correlated.

Plane or three-dimensional strain compared with other mechanical states (e.g., stress or strain energy) does not appear to be well encoded by afferents particularly sensitive to tensile loading. In isolated rat skin, the neural responses of slowly adapting type II afferents (SAIIs) to stretch were strongly directionally selective, most neural responses of slowly adapting type II afferents (SAIIs) to stretch were most highly correlated with stress (Fuller et al. 1991b; Grigg and Hoffman 1982; Khalsa et al. 1996) or strain energy density (Grigg and Hoffman 1984). In isolated rat skin, the neural responses of slowly adapting type II afferents (SAIIs) to stretch were strongly directionally selective, most highly correlated with the tensile stress along the unit's preferred direction, and poorly related to strain variables (Grigg 1996). In rat hairy skin, the neural responses of Aδ and C mechanoreceptors (putative mechano-nociceptors) to tension, compression, and combined tension and compression were more highly correlated to stress than strain (Khalsa et al. 1997). There are no reported studies of the relationship between pressure and stress share the same units (pascals in the SI system), they are in general not the same thing. Specifically, pressure is the trivial case of where the stress magnitudes are the same along all axes. Arguably, cutaneous mechanoreceptors never experience pressure during any type of real-world loadings, including indentations as performed in the current experiments.

The aim of the current study was to examine what mechanical state was encoded by SAIIs during indentation. The working hypothesis was that the neural response would be more highly correlated with compressive stress than other relevant variables (i.e., compressive force, displacement, or strain). To eliminate confounding variables due to nonlinear geometry and tension developing during indentation, we used a well-established isolated rat skin-nerve preparation that enabled us to apply compression without developing tension (Khalsa et al. 2000) and had a flat (i.e., linear) geometry.

METHODS

Isolated skin-nerve preparation

Nineteen Sprague-Dawley rats (approximately 250 g), of either sex, were used for the skin-nerve preparation similar to that previously reported in detail (Khalsa et al. 1997, 2000). Briefly, hair was removed from the medial thigh of a pentobarbital-sodium-anesthetized rat, and small markers (0.5-mm diam) were glued to the skin and their locations measured relative to one another (Fig. 1A). The patch of skin and its intact innervation were then harvested and placed in a dish containing circulated and gassed (100% O2) rodent, synthetic interstitial fluid (Koltzenburg et al. 1997). Tabs (7 × 14 mm, three tabs per side, four sides, total of 12 tabs) were cut into the margins of the skin and coupled to force transducers mounted respectively on the ends of 12 linear actuators (Fig. 1B). The skin was then stretched until the markers closely approximated their in vivo (reference) configuration. All compressive loads were subsequently applied with the skin in this reference state.

Mechanical system and measurements

Pure compressive loads were applied similarly as previously reported (Khalsa et al. 2000). Briefly, compressive loads were applied by compressing the skin between an indenter and a hard platform. The hard platform was a 15-mm-diam flat cylinder positioned just underneath the skin. Indenters were flat cylinders with radii of 1.000, 1.290, 1.580, 2.236, or 3.162 mm. The smallest diameter indenter was designed such that it would have an area much greater than the area occupied by the receptive ending of the mechanoreceptors. This ensured that shear stress (and strain) that developed around the edge of the indenter, and that was immeasurable, would not confound the “pure” compressive stress created toward the center of the indenter (Khalsa et al. 1997, 2000). The largest diameter indenter was designed so that, for a given compressive force, the compressive stress would be well encoded by afferents particularly sensitive to tensile loading. In isolated cat knee-joint capsule, the neural response of mechanoreceptors (putative mechano-nociceptors) to tension, compression, and combined tension and compression were more highly correlated to stress than strain (Khalsa et al. 1997). There are no reported studies of the relationship between pressure and stress share the same units (pascals in the SI system), they are in general not the same thing. Specifically, pressure is the trivial case of where the stress magnitudes are the same along all axes. Arguably, cutaneous mechanoreceptors never experience pressure during any type of real-world loadings, including indentations as performed in the current experiments.

The aim of the current study was to examine what mechanical state was encoded by SAIIs during static indentation. The working hypothesis was that the neural response would be more highly correlated with compressive stress than other relevant variables (i.e., compressive force, displacement, or strain). To eliminate confounding variables due to nonlinear geometry and tension developing during indentation, we used a well-established isolated rat skin-nerve preparation that enabled us to apply compression without developing tension (Khalsa et al. 2000) and had a flat (i.e., linear) geometry.

METHODS

Isolated skin-nerve preparation

Nineteen Sprague-Dawley rats (approximately 250 g), of either sex, were used for the skin-nerve preparation similar to that previously reported in detail (Khalsa et al. 1997, 2000). Briefly, hair was removed from the medial thigh of a pentobarbital-sodium-anesthetized rat, and small markers (0.5-mm diam) were glued to the skin and their locations measured relative to one another (Fig. 1A). The patch of skin and its intact innervation were then harvested and placed in a dish containing circulated and gassed (100% O2) rodent, synthetic interstitial fluid (Koltzenburg et al. 1997). Tabs (7 × 14 mm, three tabs per side, four sides, total of 12 tabs) were cut into the margins of the skin and coupled to force transducers mounted respectively on the ends of 12 linear actuators (Fig. 1B). The skin was then stretched until the markers closely approximated their in vivo (reference) configuration. All compressive loads were subsequently applied with the skin in this reference state.

Mechanical system and measurements

Pure compressive loads were applied similarly as previously reported (Khalsa et al. 2000). Briefly, compressive loads were applied by compressing the skin between an indenter and a hard platform. The hard platform was a 15-mm-diam flat cylinder positioned just underneath the skin. Indenters were flat cylinders with radii of 1.000, 1.290, 1.580, 2.236, or 3.162 mm. The smallest diameter indenter was designed such that it would have an area much greater than the area occupied by the receptive ending of the mechanoreceptors. This ensured that shear stress (and strain) that developed around the edge of the indenter, and that was immeasurable, would not confound the “pure” compressive stress created toward the center of the indenter (Khalsa et al. 1997, 2000). The largest diameter indenter was designed so that, for a given compressive force, the compressive stress would be well encoded by afferents particularly sensitive to tensile loading. In isolated cat knee-joint capsule, the neural response of mechanoreceptors (putative mechano-nociceptors) to tension, compression, and combined tension and compression were more highly correlated to stress than strain (Khalsa et al. 1997). There are no reported studies of the relationship between pressure and stress share the same units (pascals in the SI system), they are in general not the same thing. Specifically, pressure is the trivial case of where the stress magnitudes are the same along all axes. Arguably, cutaneous mechanoreceptors never experience pressure during any type of real-world loadings, including indentations as performed in the current experiments.

The aim of the current study was to examine what mechanical state was encoded by SAIIs during static indentation. The working hypothesis was that the neural response would be more highly correlated with compressive stress than other relevant variables (i.e., compressive force, displacement, or strain). To eliminate confounding variables due to nonlinear geometry and tension developing during indentation, we used a well-established isolated rat skin-nerve preparation that enabled us to apply compression without developing tension (Khalsa et al. 2000) and had a flat (i.e., linear) geometry.

METHODS

Isolated skin-nerve preparation

Nineteen Sprague-Dawley rats (approximately 250 g), of either sex, were used for the skin-nerve preparation similar to that previously reported in detail (Khalsa et al. 1997, 2000). Briefly, hair was removed from the medial thigh of a pentobarbital-sodium-anesthetized rat, and small markers (0.5-mm diam) were glued to the skin and their locations measured relative to one another (Fig. 1A). The patch of skin and its intact innervation were then harvested and placed in a dish containing circulated and gassed (100% O2) rodent, synthetic interstitial fluid (Koltzenburg et al. 1997). Tabs (7 × 14 mm, three tabs per side, four sides, total of 12 tabs) were cut into the margins of the skin and coupled to force transducers mounted respectively on the ends of 12 linear actuators (Fig. 1B). The skin was then stretched until the markers closely approximated their in vivo (reference) configuration. All compressive loads were subsequently applied with the skin in this reference state.

Mechanical system and measurements

Pure compressive loads were applied similarly as previously reported (Khalsa et al. 2000). Briefly, compressive loads were applied by compressing the skin between an indenter and a hard platform. The hard platform was a 15-mm-diam flat cylinder positioned just underneath the skin. Indenters were flat cylinders with radii of 1.000, 1.290, 1.580, 2.236, or 3.162 mm. The smallest diameter indenter was designed such that it would have an area much greater than the area occupied by the receptive ending of the mechanoreceptors. This ensured that shear stress (and strain) that developed around the edge of the indenter, and that was immeasurable, would not confound the “pure” compressive stress created toward the center of the indenter (Khalsa et al. 1997, 2000). The largest diameter indenter was designed so that, for a given compressive force, the compressive stress would be well encoded by afferents particularly sensitive to tensile loading. In isolated cat knee-joint capsule, the neural response of mechanoreceptors (putative mechano-nociceptors) to tension, compression, and combined tension and compression were more highly correlated to stress than strain (Khalsa et al. 1997). There are no reported studies of the relationship between pressure and stress share the same units (pascals in the SI system), they are in general not the same thing. Specifically, pressure is the trivial case of where the stress magnitudes are the same along all axes. Arguably, cutaneous mechanoreceptors never experience pressure during any type of real-world loadings, including indentations as performed in the current experiments.

The aim of the current study was to examine what mechanical state was encoded by SAIIs during static indentation. The working hypothesis was that the neural response would be more highly correlated with compressive stress than other relevant variables (i.e., compressive force, displacement, or strain). To eliminate confounding variables due to nonlinear geometry and tension developing during indentation, we used a well-established isolated rat skin-nerve preparation that enabled us to apply compression without developing tension (Khalsa et al. 2000) and had a flat (i.e., linear) geometry.
be an order of magnitude (10 times) smaller than that developed for the smallest diameter indenter, and the other three indenters were equally spaced between the smallest and largest indenters. Indenters were 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 (12 tensile loads, 1 compressive load, and 1 compressive displacement) was accomplished via a laboratory computer, A/D and D/A converter, and custom software. Compressive force and displacement were sampled at 500 Hz. Compressive stress was calculated from the applied force divided by the area of the indenter. The same compressive stresses could be applied for indenters of different diameter by varying the force; and applying the same compressive forces but varying the indenter diameters would achieve different compressive stresses. We did not attempt to directly measure the compressive strain because we were only comparing the correlation between neuronal response and displacement. However, for a given neuron, the correlation between the neuronal response and compressive displacement would be directly proportional to compressive strain. Skin compliance at the location of the receptive ending compressive displacement would be directly proportional to compressive stress, maintained for 5 s, and then unloaded (Fig. 2C).

**Neuron recording and classification**

Neuron recording and classification have been reported previously in detail (Khalsa et al. 2000). Briefly, the nerve innervating the skin 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 (Spiketwo, Cambridge Electronic Design, UK). Only neurons responsive to mechanical stimuli (Fig. 2) and with conduction velocities (corrected for the room temperature of the saline bath) in the Aβ fiber range (i.e., 20–60 m/s) were included in this study. The most sensitive spot (MSS) of a neuron’s receptive field was determined by use of calibrated monofilaments (Stoelting). Neurons whose MSSs were outside the makers (e.g., on the edge of the skin or in a tab) were excluded because we could not reliably control the mechanical state. While not anticipated a priori, we observed that SAs could be empirically subcategorized into two groups on the basis of where their response saturated. The saturation level was defined as the magnitude of load above which there was no significant increase in neural response or at which the neural response actually decreased for increasing load (Khalsa et al. 1996; Rossi and Grigg 1982). In the current experiments, it was determined empirically by observing when the neural response no longer increased for increasing compressive loads. For the SAs in these experiments, a clear division in saturation levels was found to occur at 10 kPa of compressive stress (Fig. 3). Hence, we categorized SAs as either having high or low saturation levels by whether their neuronal response saturated above or below 10 kPa (i.e., SA-I or -L, respectively).

**Experimental protocol**

Once a suitable afferent was identified, compressive loads were applied at its MSS by first lowering the indenter to the surface of the skin until a minimal contact force (5 mN) was detected. For some SAs, this minimal force exceeded threshold [cf. Cain et al. (2001) that reported for mouse Aβ mechanoreceptors, both SAs and RAs, a mean threshold of 2.1 mN with a range of 0.4–56.6 mN]. However, this was the smallest force that we could reliably deliver with our apparatus given the range of forces needed to fully explore their sensitivities and saturation levels. The contact force was maintained for 0.5 s, and then the load was step indented to a predetermined compressive stress, maintained for 5 s, and then unloaded (Fig. 2A).

**Data analysis**

The neuronal response was characterized using the following methods: 1) the overall mean frequency, by dividing the total number of action potentials by the duration of the constant stimulus (i.e., 5 s; Fig. 2B); 2) the early phase, by dividing the total number of action potentials during the first 2 s (of the constant stimulus by 2 s, 3) the late phase, by dividing the total number of action potentials during the last 3 s of the constant stimulus by 3 s, 4) the peak instantaneous frequency, designated as P1, and three sequential later instantaneous frequencies occurring 3.0, 3.5, and 4.0 s after the peak frequency (P1) and designated P2–P4, respectively.

Inter-trial intervals were 3 min to allow the skin to recover its prestimulus state and to allow the neurons to have similar responses for repeated simulations (Baumann et al. 1986) (Fig. 4). Ranges of loads were applied to encompass the estimated threshold to estimated saturation level for compression for each neuron (e.g., 0.25–2 kPa). Generally, trials were repeated three times at each compressive stress magnitude. After all the trials were completed for an indenter of a given diameter, then the same loading sequence (i.e., range of compressive stresses) would be repeated for an indenter of a different diameter.
Forty SAIs were isolated during 19 successful experiments. Among them, 8 neurons had their MSS outside the markers and 11 neurons stopped responding prematurely to the stimulation before the minimal data recording protocol was completed. Hence the results are based on recordings from 21 SAI afferents. Of these, nine were categorized as low saturation level SAIs (i.e., SAI-L) and 12 as high saturation level SAIs (i.e., SAI-H; Fig. 3).

The mean conduction velocity (CV) for all SAIs was 26.7 ± 1.8 (SE) m/s, while the mean CVs for the subcategories (SAI-L and SAI-H) were not significantly different ($P = 0.8$; Fig. 5). Mean skin compliance at the sites of indentation for neurons classified as SAI-L was greater than for neurons classified as SAI-H, but that difference was not significant (10.3 ± 4.1 and 1.5 ± 0.7 μm/mN, respectively, $P = 0.08$). The neural responses of seven SAIs adapted to zero before the end of the 5-s stimulus, regardless of the stimulus magnitude (Fig. 6), similar to the moderately slowly adapting (MSA) afferents reported by Pubols (1982a). Among the seven MSA, two were categorized as SAI-L and five as SAI-H. There was no significant difference ($P = 0.87$) between the mean conduction velocity of the MSAs, 27.13 ± 3.16 m/s, and that of the other SAI neurons, 26.5 ± 2.25 m/s.

The mean sensitivity for all SAIs was 6.4 ± 1.5 Hz/kPa (Fig. 7A). However, the SAI-L were almost five times more sensitive than the SAI-H (10.9 ± 2.2 vs. 2.2 ± 0.7 Hz/kPa, respectively, $P < 0.01$). At their respective saturation loads, there was no significant difference ($P = 0.90$) between the mean neural responses of SAI-L and -H (Fig. 7B).

For each neuron, the neural response to all loads (same stresses with different indenter areas) was correlated to compressive stress, force, and displacement (Fig. 8). On average, for all SAIs the neural response was significantly ($P = 0.013$, ANOVA) and substantially more highly correlated with compressive stress than force or displacement (Pearson correlation coefficients: 0.64 ± 0.05, 0.43 ± 0.07, 0.52 ± 0.07 for stress, force, and displacement, respectively; Fig. 9). For all SAI-Ls,
the mean correlation coefficient was significantly \((P = 0.013)\) and substantially greater for stress \((0.63 \pm 0.08)\) than that for force \((0.39 \pm 0.1)\) but not significantly different \((P = 0.27)\) for displacement \((0.61 \pm 0.07); \text{Fig. 9}\). For all SAI-Hs, the mean correlation coefficient was significantly \((P = 0.04)\) and substantially greater for stress \((0.65 \pm 0.05)\) than that for force \((0.42 \pm 0.09)\) or for displacement \((0.43 \pm 0.11); \text{Fig. 9}\).

There were significant differences between the early and late phases of the neuronal responses (Fig. 10). Of the different metrics we used to evaluate the neuronal responses, the least correlated (i.e., with the greatest differences) were the peak instantaneous frequency (designated as P1) and the instantaneous frequency that followed P1 by 4.0 s (designated as P4). We sought to determine if these metrics would further elaborate on the mechanical state encoded by SAIs and potential differences in the subcategories, SAI-L and SAI-H. In general, neither of these specific “snap-shot” characterizations of neuronal response were as well correlated with the stimulus as was the overall mean frequency (compare Fig. 9 with Fig. 11A). For all SAIs, compressive stress was more highly correlated with neuronal responses P1 and P4 than were force or displacement, though the differences were not significant \((P > 0.05); \text{Fig. 11A}\). For SAI-Ls, displacement was more highly correlated with P1 and P4, though the differences were not significant (Fig. 11B). For SAI-Hs, P1 and P4 were more highly correlated with stress than force, although only with P1 was the difference significant \((P < 0.05)\). Thus these findings using early and late phase metrics of the neuronal response concur with the analysis using the overall mean frequency.

**DISCUSSION**

The data from this study support the hypothesis that SAI mechanoreceptors stimulated by indentation encode compressive stress rather than force, displacement, or strain. These experiments were designed to eliminate confounding factors typically encountered during in vivo experiments such as nonlinear skin geometry and simultaneous tension with compression, and experimental design issues such as shear loading, and viscoelastic effects due to insufficient inter-trial intervals (i.e., the skin and mechanoreceptor not fully recovering). Hence, the fundamental mechanical state encoded by SAIs appears to be the same as that encoded by SAIIs (Grigg 1996), Ruffini afferents (Fuller et al. 1991a; Grigg and Hoffman 1982, 1984; Khalasa et al. 1996), rapidly adapting type II (Aβ) afferents (Prete and Grigg 1998), and Aβ and C mechanonociceptors (Khalsa et al. 1997).
The geometry of the indenters was an important design consideration in these experiments. The cross-sectional area of the smallest indenter was much larger than a single “touch-dome” of the rat hairy skin (Leon and McComas 1984) and hence greater still for a single mechanoreceptive ending within a single touch-dome. This ensured that uncontrollable (and un-measureable) shear stresses were minimized during the compression. Further, for the same applied force, the compressive stress developed with the largest diameter indenter was an order magnitude smaller than that developed with the smallest diameter indenter. This allowed us to examine whether stress or force was being encoded by these afferents. It could be argued that the question could also be examined by using indenters whose cross-sectional areas would be smaller than that of a single SAI receptive ending. There are two related problems with this later argument. First, unless it were possible to isolate a receptive ending from its encompassing extracellular matrix (cf. Bolanowski and Zwislocki 1984), then there would be no effective way to conduct such a test. In situ compression by such a small indenter would result in a non-uniform (and nonlinear) stress distribution over the membrane of the receptive ending. These stresses would not only vary spatially (Khalsa et al. 2000), but temporally due to the intrinsic viscoelasticity of the skin. Second, even if it were possible to isolate the ending, the experimental conditions would be so artificial as to make the extrapolation of the results to the in-vivo condition as nonsensical. Indeed, it could be argued a priori that receptive endings of any type of mechanically sensitive neuron, including SAI’s, could not experience force at any scale except perhaps at the single membrane-channel level.

FIG. 9. The mean NR for all SAI’s was more highly correlated with compressive stress than with compressive force or displacement ($P = 0.013$, ANOVA). Within each group (i.e., stress, force, or displacement), there was no significant difference between SAI-L and -H ($P > 0.05$). For SAI-H, the highest correlation was between NR and compressive stress ($P = 0.04$). For SAI-L, there was no significant difference in the correlations between NR and stress and NR and displacement ($P = 0.27$).

FIG. 10. Mean correlations ($n = 21$) between the different phases of the neuronal responses. The least correlated phases were P1 and P4 and were subsequently used to further examine relationships between SAI-L and -H afferents. P1, peak instantaneous frequency; P2–P4, instantaneous frequencies following P1 at 3.0, 3.5, and 4.0 s, respectively; EO, early phase, mean frequency during the 1st 2.0 s of the stimulus; LP, late phase, mean frequency during 2.0–5.0 s of the stimulus; ALL, mean frequency for the entire 5.0-s stimulus.
Characterized by P4) and displacement. Only observed for the SAI-H afferents between peak neuronal response and stress and the late phase neuronal response (as B), and all 12 SA1-Hs (subdivided by all SA1s (C), all 9 SA-1-Ls (fi), and even that is conjectural. Rather, above the single channel scale, force is always distributed as a continuum over an area of interest (e.g., the membrane or a portion thereof), and, hence, must be described as stress tensor quantity. Thus the use of indenters with relatively small cross-sectional areas would have been problematic for these experiments and, hence, were not used.

We empirically observed that we could subcategorize SAIs by whether their neural response saturated above or below 10 kPa (SAI-H or -L, respectively). For both SAI-L and -H, their overall neuronal responses were significantly and substantially more correlated with compressive stress than force. For SAI-H, their overall neuronal responses were also significantly and substantially more correlated to compressive stress than displacement, which for these experiments was directly proportional to compressive strain. However for SAI-L, there was no significant difference between the correlation of overall neuronal response and compressive stress or displacement (and hence in these experiments, strain). Characterizing the early and late phases of the neural responses and correlating those phases with the stimulus accentuated this similarity in response to stress and displacement. This again revealed no significant differences between stress and displacement for SAI-L afferents. An explanation for this difference between SAI-L and -H is due to the viscoelasticity of skin. The maximum compressive loads applied to the SAI-L were much smaller than those applied to the SAI-H. For small compressive loads, skin would tend to respond as if it was linearly elastic, and, hence, compressive stress would be linearly proportional to compressive strain (or displacement). For this case, there should be no difference in the correlation between mechanoreceptor's neural response and stress or strain, as was observed for the SAI-L. However, for large loads, skin would behave in a nonlinear viscoelastic manner (Daly 1982; Lanir 1979; Pubols 1982b) resulting in more complex relationships between stress and strain. For this case, it would be expected that the neural response should be more highly correlated to either stress or to strain but not both as was observed in the current experiments.

Our data using a rat model support the hypothesis that SAIs encode compressive stress rather than strain. This contrasts with work by Phillips and Johnson (1981), who concluded that primate SAIs encode maximal compressive strain. It is possible that there exist significant differences between SAIs in rat hairy skin and primate glabrous skin. Both types are associated with Merkel cells at their terminal endings, but the terminal endings of rat SAIs occur in touch domes in hairy skin, whereas the primate Merkel cells occur at the interface between the epidermis and dermis and not in touch domes. Primate glabrous fingertip skin also has ridges, which rat hairy skin does not, that undoubtedly influence the mechanics of load distribution. However, there are many similarities between the neural responses of these SAI types including conduction velocity, threshold, sensitivity, and saturation levels. Our experimental paradigm eliminated some of the potentially confounding variables present in Phillips and Johnson (1981) including nonlinear skin geometry, combined compression and tension, and a priori modeling assumptions.

Our results are in disagreement with the conclusions of Srinivasan and Dandekar (1996) using a two-dimensional (2D) finite-element model (FEM) of primate fingertip, but support their subsequent findings (Dandekar and Srinivasan 1995) using a three-dimensional (3D) FEM. Their 2D FEM reasonably predicted the neural responses of primate mechanoreceptors reported by Phillips and Johnson (1981), but failed to accurately represent the actual deformations of the fingertip. Their 3D FEM accurately predicted both the mechanoreceptors responses and fingertip deformations reported by Srinivasan and LaMotte (1991). Whereas, their 2D FEM found the best correlation between neural response and maximum compressive strain, their 3D FEM found the best correlation between neural response and mean compressive stress. A limitation of both of these models was that they were based on linear elasticity, whereas skin is intrinsically nonlinear viscoelastic (as well as being anisotropic). Our results suggest that the modeling assumption of linear elasticity is appropriate only for small loads and hence may only accurately represent neural responses of very low-threshold and low-saturation mechanoreceptors.

Slowly adapting cutaneous mechanoreceptors (SA) have

FIG. 11. Mean correlations among stress, force, and displacement with the neural response characterized by the peak instantaneous frequency (P1) and the instantaneous frequency at |1 + 4.0 s (P4). P1 and P4 are the least correlated of the neural response metrics examined and, hence, should show the greatest differences between SAI-L and -H afferents. The correlations are subdivided by all SA1s (A), all 9 SA-1-Ls (B), and all 12 SA1-Hs (C). Significant differences (P < 0.05) are indicated (*) and were only observed for the SAI-H afferents between peak neuronal response and stress and the late phase neuronal response (as characterized by P4) and displacement.
been categorized based on different criteria. Iggo and colleagues distinguished SAIIs from SAIIs based on SAIIs’ more regular response to static indentation and greater sensitivity to stretch (Chambers et al. 1972; Iggo and Muir 1969). Recently, Edin (2001) has introduced another category, the SAIII, which is characterized in humans by some features of SAIIs (i.e., regular response to indentation and high sensitivity to skin stretch) and some features of SAIIs (i.e., no directional sensitivity to stretch and small, clearly demarcated receptive fields). In the current study, we did not observe any SAs that exhibited significant sensitivity to stretch, and hence categorized them all as SAIIs rather than SAIIs or SAIIs. We did observe some SAs that responded similarly to the MSA as reported by Pubols (1982a) in raccoon hairy skin, but most were similar to the very slowly adapting neurons (VSA). However, the SAI-L was more sensitive to indentation than was the SAI-H, and this was analogous to the lower thresholds of VSAs compared with MSAs (Pubols 1982a). To our knowledge, this is the first report of differences in saturation levels for subcategories of SAIIs. It is not clear if these differences are due to neuron phenotypic expression, skin mechanics, or combination of the two.

We thank C. Zhang for technical assistance and P. Grigg for the generous donation of equipment used in the experiments.

This study was partially funded by The Whitaker Foundation (RG-97-0175).

REFERENCES


