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MECHANICAL PROPERTIES OF SKIN AND RESPONSIVENESS OF SLOWLY ADAPTING TYPE I MECHANORECEPTORS IN RATS AT DIFFERENT AGES

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SUMMARY

1. Slowly adapting type I (s.a. I) cutaneous mechanoreceptors were studied in young (3–4 months old) and adult (9–11 months old) rats. Trains of thirty repetitive mechanical stimuli with 0·1 s rise time, 1·9 s plateau phase, and 0·7 s interstimulus interval were applied. A feed-back mechanism maintained the force of stimulation at 20 mN during the plateau phases of stimuli and the contact force between stimuli at 0·5 mN.

2. During the first few stimuli in a train residual indentation at contact force increased rapidly. Maximal indentation required to maintain the force of stimulation of 20 mN increased as well but to a smaller extent. Thus, the stroke amplitudes of individual stimuli decreased with increasing stimulus number.

3. All displacement values in the group of adult rats were consistently reduced to $62 \pm 3\%$ of the respective values in the group of young rats, indicating a linear decrease in skin compliance in the force range of 0·5–20 mN.

4. Nervous responses to individual stimuli decreased from about 200 impulses for stimulus number 1 to about 60 impulses for stimulus number 30. Significant differences in the number of impulses between young and adult rats were observed from stimulus number 9 to number 16 only.

5. It is concluded that the design of the s.a. I receptor allows maintained high tactile sensitivity in response to force-related stimuli irrespective of age-induced changes in mechanical properties of the skin and underlying tissues.

INTRODUCTION

A sensory receptor can only encode stimuli as they actually reach the transducer. In crayfish stretch receptors a gradually adapting generator potential is elicited during stimulation with constant stretch. In this experimental situation the visco-elasticity of the preparation permits the tension of stimulation to drop. In contrast, during stimulation with constant tension the generator potential does not adapt (Nakajima & Onodera, 1969).

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The interrelationship between force and displacement during innocuous mechanical stimulation of the skin has been investigated extensively (Lindblom, 1965; Kruger & Kenton, 1973; Knibestöl, 1975; Petit & Galifret, 1978; Pubols, 1982*a*, *b*). The long time course of recovery of the skin after mechanical stimulation was emphasized by Pubols (1982*b*). The present study is concerned with the effect of changes in the mechanical properties of normal skin with age on the responsiveness of a well investigated cutaneous mechanoreceptor, the slowly adapting type I (s.a. I) receptor. The functional subunit of this type of receptor is a complex at the epidermo-dermal border formed by tactile or Merkel cells being in close contact with always one expanded terminal branch from an afferent nerve fibre (Iggo & Muir, 1969). In adult rats there are approximately ninety Merkel-type cells in each s.a. I receptor; but not each of these cells possesses a nerve terminal (Nurse & Diamond, 1984). Smith (1967) counted about twenty-five nerve terminals per s.a. I receptor in the rat.

It is known that composition and mechanical properties of the skin and subcutaneous tissue change with age (Ridge & Wright, 1966; Daly & Odland, 1979). Agache, Monneur, Leveque & De Rigal (1980) demonstrated a marked drop in stretchability of human skin around the age of 30–35 years. In the present results the responsiveness of s.a. I receptors to constant force stimuli was little affected in spite of pronounced changes in the viscoelastic properties of the skin with age. It appears that the design of the receptor allows continued high tactile sensitivity irrespective of age-related changes of the skin.

METHODS

Experimental animals

The experiments were performed on Sprague-Dawley rats. The 'young' population was fed normal rat chow (Purina). The 'adult' group was the control population for vitamin A deficient rats described in the accompanying paper (Baumann, Cheng-Chew, Hamann & Leung, 1986) and was fed a vitamin A deficient diet (Purina) supplemented with vitamin A. Until 3 months of age there was no significant difference in body weight between the two groups ($P > 0{\cdot}2$). Acute electrophysiological investigations were performed in the young group at an age of 3–4 months, whereas the rats of the adult group were between 9 and 11 months old at the time of the experiments.

Preparation for acute experiments

The animals were anaesthetized with urethane (20% w/v, 6 ml/kg, I.P.). Supplementary doses were given as required. One carotid artery and one jugular vein were cannulated allowing measurement of arterial blood pressure and venous access. The minimum mean blood pressure permitted was 80 mmHg. The body temperature was kept at 38 °C by a thermostatically controlled electric blanket. The skin was shaved closely with clippers. No depilating agent was used because it was felt that the removal of hair roots by itself could have changed the compliance of the skin. A small pool to be filled with liquid paraffin (B.P.) made from skin flaps sewn to a lead ring was formed over the dorsal gluteal region. Small strands were dissected from the exposed superficial cutaneous nerves (clunium nerves) supplying touch corpuscles in the skin of the proximal hind limb and single-unit recordings were made. Receptors were identified by (*a*) their slowly adapting response during maintained indentation; (*b*) the absence of a response to stretching the skin and (*c*) by their responsiveness to stimulation of discrete spots of skin only (Iggo & Muir, 1969; Hamann & Lee, 1982). A piece of plasticene was put underneath each axilla thus raising the thorax to avoid general movement caused by respiratory excursions. The left hind limb was kept in an extended position by being attached to the operating table with orthopaedic tape (Hexcelite). Recordings were made with bipolar Ag–AgCl hook electrodes. The nerve action potentials in response to the stimuli applied were amplified with a differential amplifier (Neurolog NL103) and displayed on an oscilloscope (Tektronix 5103N). Original recordings of nervous responses, forces and displacements of stimulation were made with a tape recorder (Hewlett Packard 3964A). Cumulative spike counts (Digitimer Spike Processor D130) during individual stimuli, displacements, and forces were recorded with a chart recorder (Lectromed).

Mechanical stimulation

A pattern of repetitive mechanical stimulation was designed using stimuli that were within the physiological range of stimuli for touch corpuscles and at the same time produced some work stress for the receptor. Both aims were achieved by trains of thirty identical stimuli. Each individual stimulus (Fig. 1) rose over 0·1 s to a force of 20 mN where it was maintained via an electronic feed-back mechanism for 1·9 s (Hamann, Baumann, Lee & Leung, 1983). Stimulation forces of 20 mN were found to produce close to maximal nervous responses (Baumann, Cervero, Hamann & Leung, 1985). This type of stimulation proved to be effective for detection of changes in responsiveness of touch corpuscles in other experimental situations (Baumann, Cheng-Chew, Hamann & Leung, 1984).

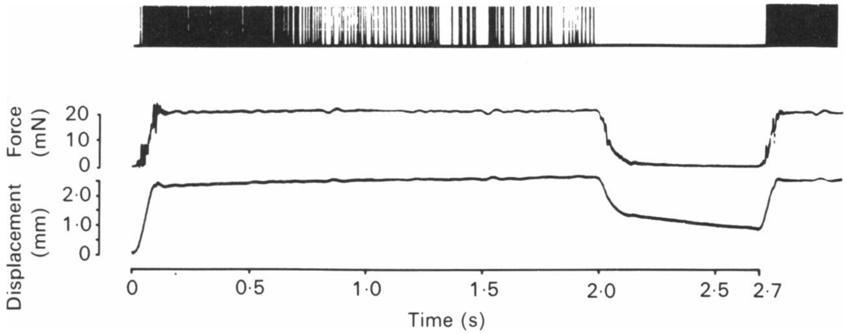


Fig. 1. Original recordings of displacement (lower trace), force (middle trace), and nervous response (upper trace) of an s.a. I receptor in a young rat during the first stimulus of a standard train. Nervous impulses are displayed as output from a Schmitt Trigger. Note the gradual increase in displacement during the plateau phase of the stimulus while the force is kept constant at 20 mN. During the interstimulus interval the force quickly drops to 0·5 mN whereas the displacement recovers more slowly and does not return to zero level (residual indentation).

The repetitive stimuli were produced by feeding RC-coupled square waves (time constant 50 ms) into the feed-back control unit of the stimulator. The relative shortness of the interstimulus intervals of 0·7 s (Fig. 2) implied that there was incomplete creep recovery of the skin during this time (Pubols, 1982*b*). Mechanical stimuli were applied to the skin with a nylon probe (diameter 1 mm, spherical tip), which was attached to a force transducer (Statham UC2). The stimulating probe was placed under visual control over the receptor in a position perpendicular to the skin. A contact force of 0·5 mN was maintained during interstimulus intervals. This force was below mechanical threshold of the touch corpuscles examined. Nervous responses were expressed as cumulative counts per stimulus of 2·0 s duration. Dynamic responses during the 0·1 s rising phase were found to be consistently in the range of 15 % of the total responses.

An electronic vibrator (Bruehl & Kjaer No. 4810) served as electro-mechanical transducer. Forces of stimulation were measured with the Statham force transducer. Displacements were obtained by measuring the drive voltages required by the vibrator. The system was calibrated after the experiments. A micrometer attached to the stimulator measured the displacements produced by standard drive voltages.

The following displacements were determined for each of the thirty successive stimuli: residual indentation at contact force just preceding each stimulus (the value before the first stimulus was taken as zero point) and maximal indentation at the end of the plateau phase. The difference of these two values represented the stroke amplitude.

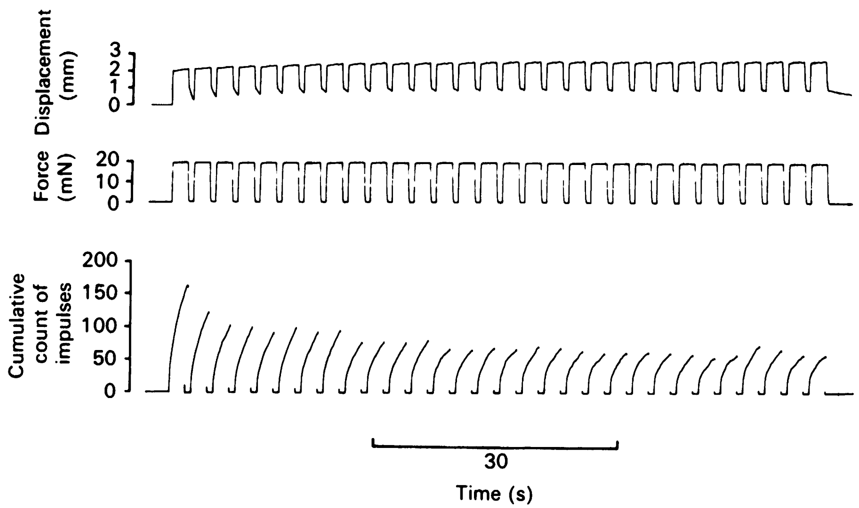


Fig. 2. Original recording of a standard train of thirty repetitive constant force stimuli to cutaneous s.a. I mechanoreceptors in a young rat. Upper trace, displacement; middle trace, force; lower trace, cumulative counts of nerve impulses per stimulus. Note the gradual increase in maximal and residual indentation with increasing stimulus number in order to maintain the stimulation force of 20 mN and the contact force of 0·5 mN.

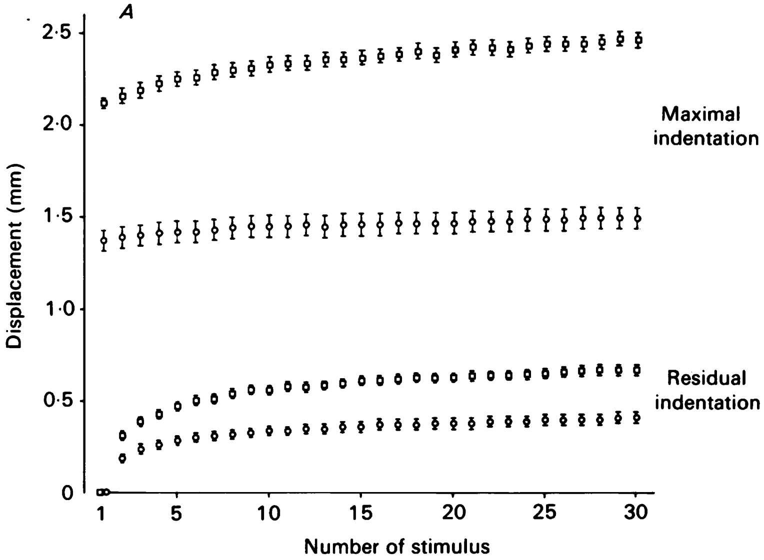
Evaluation

Individual units were subjected to several trains of thirty repetitive stimuli with a recovery time of at least 6 min between runs. In nine 'young' rats fifteen units were examined with a total number of fifty-one runs. The results were compared with those obtained from six 'adult' rats in fifteen units and forty-five runs. All results are expressed as mean ± S.E. of mean; for statistical analysis of the data Student's *t* test was used. The minimum requirement for significant differences was set at a significance level of $P \leqslant 0{\cdot}05$.

RESULTS

Displacements

On applying repetitive stimuli of constant force (Fig. 2) the maximal indentation required to maintain the same stimulation force of 20 mN generally increased with increasing stimulus number (Fig. 3*A*). Similarly, the residual indentation at a contact force between stimuli of 0·5 mN increased to an even larger extent (Fig. 3*A*). As a result the stroke amplitudes decreased throughout the train of thirty repetitive stimuli of constant force (Fig. 3*B*). Both age groups showed basically the same pattern of changes of the displacement parameters although there were great differences between the groups with respect to the absolute displacement values. The indentation at contact force before the first stimulus was taken as zero point of displacements for each individual train of repetitive stimulation. Thus, for the first stimuli the maximal indentation and the stroke amplitude were the same. Their mean value was 1·37 ± 0·06 mm in the adult rats and 2·12 ± 0·03 mm in the young rats. The mean residual indentations just preceding stimulus number 30 were 0·41 ± 0·03 mm and 0·67 ± 0·02 mm respectively. Also the maximal indentation of stimulus number 30 differed sharply with 1·50 ± 0·06 mm in the adult rats and 2·47 ± 0·04 mm in the



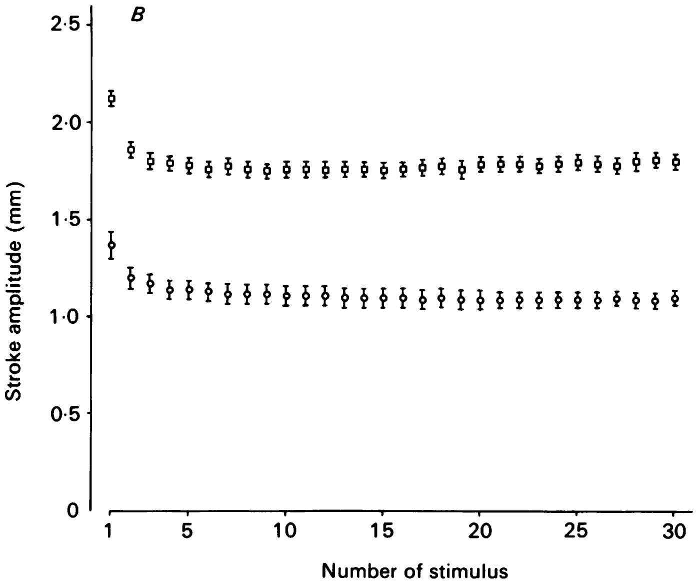


Fig. 3. Displacements needed to maintain a contact force of 0·5 mN (residual indentation) and the force of stimulation at 20 ± 0·5 mN (maximal indentation) plotted against ordinal number of stimulus for trains of thirty repetitive stimuli. *A*, residual and maximal indentation. *B*, stroke amplitude (difference between maximal and residual indentation). □, young rats (3–4 months); ○, adult rats (9–11 months). Comparison of the corresponding values between both groups are all statistically significant ($P \leqslant 0{\cdot}001$).

young group. Consequently, the resulting stroke amplitudes were 1·09 ± 0·04 mm and 1·80 ± 0·04 mm respectively. Comparing corresponding displacement values of the two age groups statistically the residual and maximal indentations as well as the stroke amplitudes throughout the entire train of thirty stimuli were different at a significance level of $P \leqslant 0{\cdot}001$ (see Fig. 3*A* and *B*).

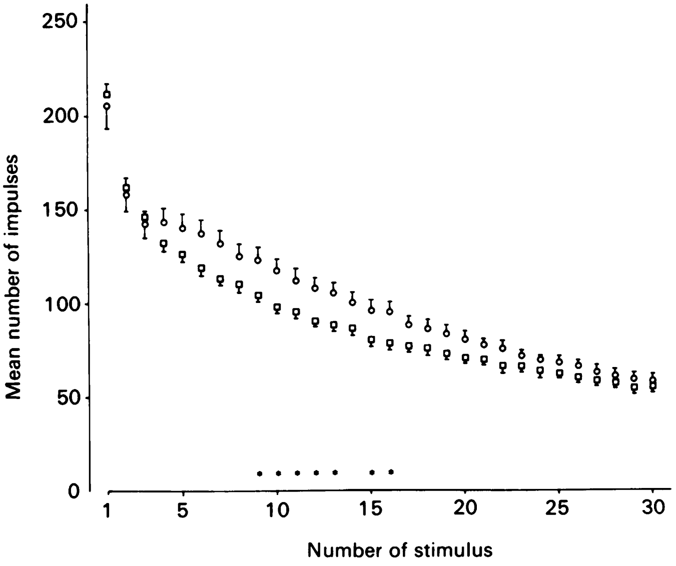


Fig. 4. Responsiveness of s.a. I receptors (in mean numbers of nerve impulses per stimulus) to trains of constant force stimuli (20 ± 0·5 mN) plotted against ordinal number of stimulus. □, young rats (3–4 months); ○, adult rats (9–11 months). Significant differences of corresponding values between groups are indicated by *; $P \leqslant 0{\cdot}05$.

Taking the values obtained for the first stimulus as 100 % the maximal indentation increased to 112 ± 3 % up to the 30th stimulus in the adult and to 117 ± 1 % in the young group, while stroke amplitudes for stimulus number 30 were 82 ± 2 % and 85 ± 1 % respectively of those for the first stimulus. There was no consistent pattern of significant differences for the relative values between the groups.

Nervous responses

In spite of the pronounced differences between the absolute displacement values obtained from both groups there were only minor differences between the nervous responses of receptors in young and adult rats (Fig. 4). Responses to the first stimulus were 206 ± 13 impulses in the adult rats and 211 ± 6 impulses in the young rats. Mean numbers of impulses decreased throughout the train of thirty stimuli in both groups in a very similar manner. For the last stimulus the mean responses were 58 ± 4 and 56 ± 3 impulses respectively. Significant differences at a level of $P \leqslant 0{\cdot}05$ between the two age groups could only be observed for the responses to stimuli number 9 to 13, 15 and 16.

DISCUSSION

In the present study slowly adapting type I cutaneous mechanoreceptors were subjected to trains of thirty feed-back controlled constant force stimuli. This mode of stimulation allowed determination of the displacement needed to maintain the force of individual stimuli at 20 mN as well as of the residual indentation of the skin at a low contact force of 0·5 mN between stimuli. The present results confirmed observations by Pubols (1982*b*) that residual indentation of the skin increased with increasing stimulus number. The largest increase in residual indentation during repetitive stimulation occurred during the first few stimuli. There was also a progressive but less pronounced increase in the maximal indentation. As a result the stroke amplitude decreased during the first few cycles rapidly reaching a nearly steady level around stimulus number 10. Previous investigators (Catton, 1970; Pubols, 1982*b*; Barker, Shepard & McDermott, 1982; Pubols & Maliniak, 1984) described a decrease in the responsiveness of cutaneous mechanoreceptors with increasing stimulus number during repetitive stimulation. This phenomenon was also observed with the pattern of stimulation employed in this study.

In the present results the maximal indentations increased in both age groups by about 15% from the first to the last stimulus in order to maintain the stimulation force of 20 mN. The residual indentations and stroke amplitudes showed similar relative changes throughout the train of thirty stimuli without significant differences between groups. The displacement values obtained in the adult rats were consistently reduced to $62 \pm 3\%$ of the respective values obtained in the young rats. These results indicate a linear decrease in skin compliance with age in the force range from 0·5 to 20 mN. In contrast, conditions other than ageing that alter the viscoelastic properties of the skin may not affect all displacement parameters to the same extent. For example in vitamin A deficiency, as described in detail in the accompanying paper (Baumann *et al.* 1986), the compliance of the skin was larger than in the (adult) control rats. The increase in residual indentation at 0·5 mN contact force was very close to the values obtained in the young rats in this study. But with the stimulation force of 20 mN the increase in compliance was less pronounced, and the maximal displacement was only about 40% above the control values and thus significantly below the corresponding values obtained in the young rats.

The group of adult rats in this study had an age of 9–11 months and can thus not be regarded as senile. The median life span of Sprague–Dawley rats is about 24 months (Curcio, McNelly, & Hinds, 1984). The observed changes in skin compliance in early adulthood were well in line with those reported in the literature. Agache *et al.* (1980) observed that stretchability of human skin *in vivo* decreases around the age of 30–35 years. Reduced stretchability of aged skin has been attributed to an extensive elastogenesis with the histological finding of large amounts of microfibrils in the papillary dermis. There is also a flattening of the interdigitations at the epidermo-dermal interface (Lavker, 1979). A decrease in the content of hyaluronic acid in the dermal extracellular space may be the reason for the reduced water-binding capacity of aged skin (Carlisle & Montagna, 1979).

In the present study the profound differences in some mechanical properties of the skin between young and adult rats were not matched by differences in nervous responsiveness of s.a. I receptors. This finding is a good complement to morphological studies in man showing only negligible changes in cutaneous nerves as well as appearance and number of Merkel discs (Sinclair, 1973). Furthermore, the present results are well in line with observations by Verrillo (1979), who reported unchanged sensitivity to low frequency stimulation in elderly as compared with young human subjects in contrast to a marked decrease in sensitivity in the high frequency range thought to be perceived through Pacinian corpuscles (Burgess & Perl, 1973).

Thus, it may be concluded that the design of touch corpuscles secures continued high responsiveness to force-related stimuli in spite of pronounced age-induced changes in the viscoelastic properties of the skin and underlying tissue.

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